A Model System based on Photodecompositions of Alkylcobaloximes for the Conversion of 1,2-Diols to Aldehydes catalysed by Diol Dehydrase

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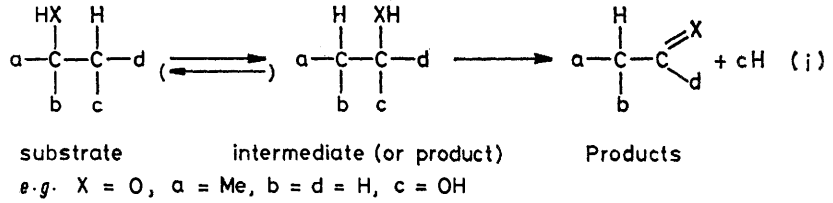
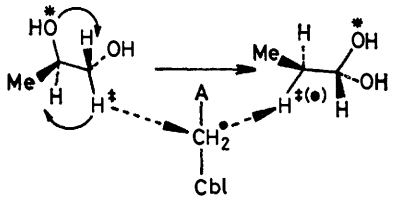
Attempts to model the conversion of 1,2-diols to aldehydes catalysed by the adenosylcobalamin-dependent enzyme diol dehydrase utilising the photohomolysis of alkylcobaloximes, have been carried out in two ways. First, the alkyl radical released from such photolysis has been shown to convert ethane-1,2-diol to CH_3CHO at pH 2 with an efficiency of *ca.* 10%. Second, photolysis of several compounds of the type $HOCH_2CHOH[CH_2]_n$-$Co(dmgH)_2(C_5H_5N)$ yields products (when n = 3 or 4) suggestive of the sequence: (a) photohomolysis followed by (b) an intramolecular 1,5-hydrogen shift (n = 3 or 4) or 1,6-hydrogen shift (n = 4) to produce a 1,2-dihydroxyalkyl radical which in turn undergoes (c) further transformations, including either an acid-catalysed 1,2-hydroxy shift [to give a 1-(dihydroxymethyl)alkyl radical], or acid-catalysed dehydration to $R^1\dot{C}HCOR^2$ (R^1 = Pr^n, R^2 = H, R^1 = H, R^2 = Bu^n, or R^1 = Bu^n, R^2 = H), finally yielding $R^1CH_2COR^2$. When n = 3, the exclusive carbonyl-containing product from the dihydroxyalkyl group is pentanal (*via* a 1,5-hydrogen shift). These results enable us to counter claims that radical chemistry cannot simulate the diol dehydrase reactions, and to provide a critique of the present discussion of models for adenosylcobalamin-dependent enzymatic reactions.

ENZYMES requiring adenosylcobalamin (AdoCbl,* a corrinoid coenzyme) as cofactor catalyse transformations of the type (i).[1] For diol dehydrase, the substrate is a 1,2-diol which is converted *via* a 1,1-diol to an aldehyde[2] [*e.g.* (*R*)- or (*S*)-propane-1,2-diol → propanal]. The mechanisms of these reactions, which we now summarize, are a subject of immense interest and persistent controversy.



(i)



SCHEME 1 1,2-Shifts in the conversion of propane-1,2- to -1,1-diol [detected by labelling experiments with ^{18}O(*) and ^2H or ^3H (‡ or ●)]

The results of some isotopic labelling studies[1-3] with diol dehydrase are shown in Scheme 1. As a consequence of these and other investigations, particularly those suggestive of intermediacy of radicals,[1] a pathway can be proposed for the reactions catalysed by diol dehydrase which is illustrated in Scheme 2 for propane-1,2-diol.[4]

The first step of this pathway; homolysis of the cobalt–carbon bond of AdoCbl to generate Cbl^{II} and an adenosyl radical, is a process known[5] to occur in the photolysis of AdoCbl and other alkylcobalamins. For this step to occur under physiological conditions, it is necessary to explain how the activation energy for cleavage of the Co–C bond of AdoCbl is sufficiently reduced. One possible explanation is that the enzyme acts like a rack, forcing the cleavage of the relatively weak Co–C bond in return for improved binding between the components of the cleaved coenzyme and enzyme. This action might be stimulated by the arrival of substrate at the active site. The resulting adenosyl radical then abstracts a hydrogen atom from a substrate molecule. Alternatively, the presentation of substrate at the active site might bring about a hydrogen transfer from the substrate to the adenosyl 5′-methylene group concerted with cleavage of the Co–C bond, thereby combining steps 1 and 2 (Scheme 2). The conversion of the substrate-derived radical ($CH_3CHOH\dot{C}HOH$ in Scheme 2) to product-related radical [$CH_3\dot{C}HCH(OH)_2$] is an imperfectly understood step for which several mechanisms have been proposed (*cf.* Scheme 3). Mechanism I[6] invokes organocobalt intermediates (A) and (B) (arising

* See *Biochemistry*, 1974, **13**, 1555 for abbreviations for corrinoids.

[1] Reviewed by R. H. Abeles and D. Dolphin, *Accounts Chem. Res.*, 1976, **9**, 114.

[2] B. Zagalak, P. A. Frey, G. L. Karabatsos, and R. H. Abeles, *J. Biol. Chem.*, 1966, **241**, 3028.

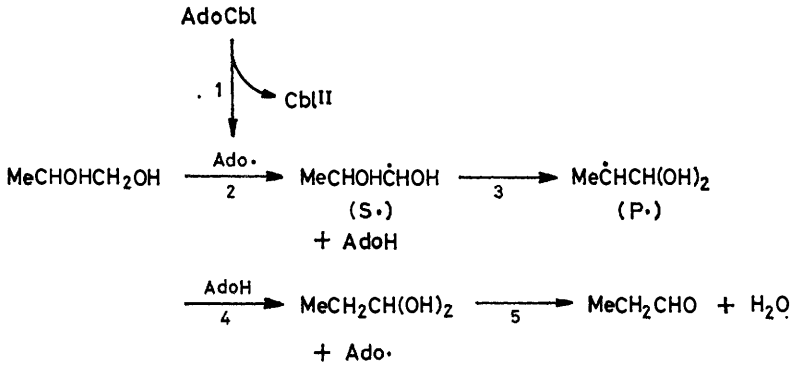
[3] J. Retey, A. Umani-Ronchi, and D. Arigoni, *Experientia*, 1966, **22**, 72; J. Retey, A. Umani-Ronchi, J. Seibl, and D. Arigoni, *ibid.*, p. 502.

[4] T. H. Finlay, J. Valinsky, K. Sato, and R. H. Abeles, *J. Biol. Chem.*, 1972, **247**, 4197.

[5] H. P. C. Hogenkamp, P. J. Vergamini, and N. A. Matwiyoff, *J.C.S. Dalton*, 1975, 2628.

[6] R. B. Silverman, D. Dolphin, and B. M. Babior, *J. Amer. Chem. Soc.*, 1972, **94**, 4028.

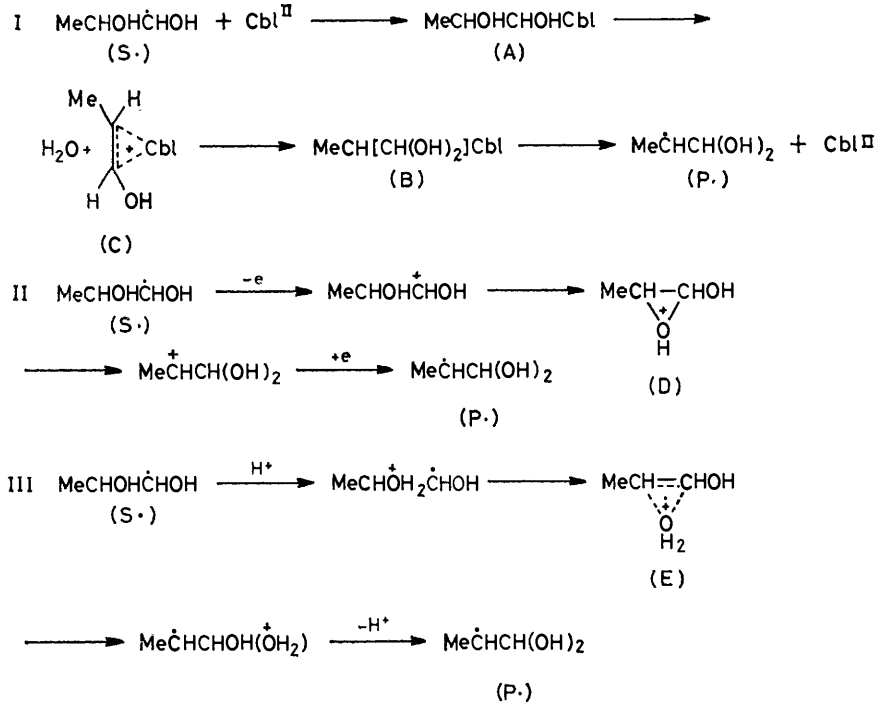
from the combination of S· and P· respectively, with Cbl^{II}) which are interconverted *via* a π-complex (C). species E as a possible intermediate connecting protonated S· and P·.



