Mechanism of Action of the Vitamin B_{12}-dependent Enzyme Dioldehydrase: Refinement of a Model System Involving Photodecomposition of Dihydroxyalkylcobaloximes

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Summary Anaerobic photolysis of the following cobaloximes in 0·1 M acetic acid gives the carbonyl-containing product(s) in parentheses: 3,4-dihydroxybutyl(pyridine)-cobaloxime (none), 4,5-dihydroxypentyl(aquo)cobaloxime (10% pentanal), 5,6-dihydroxyhexyl(pyridine)cobaloxime (16% hexan-2-one + *ca.* 4% hexanal); these findings strengthen a model system proposed for the vitamin B_{12}-dependent enzyme dioldehydrase.

WE reported[1] that anaerobic photolysis of 4,5-dihydroxypentyl(pyridine)cobaloxime† (**2a**) in 0·1 M acetic acid gives pentanal among other products.‡ This transformation was proposed as a model system for the conversion of certain 1,2-diols into aldehydes catalysed by dioldehydrase, a vitamin B_{12}-dependent enzyme.[2]§ The regiospecificity observed in the enzymatic reaction (initial attack at C-1 of substrate[2]) was supposed to be reproduced in the model by a specific 1,5-H transfer which leads from the 4,5-dihydroxy-

† Alkyl(base)cobaloximes are octahedrally co-ordinated cobalt complexes: $RCo(dmgH)_2L$ [where R = σ-alkyl group, dmgH = monoanion of dimethylglyoxime and L = Lewis base (R and L are *trans*)] discovered by G. N. Schrauzer (*cf. Accounts Chem. Res.*, **1968**, **1**, 97) and used as models for cobalamins.

‡ The optimised yield of pentanal from (**2a**) is 10·1% (anaerobic photolysis through a Pyrex filter). Other products from the cobalt-dihydroxypentyl group are pentane-1,2-diol (20%), pent-4-ene-1,2-diol (30%), and decane-1,2,9,10-tetraol (20%).

§ In this reaction and related enzymatic reactions (see ref. 2), vitamin B_{12} is activated as its coenzyme form adenosylcobalamin.

pentyl radical to the 1,2-dihydroxypentyl radical and eventually to pentanal. Pentan-2-one, which could arise from the 4,5-dihydroxypentyl radical *via* a 1,4-H shift giving the 1-hydroxy-(1-hydroxymethyl)butyl radical, was not detected. To confirm our ideas, we have now studied 3,4-dihydroxybutyl(pyridine)cobaloxime (**1**), 4,5-dihydroxypentyl(aquo)cobaloxime (**2b**), and 5,6-dihydroxyhexyl(pyridine)cobaloxime (**3**). The cobaloxime (**1**) is *not* expected efficiently to yield any carbonyl-containing products from its dihydroxybutyl group on photolysis in 0·1 M acetic acid, whilst (**2b**) should give pentanal in a similar manner to (**2a**), if the axial ligands water and pyridine play no part in the transformation. The cobaloxime (**3**) is expected to give predominantly, if not exclusively, hexan-2-one (rather than hexanal) by the route shown in the Scheme.

$RCo(dmgH)_2L$

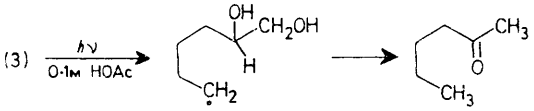
dmgH = dimethylgloxime monoanion; py = pyridine

(**1**) R = $CH_2CH_2CHOHCH_2OH$, L = py
(**2a**) R = $[CH_2]_3CHOHCH_2OH$, L = py
(**2b**) R = $[CH_2]_3CHOHCH_2OH$, L = H_2O
(**3**) R = $[CH_2]_4CHOHCH_2OH$, L = py
(**4**) R = Pr^n, L = H_2O

[Compounds (**1**)—(**3**) are racemates]

The crystalline, analytically pure cobaloximes (**1**), (**2b**), and (**3**) were synthesised by the reaction of the corresponding toluene-*p*-sulphonate of 2,2-dimethyl-4-(*n*-hydroxyalkyl)-1,3-dioxolan (*n* = 2, 3, or 4; alkyl = Et, Pr, or Bu, respectively) with a 50% molar excess of the appropriate cobaloxime(I) nucleophile,[3] followed by hydrolysis of the resulting acetal with 0·1 M HCl in ethanol–water (1:1; 2 h, room temp.).