SCHEME 2 Pathway for AdoCbl-dependent enzymatic reactions (*cf.* ref. 5)

Mechanism II [7] requires electron transfer steps, which interconvert radicals S· and P· with carbocations S^+ and

Other mechanisms for diol dehydrase have been put forward by both Schrauzer [11] and Corey.[12] Their



SCHEME 3 Possible mechanisms I—III for the conversion of substrate-derived radical (S·) to product-related radical (P·)

P^+ respectively. S^+ could be transformed to P^+ *via* the protonated hydroxyepoxide (D). Mechanism III [8] regards the conversion of S· to P· as analogous to certain non-enzymatic reactions of radicals,[9] *e.g.* reaction

$$HOCH_2\dot{C}HOH \xrightarrow{H^+} \dot{C}H_2CHO + H_2O \qquad \text{(ii)}$$

(ii). We have discussed this mechanism in detail elsewhere [10] and have put forward the protonated bridged proposals (see below) avoid postulating radical intermediates since they regard these species as experimentally unproven in corrinoid-dependent enzymatic reactions. Model studies reported in this paper are relevant to the mechanism of action of diol dehydrase. We demonstrate (1) non-enzymatic transformations analogous to those catalysed by diol dehydrase, *i.e.* (iii), and (2) simulation of the regiospecific attack by diol dehydrase

[7] *Cf.* J. Halpern, *Ann. New York Acad. Sci.*, 1974, **239**, 2.
[8] B. T. Golding and L. Radom, *J.C.S. Chem. Comm.*, 1973, 939.
[9] B. C. Gilbert, J. P. Larkin, and R. O. C. Norman, *J.C.S. Perkin II*, 1972, 794; K. M. Bansal, M. Gratzel, A. Henglein, and E. Janata, *J. Phys. Chem.*, 1973, **77**, 16.
[10] B. T. Golding and L. Radom, *J. Amer. Chem. Soc.* 1976, **98**, 6331.
[11] G. N. Schrauzer and J. W. Sibert, *J. Amer. Chem. Soc.*, 1970, **92**, 1022.
[12] E. J. Corey, N. J. Cooper, and M. L. H. Green, *Proc. Nat. Acad. Sci., U.S.A.*, 1977, **74**, 811.

at C-1 of 1,2-diols. Preliminary accounts of part of this work have been published.[13] We have used alkylcobaloximes (1) (undergoing photohomolysis under

$$RCHOHCH_2OH \longrightarrow RCH_2CHO\ (+H_2O) \qquad (iii)$$

various conditions of pH) as models for alkylcobalamins in order to probe features of the pathway given in Scheme 2, having previously noted[13a] the similarity between the reactions catalysed by diol dehydrase and radical reactions of 1,2-diols initiated by hydroxyl radicals,[9] *e.g.* (iv).

$$HOCH_2CH_2OH \xrightarrow{HO\cdot} H_2O + HOCH_2\dot{C}HOH \longrightarrow H_2O + \dot{C}H_2CHO \qquad (iv)$$

It was anticipated that similar reactions could be brought about by the attack of alkyl radicals on 1,2-diols in view of the reactivity of Me· towards activated C–H bonds. If the derived 1-formylalkyl radical abstracted a hydrogen atom from a suitable source, the conversion of diol to aldehyde would be similar to the pathway supposed to operate with diol dehydrase. Schrauzer[14] has reported that thermolysis or photolysis of alkylcobalt compounds in the presence of ethane-1,2-diol produces erythritol but not acetaldehyde. These experiments purported to test the pathway of Scheme 2, and the failure to detect acetaldehyde was thought to be evidence against this pathway. However, all that these experiments actually show is that dimerisation of the radical $HOCH_2\dot{C}HOH$ under neutral conditions is faster than its conversion to $\dot{C}H_2CHO$. Others have shown this conversion to be both acid- and base-catalysed.[9] Law and Wood[15] have claimed that photolysis of AdoCbl in pH 7.4 buffer containing ethanolamine converts the latter to >80% acetaldehyde.

RESULTS AND DISCUSSION

Non-enzymatic Conversion of 1,2-Diols to Aldehydes initiated by Alkyl Radicals from Alkylcobaloximes.— Photolysis (λ 380 ± 15 nm) of methyl(aquo)cobaloxime (1a) (4×10^{-3}M) in the presence of molar ethane-1,2-diol

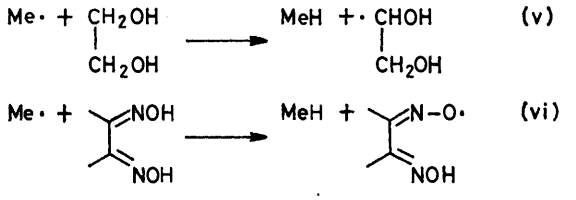
$$RCo(dmgH)_2L$$
(1)

a; R = Me, L = OH_2
b; R = $[CH_2]_3CHOHCH_2OH$, L = py
c; R = $[CH_2]_3CHOHCH_2OH$, L = OH_2
d; R = $[CH_2]_4CHOHCH_2OH$, L = py
e; R = $[CH_2]_2CHOHCH_2OH$, L = py
f; R = $[CH_2]_9CHOHCH_2OH$, L = py

dmgH = dimethylglyoxime monoanion; py = pyridine

at pH 2 (aqueous KCl–HCl) does indeed yield acetaldehyde as demonstrated (i) by colorimetric assay [*N*-methylbenzothiazolone (MBTH reagent)] or (ii) more reliably (see below) by addition of acidic 2,4-dinitrophenylhydrazine followed by isolation of acetaldehyde dinitrophenylhydrazone (DNP) by extraction and chromatography. The yields of aldehyde indicated by MBTH assay were variable up to 33% based on (1a) (see Experimental section). The yield of acetaldehyde DNP estimated from a ^{1}H n.m.r. spectrum of crude DNP (immediately after extraction with CH_2Cl_2) was *ca.* 7%. (For this particular experiment, a MBTH assay gave 15—20%.) The yield of pure isolated acetaldehyde DNP was 5% (estimated by u.v.–visible spectroscopy); MBTH assay showed that with a fixed concentration of (1a) (4×10^{-3}M), as the concentration of ethane-1,2-diol was increased, so did that of product acetaldehyde, presumed to be the only aldehyde formed (see Experimental section). DNP assay showed that with 0.1M-ethane-1,2-diol and 4×10^{-3}M (1a) practically no acetaldehyde was formed. Examination by t.l.c. of the crude DNPs extracted after addition of reagent at the end of a photolysis showed, besides a spot corresponding to acetaldehyde DNP, a spot of slightly higher R_F. This substance was isolated by preparative layer chromatography and shown to be the DNP of biacetyl monooxime (see Experimental section), originating presumably from dimethylglyoxime (dmgH) released in the photolysis.

It is known that alkylcobaloximes efficiently decompose to an alkyl radical, aquated cobalt(II), and free dimethylglyoxime on photolysis under acidic conditions [*e.g.* ϕ_{380nm} 0.17 for (1a) at pH 2].[16] The alkyl radical attacks ethane-1,2-diol, giving methane and $HOCH_2\dot{C}HOH$, which is converted to $\dot{C}H_2CHO$ in an acid-catalysed manner. The radical $\dot{C}H_2CHO$ then abstracts a hydrogen atom from a suitable source. This source is either ethane-1,2-diol or dmgH (possibly unco-ordinated): the latter is expected to be particularly reactive towards alkyl radicals because the hydrogen abstraction product is a conjugated nitroxide radical. We have previously shown[16] irradiation of (1a) in D_2O at pD 2 gives exclusively CH_3D from attack of Me· on dmgD. The high reactivity of oximes towards one-equivalent oxidants such as Ce^{IV} is well established.[17] The competition between steps (v) and (vi) prevails in all our experiments.



k_{vi} is probably near the value for diffusion control, whilst k_v may be *ca.* 10^7 dm^3 mol^{-1} s^{-1} judging by the data for Me· oxidation of other organic substrates.[18]

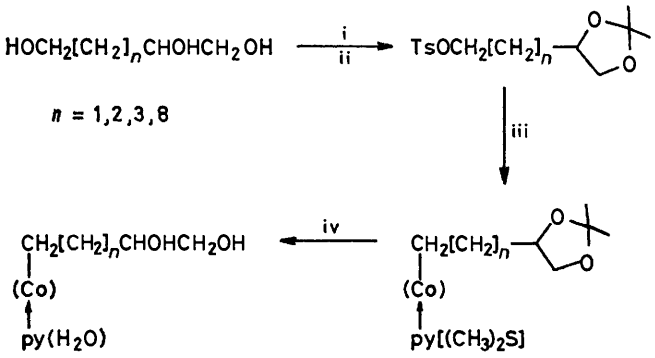
[13] (*a*) B. T. Golding, T. J. Kemp, E. Nocchi, and W. P. Watson, *Angew. Chem. Internat. Edn.*, 1975, **14**, 813; (*b*) B. T. Golding, C. S. Sell, and P. J. Sellars, *J.C.S. Chem. Comm.*, 1976, 773.
[14] G. N. Schrauzer, W. J. Michaely, and R. J. Holland, *J. Amer. Chem. Soc.*, 1973, **95**, 2024.
[15] P. Y. Law and J. M. Wood, *Biochim. Biophys. Acta*, 1973, **321**, 382.
[16] B. T. Golding, T. J. Kemp, P. J. Sellars, and E. Nocchi, *J.C.S. Dalton*, 1977, 1266.
[17] J. R. Thomas, *J. Amer. Chem. Soc.*, 1964, **86**, 1446.
[18] B. C. Gilbert, R. O. C. Norman, G. Placucci, and R. Sealy, *J.C.S. Perkin II*, 1975, 885.

Consequently [ethane-1,2-diol] needs to be *ca.* 10^3 [oxime] to give even 50% of aldehyde precursor radical based on (1a). The presence of the oxime as an effective hydrogen-atom source curtails the effect of the chain process (vii) + (viii).[19]

$$\dot{C}H_2CHO + HOCH_2CH_2OH \longrightarrow CH_3CHO + HO\dot{C}HCH_2OH \quad (vii)$$

$$HO\dot{C}HCH_2OH \longrightarrow \dot{C}H_2CHO, \textit{ etc.} \quad (viii)$$

In order to improve the efficiency of the transfer of a hydrogen atom from 1,2-diol to alkyl radical and thereby increase the yield of aldehyde, we have synthesized alkylcobaloximes which contain a 1,2-diol as part of the alkyl group. Now, after photocleavage of the Co–C bond, hydrogen transfer from diol to alkyl radical can take place intramolecularly. Providing that there are no stereoelectronic constraints, this hydrogen transfer could be a very efficient process. Five dihydroxyalkylcobaloximes (1b—f) have been prepared by the general method shown in Scheme 4. It was convenient to synthesize these cobaloximes with L = pyridine. To show that pyridine did not influence the outcome of photodecompositions, the aquocobaloxime (1c) was also prepared for comparison with (1b).

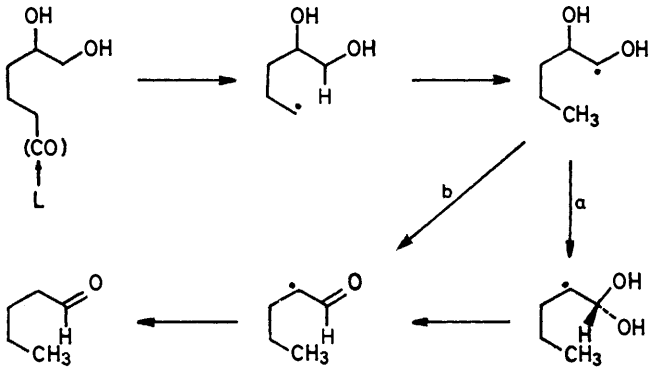


SCHEME 4 Preparative route to cobaloximes (1b—f). Reagents: i, Me_2CO–H^+; ii, TsCl–py; iii, cobaloxime(I); iv, aqueous HCl

Photodecompositions of Cobaloximes (1b *and* c) *under Acidic Conditions.*—By analogy with the behaviour of simple alkylcobaloximes,[16] it was expected that photolyses of cobaloximes (1b and c) under neutral conditions would afford the corresponding alkene 4,5-dihydroxypent-1-ene, whilst under acidic conditions this alkene might accompany pentane-1,2-diol and decane-1,2,9,10-tetraol. It was also anticipated (see above) that intramolecular hydrogen transfer in the 4,5-dihydroxypentyl radical could eventually yield pentanal (from 1,5-hydrogen transfer) and/or pentan-2-one (from 1,4-hydrogen transfer).

Initial photolyses of (1b) were carried out using filtered light (λ 380 $\pm$ 15 nm). However, Pyrex-filtered light gave the same products from (1b) in a much shorter time and was therefore used for the majority of photolyses. Unless mentioned otherwise, all photolyses were performed anaerobically.

The photolysis of (1b) in 0.1M-acetic acid yielded four products based on the dihydroxypentyl group: pentanal (isolated and characterised as its DNP derivative; see Experimental section), 4,5-dihydroxypent-1-ene (30%), pentane-1,2-diol (20%), and decane-1,2,9,10-tetraol (20%). A possible mode of formation of pentanal is shown in Scheme 5. The initial photohomolysis of the



SCHEME 5 Possible mechanisms for the formation of pentanal from 4,5-dihydroxypentylcobaloximes

Co–C bond of (1b) is followed by isomerisation of the resulting 4,5-dihydroxypentyl radical to a 1,2-dihydroxypentyl radical. This step takes place *via* an intramolecular 1,5-hydrogen shift, a process evidently preferred to a 1,4-hydrogen shift as no pentan-2-one is formed (*cf.* Figure which shows no peak at δ 2.02 corresponding to the 1-methyl group of the major isomer of pentan-2-one DNP). Further transformation of the 1,2-dihydroxypentyl radical could involve either an acid-catalysed 1,2-hydroxy shift to yield the 1-(dihydroxymethyl)butyl radical (pathway a, Scheme 5) or acid-catalysed elimination of water to give the 1-formylbutyl radical (pathway b). The terminal radical then abstracts a hydrogen atom from its environment to give either pentane-1,1-diol or pentanal. Which of the pathways (a or b) is correct can, in principle, be established by an oxygen-labelling experiment. In pathway a, an oxygen label at C-2 of 1,2-dihydroxypentyl will be transferred to C-1 and therefore half retained in the product pentanal; in pathway b, the label will be lost to water. As discussed elsewhere,[10] a mechanism involving an acid-catalysed 1,2-hydroxy shift is an attractive possibility for diol dehydrase in accord with experimental evidence obtained with the enzyme (see above).