Irradiation (Pyrex filter) of an oxygen-free 2×10^{-3} M solution of (**1**) in 0·1 M acetic acid caused decomposition of the cobaloxime (50 mg) within 10 min, but neither butanal nor butanone could be detected as their 2,4-dinitrophenylhydrazones (DNP's) among the products. A similar photolysis of (**2b**) gave 11% of pentanal‡ (as its DNP) showing that the effect of quite dissimilar axial ligands [water in (**2b**), pyridine in (**2a**)] on carbonyl-containing product(s) is negligible and may be excluded from the proposed mechanism (*cf.* Scheme and ref. 1). Subjecting (**3**) to a similar photolysis as (**1**) gave hexane-1,2-diol, hex-5-ene-1,2-diol, dodecane-1,2,11,12-tetraol, and hexan-2-one as major products from the cobalt-dihydroxyalkyl group. The hexan-2-one was isolated as its DNP in 16% yield (4 expts.) and was identified by t.l.c., h.p.l.c., mass spectrometry, and i.r. and ^1H n.m.r. spectroscopy. These techniques show that the crude hexan-2-one DNP is contaminated with another DNP, which is probably derived from hexanal (a ^1H n.m.r. spectrum indicates the admixture of *ca.* 20% of an aldehyde DNP from integration of the -CH=N signal). G.l.c. of a pentane extract of the photolysed reaction mixture showed hexan-2-one and hexanal in a ratio of 3·8 ± 0·3:1.



+ Co^{2+} + 2dmgH + Py

SCHEME

The above results support the mechanism we proposed[1] to explain the formation of pentanal from photolysis of (**2a**) in 0·1 M acetic acid. The order of preference of 1,*n*-hydrogen shifts in the dihydroxyalkylcobaloximes examined is 1,5- > 1,6- > 1,4-shift (the latter not being observed at all). Of additional significance are the observations that irradiation of (**2a**) or (**3**) in de-oxygenated water gives < 0·05% aldehyde (or ketone) DNP, whilst anaerobic photodecomposition of 2×10^{-3} M propyl(aquo)cobaloxime (**4**) in 0·1 M acetic acid containing 2×10^{-3} M of pentane-1,2-diol did not give detectable pentanal (as its DNP). Our findings strengthen the idea[2] that the mechanism of dioldehydrase involves the interaction between the adenosyl radical and substrate (*e.g.* propane-1,2-diol) to give 5′-deoxyadenosine and a substrate-derived radical (S·, *e.g.* MeCHOHĊHOH), which is transformed[4] to a product-related radical [P· *e.g.* MeĊHCH$(OH)_2$] and thence to product aldehyde (*e.g.* propionaldehyde). In the model systems described we have no evidence that Co^{II} plays a part in the transformation of the radicals to hexan-2-one or pentanal. Note that once homolysis of the Co–C bond has occurred under acidic conditions, the derived cobaloxime(II) species very rapidly degrades to aquated Co^{2+}, free dimethylglyoxime (and its hydrolysis products), and pyridine (mainly protonated).[5] On the contrary, under neutral conditions (where hexan-2-one or pentanal are not formed) the alkyl radical from Co–C bond homolysis probably remains in intimate contact with a cobaloxime(II) complex for a relatively long time before decomposing to an alk-1-ene.[5] We infer that the enzymatic conversion of S· into P· (see above concerning terminology) need not necessarily occur *via* organocobalt intermediates as favoured by some authors.[6] It may suffice for the radical intermediates to be 'held' by the protein, which may also mediate their interconversion.

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[1] B. T. Golding, T. J. Kemp, E. Nocchi, and W. P. Watson, *Angew. Chem. Internat. Edn.*, 1975, **14**, 813.
[2] R. H. Abeles and D. Dolphin, *Accounts Chem. Res.*, 1976, **9**, 114.
[3] G. N. Schrauzer, *Inorg. Synth.*, 1968, **11**, 61.
[4] Possible mechanisms for this step are discussed in ref. 2. See also B. T. Golding and L. Radom, *J. Amer. Chem. Soc.*, 1976, in the press.
[5] B. T. Golding, T. J. Kemp, E. Nocchi, P. J. Sellars, and W. P. Watson, unpublished results.
[6] See *e.g.* K. L. Brown and L. L. Ingraham, *J. Amer. Chem. Soc.*, 1975, **96**, 7681, and references cited therein. There is no evidence yet (*cf.* ref. 2) for alkylcobalamins (formed by covalent association of vitamin B_{12r} with substrate-derived or product-related radicals) as intermediates in this or any other adenosylcobalamin-dependent enzymatic reaction.