Pentanal was always detected as a product in a large number of photolyses of (1b and c) and its yield was reproducible under standardised conditions. The results of a study of the effects of changing buffer, concentration of (1b and c), temperature, and pH on the yield of pentanal are given in Table 1. The yield of pentanal is diminished by increasing concentration of (1c) [this may selectively favour one of the competitive pathways for disappearance of the 4,5-dihydroxypentyl radical, *i.e.* dimerisation to decane-1,2,9,10-tetraol], decreasing

[19] P. J. Venter, H. J. van der Linde, and R. Basson, *J.C.S. Chem. Comm.*, 1972, 187.

temperature, changing from acetate to citrate–phosphate buffer, and raising pH. The production of pentanal is not appreciably subject to general acid catalysis because

TABLE 1

Effect of varying buffer, concentration, pH, and temperature on yield of pentanal from photolysis of (1b or c)

Concentration (mM)	T/°C	Buffer	pH	Yield of pentanal (%) [a]
5.82	Room	0.1M-HOAc	2.89	4.1
5.79	Room	0.1M-HOAc	2.89	3.2
2.42	Room	0.1M-HOAc	2.89	12.0
2.46	Room	0.1M-HOAc	2.89	10.0
2.28	Room	0.1M-HOAc	2.89	11.3
2.40	0	0.1M-HOAc	2.89	6.4
2.56	45	0.1M-HOAc	2.89	10.6
2.10	Room	0.1M-HOAc	2.89	9.9
2.12	Room	0.1M-HOAc	2.89	10.2
2.12	Room	0.1M-HOAc	2.89	10.2 [b]
1.88	Room	1.0M-HOAc	2.37	4.0
2.32	Room	1.0M-HOAc	2.37	5.5
2.01	Room	Citrate–phosphate	4.25	8.2
2.38	Room	Citrate–phosphate	3.62	5.6
2.32	Room	Citrate–phosphate	2.92	6.1
1.87	Room	Citrate–phosphate	2.48	4.5
2.31	Room	0.1M-HOAc–NaOAc	4.14	4.6
2.18	Room	0.1M-KCl–HCl	2.89	†
0.96	Room	0.2M-KCl–HCl	2.00	9.6

[a] Yields are not corrected for efficiency of recovery (70—80%). [b] Irradiation time 2 h [*ca.* 12 × that required to completely decompose (1b)].

† Inadequate buffer.

increasing the concentration of acetic acid actually lowers the yield of pentanal.

Control experiments showed the efficiency of recovery of pentanal as its DNP to be 70—80% (yields have *not* been corrected for this factor). Numerous control experiments (see Experimental section and ref. 3*a*) to test for alternative routes to pentanal failed to reveal pentanal DNP (>0.2% yield of DNP would have been detected by t.l.c.). The possibility that pentanal arose by acid-catalysed, pinacol-like rarrangement of pentane-1,2-diol [a product of photolysis of (1b or c), see below] was eliminated by incubating pentane-1,2-diol with 0.1M-acetic acid and then treating with DNP reagent for times at least as long as those used in photolyses. Repetition of this experiment in the presence of cobalt(II) ion and dimethylglyoxime also gave no pentanal. Photolysis of n-propyl(aquo)cobaloxime (1g) in 0.1M-acetic acid containing pentane-1,2-diol (3×10^{-3} or 1.00M) did not give pentanal. The first of these experiments simulates the situation during photolysis of (1c) whereby some (1c) is being decomposed in an environment containing a low concentration of pentane-1,2-diol. Formation of pentanal by this intermolecular route should be inefficient by analogy with the photodecomposition of (1a) in ethane-1,2-diol. Note that none of the routes to pentanal alternative to Scheme 5 explain why pentanal production is not accompanied by pentan-2-one, yet hexan-2-one is the predominant product from (1d) (see below).

When (1b) was irradiated in 0.1M-acetic acid in the presence of oxygen or hydroquinone, the yield of pentanal fell significantly. These radical scavengers presumably compete for the 4,5-dihydroxypentyl radical.

Photodecomposition of (1d—f).—Photolysis of a 2×10^{-3}M solution of (1d) in 0.1M-acetic acid gave a mixture of hexanal DNP and hexan-2-one DNP in a total yield of 20% (u.v.–visible assay). The ^{1}H n.m.r. spectrum of this mixture is very similar to a spectrum of authentic hexan-2-one DNP. In another experiment, the photolysate from (1d) was extracted with pentane and the extract was examined by g.l.c. (QF1 column). This showed two peaks in the ratio $(3.8 \pm 0.3) : 1$. The more intense peak corresponded to hexan-2-one, whilst the smaller peak matched hexanal. In a further experiment, the photolysate from (1d) was extracted with CCl_4. The i.r. spectrum of this solution was compared with spectra of standard solutions of hexanal and hexan-2-one in CCl_4, indicating a combined yield of 20%. Photolysis of (1d) at pH 3 also yielded hexane-1,2-diol and 5,6-dihydroxyhex-1-ene (identified by ^{1}H n.m.r. spectroscopy), whilst at pH 7 only 5,6-dihydrohex-1-ene was obtained (*no* hexanal or hexan-2-one).

The experiments with cobaloximes (1b—d) indicate that in radicals of the type $\dot{C}H_2[CH_2]_nCHOHCH_2OH$, the 1,5-hydrogen shift is preferred over the 1,6 shift, which is favoured over the 1,4 shift. It could, therefore, be predicted that (1e) might not yield any carbonyl product derived from its dihydroxybutyl group. Indeed, subjecting (1e) to the usual conditions of photolysis did not give detectable butanal (product formed *via* 1,4-hydrogen shift) or butan-2-one (product formed *via* 1,3-hydrogen shift). Finally, (1f) was examined and, as expected, neither undecanal or undecan-2-one could be detected after its photolysis in 0.1M-acetic acid. In this case, hydrogen transfer is much less favourable because it requires a high negative entropy of activation and the twelve-membered ring transition state will be destabilised by torsional interactions.

Our observations about intramolecular hydrogen transfers in the dihydroxyalkyl radicals derived from (1b—f) parallel the results of studies with other radicals where 1,*n*-hydrogen shifts can occur.[20] The preference for 1,5-hydrogen shifts in such systems allows us to mimic the regiospecificity shown by diol dehydrase towards 1,2-diols (exclusive abstraction of a hydrogen atom from C-1). Note that hydroxyl radicals attack propane-1,2-diol at all three positions,[9] whereas the 4,5-dihydroxypentyl radical from (1b—c) rearranges specifically to the 1,2-dihydroxypentyl radical.

Photolysis of (1b) at pH 7 or 9 failed to give pentanal. At pH 7, 4,5-dihydroxypent-1-ene (*ca.* 30%) (but neither pentane-1,2-diol nor decane-1.2,9,10-tetraol) was formed.

[20] R. Kh. Freidilina and A. B. Terent'ev, *Accounts Chem. Res.*, 1977, **10**, 9.

This behaviour parallels that of simple alkyl(aquo)-cobaloximes [16] which give at pH 7 exclusively alk-1-ene, hydrogen and bis(aquo)cobaloxime(II). The latter two products are believed to be derived from hydrido(aquo)-cobaloxime, which is formed directly from the excited alkyl(aquo)cobaloxime. Evidently, only in the presence of acid is the initial excited state converted to a ' radical-pair ' configuration which breaks down with *ca.* 20% efficiency to radical-derived products.

Concluding Comments.—The experiments reported in this paper are offered as a model system for the AdoCbl-dependent enzyme diol dehydrase. In particular, the photoconversions of (1b and c) to pentanal at pH 3 simulate many of the features of the mechanism for diol dehydrase given in Scheme 1. The primary radical 4,5-dihydroxypentyl released from (1b or c) is visualised as playing a role analogous to the adenosyl radical in diol dehydrase, *viz.* effecting selective hydrogen atom abstraction from C-1 of a 1,2-diol. The occurrence of 1,5-hydrogen shifts in the conversions of (1b and c) to pentanal and (1d) to hexan-2-one are supported by deuterium-labelling studies.[21] The 1,2-dihydroxypentyl radical derived from 4,5-dihydroxypentyl is converted to either 1,1-dihydroxypentyl or 1-formylbut-1-yl (*cf.* Scheme 5) and thence to pentanal (deuterium-labelling studies show that the hydrogen atom acquired by 1-(dihydroxymethyl)butyl or 1-formylbutyl is derived from water *via* dmgH).[22] Detailed understanding of these steps must await further studies.

Schrauzer [23] has frequently questioned the so-called ' free radical mechanism ' discussed in this paper, and has suggested, on the basis of his model studies, an alternative mechanism for diol dehydrase, involving cobalamin(I) as an intermediate. Recently, Corey *et al.*[12] have adopted a ' sceptical position ' with regard to the ' free radical mechanism ' and have proposed a multi-step mechanism for diol dehydrase which features an assortment of σ-alkyl and hydridocobalt intermediates. However, one step in this scheme does not accommodate the fact that butane-2,3-diol is a substrate for diol dehydrase.[24] Furthermore, there is no experimental evidence for the intermediacy of σ-alkyl or hydridocobalt species in AdoCbl-dependent reactions.

The terminology ' free radical mechanism ' in the context of AdoCbl-dependent reactions is simplistic, for it implies the dissociation of radical intermediates from the enzyme, which is not envisaged by its proponents. It is presumed that the radicals are bound by the protein, *e.g.* by hydrogen-bonding interactions, and are possibly engaged in a long-range stabilising interaction with cobalamin(II). Notwithstanding criticisms, we believe that the radical mechanism of Scheme 1 provides the best interpretation of available experimental evidence and is supported by the model studies described in this paper.

EXPERIMENTAL

All solvents were AnalaR or redistilled laboratory reagents. Ether, dichloromethane, and light petroleum (b.p. 40—60°) used for chromatography of DNPs were freed from carbonyl-containing impurities by shaking 1 dm^3 of solvent with acidic 1% 2,4-dinitrophenylhydrazine solution (2 × 25 cm^3), water (25 cm^3), and brine (25 cm^3). After drying and distillation, each solvent was stored in a stoppered dark glass bottle.

0.4% Dinitrophenylhydrazine solution was made up from 2,4-dinitrophenylhydrazine (2 g, twice recrystallised from AnalaR ethyl acetate) in AnalaR concentrated H_2SO_4 (10 cm^3) diluted to 500 cm^3 with doubly distilled water. The resulting solution was stored in darkness and filtered immediately before use.

The most active form of silica gel for column chromatography was Merck 7754 purified by heating the commercial sample (100 g) twice with AnalaR concentrated HCl (250 cm^3) on a steam-bath for 4 h. After washing with water (1.5 dm^3), methanol (250 cm^3), and chloroform (250 cm^3), the product was activated by heating at 150° for >24 h just before use.

Kieselgel plates were used for t.l.c. with the following solvent systems: 1, 10% methanol in CH_2Cl_2; 2, 1 : 1 ether–light petroleum (b.p. 40—60°).

DNPs were visualised at 254 nm (Camag TL 900/u u.v. lamp). Other spots were detected by spraying with 35% H_2SO_4 and charring. Buffer solutions were prepared in doubly distilled water. Their pH was determined with a Radiometer 4 pH meter.

^1H N.m.r. spectra were recorded either at 60 MHz (Perkin-Elmer R12, 37°) for *ca.* 0.3M solutions or at 90 MHz (Bruker WH 90) for more dilute solutions. I.r. spectra were recorded on a Perkin-Elmer 257 instrument, using 0.2 mm NaCl cells and a polystyrene film for calibration. Absorbances (u.v.–visible) of DNPs were measured on a calibrated Unicam SP 500 spectrometer.

Pentane-1,2-diol.—This was prepared by treating pent-1-ene with 50% hydrogen peroxide and a catalytic amount of osmium tetraoxide in t-butyl alcohol (essentially in the manner described for dihydropyran).[25] (*N.B.* There is no need to employ anhydrous H_2O_2 in t-butyl alcohol.) After removing solvent, the residue was fractionally distilled to afford pentane-1,2-diol (35%), b.p. 97° at 10 mmHg (lit.,[26] 109° at 14 mmHg). A further fractional distillation gave pentane-1,2-diol pure by ^1H n.m.r. spectroscopy.

Decane-1,2,9,10-tetraol.—Deca-1,9-diene was converted (2 mol. equiv. *m*-chloroperbenzoic acid in CH_2Cl_2 for 16 h at room temperature) to 1,2,9,10-diepoxydecane (95%), b.p. 66° at 0.02 mmHg (lit.,[27] b.p. 150° at 30 mmHg). Hydrolysis of the di-epoxide in THF–aqueous $HClO_4$ (based on the procedure in ref. 28) gave *decane-1,2,9,10-tetraol* as a waxy solid (81%). Recrystallisation from methanol and then chloroform gave an analytical sample, plates, m.p. 112°, δ(CD_3OD) 1.39br (12 H, s, 6 × internal CH_2), and 3.46 (6 H, s + other nearby peaks, 2 × C*H*OH

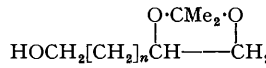
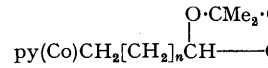
[21] B. T. Golding, C. S. Sell, and P. J. Sellars, *J.C.S. Chem. Comm.*, 1977, 693.
[22] P. J. Sellars, unpublished result.
[23] G. N. Schrauzer, *Angew. Chem. Internat. Edn.*, 1977, **16**, 233.
[24] W. W. Bachovchin, R. G. Eagar, K. W. Moore, and J. H. Richards, *Biochemistry*, 1977, **16**, 1082.
[25] C. D. Hurd and C. D. Kelso, *J. Amer. Chem. Soc.*, 1948, **70**, 1484.
[26] A. Copet, P. Fierens-Snoeck, and H. van Risseghem, *Bull. Soc. chim. France*, 1951, 902.
[27] J. L. Everett and G. A. R. Kon, *J. Chem. Soc.*, 1950, 3131.
[28] R. J. Anderson, C. A. Henrick, J. B. Siddall, and R. Zurfluh, *J. Amer. Chem. Soc.*, 1972, **94**, 5379.

and CH_2OH) (Found: C, 58.15; H, 10.75. $C_{10}H_{22}O_4$ requires C, 58.2; H, 10.75%). This substance, at least in crude form, is probably a mixture of diastereoisomers.

Alkane-1,2,n-*triols* (n = 4—6 *or* 11).—Butane-1,2,4-triol and hexane-1,2,6-triol were commercially available materials used directly. Pentane-1,2,5-triol was prepared from 2-hydroxymethyltetrahydrofuran.[29] It was found that 50(H) (2 g, 2 × excess) was added and the mixture was stirred until neutral (10 min). Filtration and removal of solvent gave a residual oil which was fractionally distilled to afford pentane-1,2,5-triol (11.3 g, 73%), b.p. 134—140° at 0.08—0.11 mmHg (lit.,[30] 167—170° at 0.5—1 mmHg). Undecane-1,2,11-triol was prepared from 11-hydroxyundec-1-ene essentially as described for the hydroxylation of

TABLE 2

Spectral and analytical data for cobaloximes (1b and d—f) and their precursors

				py(Co)$CH_2[CH_2]_n$-$CHOHCH_2OH$
$n = 1$	47%; b.p. 114° at 27 mmHg (lit.,[31] 109—110° at 20 mmHg)	67%; oil; δ (neat) 1.24 (6 H, s, Me_2), 1.83 (2 H, q, $CHCH_2CH_2$), 2.37 (3 H, s, MeAr), 3—4.5 (5 H, m, $CHCH_2$ and CH_2OSO_2), 7.39 and 7.83 (4 H, ABq, 4 × HAr)	65%; recryst. from $CHCl_3$–hexane (1 : 2); δ 1.30 (6 H, s, Me_2), 1—2 (4 H, m, $CoCH_2CH_2$), 2.14 (12 H, s, dmgMe), 3—4.1 (3 H, m, $CHCH_2$) + 5 pyH (Found: C, 47.95; H, 6.55; N, 13.8. $C_{20}H_{34}CoN_5O_6$ requires C, 48.3; H, 6.48; N, 14.1%)	81%; recryst. from ethanol; δ (CD_3OD) 2.10 (12 H, s, dmgMe), 3—3.5 (3 H, m, $CHCH_2$) + 5 pyH (Found: C, 44.35; H, 6.2; N, 15.3. $C_{17}H_{28}CON_5O_6$ requires C, 44.65; H, 6.17; N, 15.3%)
$n = 2$	See text	88%; oil; δ 1.31, 1.35 (6 H, 2 × s, Me_2), 1.65 (4 H, m, 1′ and 2′-CH_2), 2.44 (3 H, s, MeAr), 3.3—4.3 (5 H, m, ring CH, CH_2, CH_2OSO_2), 7.35 and 7.80 (4 H, ABq, 4 × HAr)	70%; recryst. from CH_2Cl_2–cyclohexane; δ 1.32 and 1.36 (6 H, 2 × s, Me_2), 1—2 (6 H, m, $CoCH_2CH_2$-CH_2), 2.10 (12 H, s, dmgMe), 3—4.1 (3 H, m, $CHCH_2$) + 5 pyH	65%; recryst. from CH_2Cl_2; δ 1.0 (2 H, m, $CH_2CH_2CH_2$), 1.32 (2 H, t, CH_2CHOH), 1.61 (2 H, t, $CoCH_2$), 2.13 (12 H, s, dmgMe), 3.2—3.8 (3 H, m, $CHOHCH_2OH$) + 5 pyH (Found: C, 45.75; H, 6.4; N, 14.8. $C_{18}H_{30}CoN_5O_6$ requires C, 45.85; H, 6.4; N, 14.85%)
$n = 3$	92%; b.p. 63° at 0.01 mmHg (lit.,[33] 118° at 5 mmHg)	79%; oil; δ 1.32, 1.35 (6 H, 2 × s, Me_2), 1.1—1.6 (6 H, m, $CH_2CH_2CH_2$), 2.43 (3 H, s, MeAr), 3.3—4.3 (5 H, m, ring CH, CH_2, CH_2OSO_2), 7.35 and 7.79 (4 H, ABq, 4 × HAr)	97%; δ 1.36 (6 H, s, Me_2), 0.7—1.8 (8 H, m, 4 × CH_2) 2.13 (12 H, s, dmgMe), 3.45 (1 H, t, ring CH), 3.98 (2 H, m, ring CH_2) + 5 pyH (Found: C, 50.15; H, 6.95; N, 13.2. $C_{22}H_{38}CoN_5O_6$ requires C, 50.3; H, 6.9; N, 13.35%)	97%; recryst. from $CHCl_3$–hexane (1 : 2); δ 0.9br (2 H, $COCH_2CH_2$), 1.3br (4 H, 3- and 4-CH_2), 2.11 (12 H, s, dmgMe), 3.5 (C H, m, CHOH and CH_2OH) + 5 pyH (Found: C, 47.0; H, 6.7; N, 14.25. $C_{19}H_{32}CO$-N_5O_6 requires C, 47.0; H, 6.64; N, 14.45%)
$n = 8$	78%; solid used directly in tosylation	80%; m.p. 44—45° [from light petroleum (b.p. 40—60°)]; δ (CCl_4) 1.25 and 1.31 (6 H, 2 × s, Me_2), 1.2—1.9br (16 H, m, 8 × CH_2), 2.44 (3 H, s, MeAr), 3.4 (1 H, d, CHO), 3.97 (4 H, t, 2 × CH_2O), 7.37 and 7.98 (4 H, ABq, 4 × HAr) (Found: C, 62.85; H, 8.45; S, 8.2. $C_{21}H_{34}O_5S$ requires C, 63.2; H, 8.5; S, 8.1%)	Crude product used directly for hydrolysis	20%; recryst. from CH_2Cl_2; δ 0.6—1.9 (16 H, m, 8 × CH_2), 2.05 (12 H, s, dmgMe), 3.3—3.9 (3 H, m, $CHCH_2$) + 5 pyH (Found: C, 51.95; H, 7.45; N, 11.95. $C_{24}H_{42}CoN_5O_6$ requires C, 51.9; H, 7.6; N, 12.6%)

* Proton resonances for pyridine and hydroxy groups are not given. Typical values are [(1b) in $CDCl_3$]: δ 7.31 (2 H, t, 2 × H-3), 7.73 (1 H, t, H-4), 8.57 (2 H, d, 2 × H-2), 17.8br (*ca.* 2 H, bridging OH), position of side-chain OH groups uncertain. {^{1}H} ^{13}C N.m.r. spectrum of (1b) in $CDCl_3$; δ 12.09 (dmgMe), 26.26 ($CH_2CH_2CH_2$) 34.19 ($CH_2CH_2CH_2$), 66.62 (CH_2OH), 71.63 (CHOH), 125.25, 137.53, 149.62, and 150.01 (pyC and dmgC).

saponification of 1,2,5-triacetoxypentane could be more efficiently carried out using catalytic sodium methoxide in methanol rather than the procedure described.[29a] Thus, the triacetate (31.8 g, 0.129 mol) in anhydrous methanol (250 cm^3) was treated with 2M-sodium methoxide in methanol (5 cm^3). After 72 h at room temperature Amberlite IRC dihydropyran.[25] The crude triol from evaporation of the reaction mixture crystallised and was recrystallised from ethyl acetate (5 cm^3 g^{-1}) to give the triol (78%), δ(CD_3OD) 1.1—1.8br (8 × CH_2) and 3.2—3.7 (m, CHOH and 2 × CH_2OH).

Preparation of Dihydroxyalkyl(pyridine)cobaloximes (1b *and* d—f).*—*Typical procedure.* 4-(3-Hydroxypropyl)-2,2-

* See Table 2 for spectroscopic data and other information not given in the text.

[29] (*a*) C. L. Wilson, *J. Chem. Soc.*, 1945, 48; (*b*) H. Hayashi and K. Nakanishi, *J. Amer. Chem. Soc.*, 1973, **95**, 4081.

[30] O. Grummitt, J. A. Stearns, and A. A. Arters, *Org. Synth.*, 1955, Coll. Vol. III, p. 833.

[31] D. Bertini and J. Perronnet, *Bull. Soc. chim. France*, 1964, 564.

dimethyl-1,3-dioxolan. Pentane-1,2,5-triol (11.7 g, 0.0975 mol) and toluene-*p*-sulphonic acid (0.3 g) in acetone–light petroleum (b.p. 40—60°) (1 : 1; 60 cm³) was refluxed in a Dean–Stark apparatus for 24 h after which all water had been azeotroped. The solvents were removed and the residue was taken up in ether. The ethereal solution was washed with aqueous sodium carbonate, dried (Na_2CO_3), and the ether was removed. The residual oil was distilled to give the acetal (10.7 g, 69%), b.p. 64—65° at 0.06 mmHg (lit.,[32] 117—118° at 12 mmHg).

2,2-Dimethyl-4-(3-*p*-tolylsulphonyloxy)-1,3-dioxolan. To the above alcohol in dry pyridine (3 ml per g alcohol) at −15° was added dropwise purified [33] toluene-*p*-sulphonyl chloride (1.1 molar excess) in dry pyridine (2.5 cm³ per g chloride) with stirring. The cooling bath was removed, and the mixture was stirred at room temperature until precipitation of pyridinium hydrochloride ceased (*ca.* 1 h). The mixture was poured into ice-cold 2M-hydrochloric acid (final pH 1) and the tosylate was immediately extracted with ether. The combined ether extracts were washed with 2M-hydrochloric acid, aqueous sodium carbonate, and brine, and then dried. Removal of the ether gave the tosylate as an oil (88%) which was used directly in the next stage.

4,5-Dihydroxypentyl(pyridine)cobaloxime. A stirred mixture of cobalt(II) chloride hexahydrate (3.0 g, 12.5 mmol) and dimethylglyoxime (2.90 g, 25 mmol) in methanol (35 cm³) at room temperature was deoxygenated by argon bubbling. The reaction vessel was a three-necked round bottomed flask fitted with a gas inlet tube, serum cap, and air-condenser with gas bubbler. Whilst maintaining an argon atmosphere, 50% (w/v) deoxygenated aqueous sodium hydroxide (1.32 cm³, 25 mmol NaOH) and pyridine (0.80 ml, 0.75 g, 12.5 mmol) were added by syringe. After cooling the resulting dark brown solution to −10°, sodium borohydride (0.077 g, 2.15 mmol) in deoxygenated water (1 cm³) and 50% aqueous NaOH (0.66 cm³) was added, followed by a deoxygenated solution of the above tosylate (2.50 g, 8.0 mmol) in methanol (2.5 cm³). The mixture was allowed to warm to room temperature and was stirred overnight. Air was bubbled through the resulting mixture for 1 h partly to remove methanol. The reaction was poured into water (200 cm³) and the orange precipitate was filtered off, washed with water and dried to give the dimethyl acetal of (1b), pure enough (2.85 g, 70%) [^{1}H n.m.r. (see Table 2) and t.l.c.] to be used directly in the next step. An analytical sample of the acetal was prepared by recrystallisation twice from dichloromethane–cyclohexane (see Table 2).

Hydrolysis of the acetal. The acetal (2.6 g, 5.1 mmol) was stirred in 0.1M-hydrochloric acid (75 cm³) for 4 h, at which time all the solid had dissolved and t.l.c. (system 1) showed the absence of acetal (some hydrolyses of acetals were carried out in 1 : 1 ethanol–water). Water and hydrogen chloride were removed under reduced pressure (bath temperature *ca.* 27°). The residue was chromatographed on Kieselgel MN (20 g per g cobaloxime, elution under suction with 10%methanol in dichloromethane). To the main yellow eluate was added pyridine (3 cm³) and the solvents and excess pyridine were removed. Recrystallisation from dichloromethane (*ca.* 60 ml boiling solvent per g cobaloxime; after filtration the solution concentrated to half volume and stored at −20° overnight) gave pure (^{1}H n.m.r.) 4,5-dihydroxypentyl(pyridine)cobaloxime. A further recrystallisation from dichloromethane gave an analytical sample after pumping for 24 h at 0.1 mmHg and room temperature to remove solvated dichloromethane (see Table 2 for ^{1}H n.m.r. and analytical data). Twice recrystallised (1b) was used in photochemical studies.

Preparation of Dihydroxypentyl(aquo)cobaloxime (1c).—This was carried out in a similar manner to the preparation of (1b) except that dimethyl sulphide was substituted for pyridine in the step producing cobaloxime (*cf.* ref. 34). Hydrolysis of the acetal in the usual manner also replaced dimethyl sulphide by water to afford (1c) (37%), δ(CD_3OD) 0.5—1.9br (6 H, m, $CH_2CH_2CH_2$), 2.23 (12 H, s, dmg Me), 3.2—3.8br (3 H, $CHOHCH_2OH$) (Found: C, 38.05; H, 6.65; N, 13.65. $C_{13}H_{27}N_4O_7Co$ requires C, 38.0; H, 6.50; N, 13.65%). This compound underwent slow dehydration on storing at room temperature to give a product, much less soluble in water and 0.1M-acetic acid, which is probably a dimer (Found: C, 39.6; H, 6.45; N, 14.1. $C_{26}H_{50}O_{12}Co_2$ requires C, 39.8; H, 6.4; N, 14.3%) [*cf.* dimer from methyl(aquo)cobaloxime [35]].

Mono-2,4-dinitrophenylhydrazone of Biacetyl Monooxime.—Dimethylglyoxime was stirred in KCl–HCl buffer (pH 2) for 2 h. Excess of dimethylglyoxime was filtered off and to the filtrate was added acidic 2,4-dinitrophenylhydrazine. After 30 min, work up in the usual manner gave a product which was recrystallised from CH_2Cl_2–light petroleum (1 : 2). The resulting yellow crystals of the title compound had m.p. 245° (Found: C, 42.7; H, 3.95; N, 24.6. Calc. for $C_{10}H_{11}N_5O_5$: C, 42.7; H, 3.9; N, 24.9%).

Photodecomposition of Methyl(aquo)cobaloxime in the Presence of Ethane-1,2-diol at pH 2.—Photolyses with methyl(aquo)cobaloxime were carried out at 380 ± 15 nm in the manner described in ref. 16. KCl–HCl buffer (pH 2.0) (20.0 cm³) containing methyl(aquo)cobaloxime (4×10^{-3}M) and ethane-1,2-diol (1.00M) was degassed with argon and irradiated until coloured faint pink (45 min). An identical solution was stored in darkness during this time. MBTH assay on a 0.25 cm³ portion of the irradiated solution, using the un-irradiated solution as a blank, indicated that 15—20% acetaldehyde had been formed (assuming it to be the only aldehyde formed). The remainder of the irradiated solution was treated with DNP reagent (25 cm³). After standing for 30 min the mixture was extracted with CH_2Cl_2 (3×25 cm³). The combined extracts were dried and the solvent removed. The residue was extracted with ether. The ethereal extract was diluted with light petroleum (b.p. 40—60°) to give an ether–light petroleum ratio of 3 : 7, and the solution was filtered through silica gel (5.0 g) under suction, eluting with more ether–light petroleum (3 : 7). The yellow eluate before elution of 2,4-dinitrophenylhydrazine was collected and evaporated to give product A. An identical procedure was applied to the un-irradiated reaction mixture to give product B. T.l.c. (system 2) and ^{1}H n.m.r. showed that A, *but not B*, contained acetaldehyde DNP. Comparison of the n.m.r. spectrum of A with the spectrum of a standard solution of acetaldehyde DNP showed the yield of acetaldehyde to be *ca.* 7%. Product A was purified by p.l.c. [Kieselgel MN, ether–light petroleum (4 : 6)] to give a homogeneous acetaldehyde DNP (5%; estimated by u.v.–visible spectroscopy), identified by

[32] R. Paul and S. Tchelitcheff, *Bull. Soc. chim. France*, 1948, 197.

[33] L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' Wiley, London, 1967, vol. 1, p. 1180.

[34] G. N. Schrauzer, *Inorg. Synth.*, 1968, **11**, 61.

[35] L. M. Ludwick and T. L. Brown, *J. Amer. Chem. Soc.*, 1969, **91**, 5188.

i.r. spectroscopy (comparison with authentic acetaldehyde DNP).

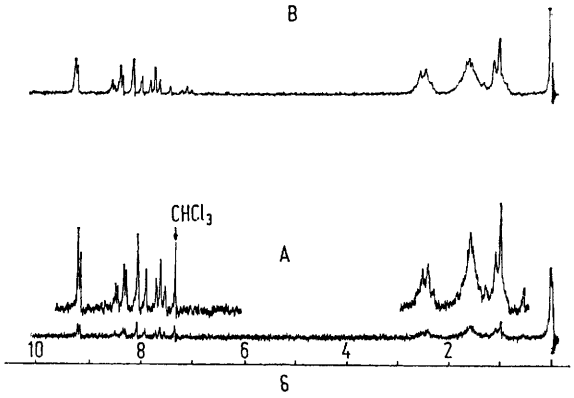
Photolysis of methyl(aquo)cobaloxime (4×10^{-3}M) in KCl–HCl buffer containing 0.1M-ethane-1,2-diol did not give detectable acetaldehyde DNP. From this experiment was isolated a DNP which is formed in the irradiation of all alkylcobaloximes under acidic conditions. The procedure, starting from a solution (19.25 cm^3) of the aforementioned components, was essentially that described for the isolation of acetaldehyde DNP (see above) and gave, after recrystallisation from ethanol, orange crystals (7 mg). On t.l.c. (system 2) this substance moves slightly ahead of acetaldehyde DNP. It is identified as the 2,4-dinitrophenylhydrazone of biacetyl mono-oxime from its spectra data and comparison (t.l.c. system 2) with an authentic standard (see above).

Photolyses of (1b—f) *at* pH 3.—Samples for photolysis were prepared by dissolving the particular cobaloxime (35—50 mg) in the appropriate solvent (1 cm^3 per mg of cobaloxime) in a small flask stoppered with a serum cap. The solutions were deoxygenated by bubbling Ar (prewashed with Cr^{II}, NaOH, and solvent) for 30 min, the flask transferred to a Pyrex water-bath, and the solution photolysed with constant stirring. Completion of photolysis was noted by a complete absence of yellow colour in the solution (*ca.* 7—10 min). The solution was then poured into 0.4% 2,4-dinitrophenylhydrazine solution (50 cm^3), stirred for 25 min, and the DNPs produced were extracted with CH_2Cl_2 (3×25 cm^3). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated to dryness. Excess of reagent was removed by triturating the residue with ether (2 cm^3), diluting with light petroleum (2 cm^3) and filtration through silica gel (5 g) using 1 : 1 ether–light petroleum (125 cm^3). The filtrate was evaporated to dryness and then chromatographed on silica gel (55 g; 280 × 23 mm) using chloroform as eluant. Three bands separated. Pentanal DNP [from (1b or c); hexan-2-one DNP and hexanal DNP from (1d)] was the first to emerge, followed by propanone DNP, and finally the DNP of biacetyl mono-oxime. Pentanal DNP [from (1b); hexanone or hexanal DNP from (1d)] band was collected, evaporated, and dissolved in spectroscopic ethanol. The absorbance maximum of this solution was measured at 358 nm (ε 2.2×10^4 l mol^{-1} cm^{-1}) on a calibrated Unicam SP 500 spectrometer. Propanone DNP arises from traces of propanone in the water used. Some early photolyses were carried out at 380 ± 15 nm in the manner described in ref. 3*a*. However, the above procedure gave identical products and yields in a much shorter time, and was used for the majority of photolyses.

Yields of pentanal obtained from (1b or c) by the above procedure are given in Table 1. Pentanal DNP was identified by comparison with an authentic sample [^{1}H n.m.r. spectra (Figure), t.l.c. in system 2 and also benzene, electron impact mass spectra, i.r., u.v.–visible spectra]. Identification of hexanal–hexan-2-one is described in the text. Isolation and characterisation of diols and tetraol is described below.

Photolyses at pH 7 *and* 9.—Under these conditions photodecompositions of (1b and d) are much slower than at pH 3 and afford a cobaloxime(II) product. Thus, in a typical experiment a degassed solution of (1b) (150 mg in 150 cm^3 water) was photolysed for 2.5 h. The resulting clear brown solution was exposed to the atmosphere resulting in a further darkening in colour due to oxidation of Co^{II}. The photolysate was analysed for aldehyde–ketone as described above and for diol(s)–tetraol as described below. At pH 7 or 9, (1b) did not give detectable pentanal. Similarly, (1d) at pH 7 gave no detectable hexanal–hexan-2-one. In each case $\geqslant 0.2\%$ aldehyde–ketone would have been readily detected by t.l.c.

Isolation of Diol(s) and Tetraol from Photolyses.—The aqueous photolysate (pH 3) from (1b) usually after treatment with DNP reagent and extraction with dichloromethane, was continuously extracted (ether for 82 h). The ether extract was concentrated and examined by t.l.c. (methyl acetate) showing a spot (R_F 0.5) corresponding to C-5 diols (comparison with authentic pentane-1,2-diol) and another spot (R_F 0.05) corresponding to decane-1,2,9,10-tetraol (comparison with authentic material). The latter was more efficiently extracted by ethyl acetate (continuous extraction for 24 h). Photolyses at pH 7 yielded no detectable decane-1,2,9,10-tetraol. Diol(s) and tetraol were isolated by column chromatography on silica gel (elution of



^{1}H N.m.r. spectra of authentic pentanal DNP (spectrum 1B) and pentanal DNP from photolysis of (1c) (spectrum 1A) (in $CDCl_3$)

diols with methyl acetate, tetraol with methanol). The proportions of pentane-1,2-diol and 4,5-dihydroxypent-1-ene (which did not separate under these conditions) were established by ^{1}H n.m.r. spectroscopy. Photolyses at pH 3 gave both diols (ratio of saturated : unsaturated diol *ca.* 2 : 3 from integrals), whilst at pH 7 only the unsaturated diol was obtained. A similar procedure was used for the isolation of C-6 diol(s) from (1d). Yields of C-5 diols and decane-1,2,9,10-tetraol (see text) were assayed by oxidation with neutral sodium periodate and estimation of the excess of periodate by back titration with iodine–sodium arsenite.

G.l.c. determination of the diols and tetraol either directly (Chromosorb 101; 195°) or as their acetates (20% DEGS; 70°) gave irreproducible results.

Recovery of DNPs (cf. *ref.* 36).—Blank experiments were carried out to assess the efficiency of recovery of aldehyde and ketone products as their DNPs. Two solutions were prepared: A, pentanal (107.8 mg, 1.26 mmol) in 2 drops of methanol (to solubilise) added to 0.1M-acetic acid (50 cm^3); B, methyl(pyridinato)cobaloxime (32 mg, 0.084 mmol) in 0.1M-acetic acid (39 cm^3) degassed with argon and photolysed by the standard procedure. The resulting solution contains similar molar concentrations of Co^{II}, pyridine, and dimethylglyoxime as photolyses of (1b).

Solution A (0.3 cm^3) was added to solution B followed by

[36] *Cf.* A. I. Vogel, ' Elementary Practical Organic Chemistry,' Longman, London, 1958, part 3, p. 739.

DNP reagent (50 cm³). After stirring for 30 min at room temperature, DNPs were extracted with dichloromethane (3 × 25 cm³). Further treatment was carried out as described above giving pentanal DNP (80% recovery). Stirring for 5 or 60 min gave lower recoveries (*ca.* 60% in each case). Addition of pentanal (1 μl), hexanal, or hexan-2-one to DNP reagent (50 cm³) followed by stirring for 30 min and work-up as previously gave the corresponding DNP with *ca.* 70% recovery.

Photolysis of n-Propyl(aquo)cobaloxime in the Presence of Pentane-1,2-diol.—n-Propyl(aquo)cobaloxime (38.5 mg, 0.11 mmol) in 0.1M-acetic acid (39.5 cm³) containing pentane-1,2-diol (0.11 mmol) was degassed and photolysed in the standard manner. Treatment with DNP reagent, extraction, and chromatographic separation of DNPs in the usual way gave no pentanal DNP. Repetition of this experiment with a molar concentration of pentane-1,2-diol gave $<0.05\%$ pentanal DNP.

MBTH Assay.[37]—*N*-Methylbenzothiazolone hydrochloride was recrystallised twice from water. A freshly prepared 1% (w/v) aqueous solution of this material, freshly made up 0.4% (w/v) aqueous iron(III) chloride, and AnalaR acetone were used in the colourimetric assay (readings at 670 nm with Bausch and Lomb colorimeter).

Dependence of Acetaldehyde Yield on Concentration of Ethane-1,2-diol.—Four photolyses (40 min each) were carried out on 4×10^{-3}M-solutions of methyl(aquo)-cobaloxime in KCl–HCl buffer (pH 2.0) (20 cm³) containing varying concentrations of ethane-1,2-diol (0.02, 0.20, 0.50, and 1.00M). Three portions from each reaction were analysed by MBTH assay, giving % yields acetaldehyde (assumed to be the only aldehyde formed) corresponding to the above concentrations of *ca.* 0, 8, 18, and 33 (all ±5%).

Detection by G.l.c. of Hexanal and Hexan-2-one from Photolyses of (1d).—5,6-Dihydroxyhexyl(pyridine)-cobaloxime (40.7 mg, 0.07 mmol) in 0.1M-acetic acid (40 cm³) was degassed and photolysed until colourless. Extraction with pentane (3 × 2 cm³) followed by careful evaporation of the combined extracts to a small volume gave a residual solution which was examined by g.l.c. (20% diethyleneglycol succinate on Chromosorb W-HP at 70° or 4% QF-1 on Chromosorb W at 30°) showing a ratio of hexan-2-one to hexanal of 3.8 ± 0.3 : 1 (comparison with standard mixtures). On the QF-1 column (nitrogen 7 lb in^{-2}) R_t (hexanal) = 9.3, R_t (hexan-2-one) = 10.4 min.

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[37] M. A. Paz, O. O. Blumenfeld, M. Rojkind, E. Henson, C. Furfine, and P. M. Gallop, *Arch. Biochem. Biophys.*, 1965, **109**, 548.