An Improved Model for B_{12}-Dependent Diol Dehydrase

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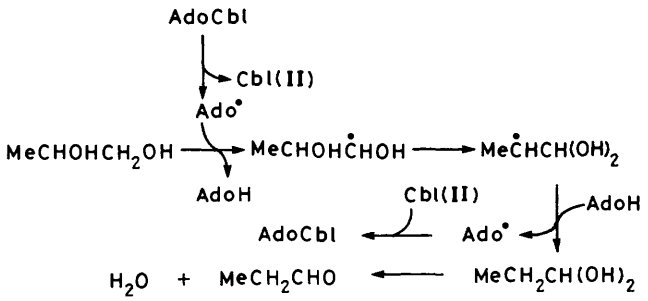
Thermolysis or photolysis of 4,5-dihydroxy-2,2-dimethylpentyl(pyridine)cobaloxime (**1a**) at either pH 3 or 9 affords 4,4-dimethylpentanal (15—45%) in a model system for B_{12}-dependent diol dehydrase.

The 'bound radical' hypothesis[1,2] for the mechanism of action of the coenzyme B_{12}-dependent diol dehydrase invokes protein-bound free radical intermediates, with cobalt [in cob(II)alamin] as a 'spectator'.[3] Thus, the adenosyl radical derived from homolytic fission of the coenzyme's cobalt–carbon σ-bond abstracts a hydrogen atom from a substrate molecule (1,2-diol) leading to deoxyadenosine and a 1,2-dihydroxyalkyl radical, which rearranges (1,2-hydroxy shift) to a 2,2-dihydroxyalkyl radical. The latter species abstracts a hydrogen atom from the methyl group of deoxyadenosine to give a geminal diol, which loses water to yield product aldehyde (see Scheme 1 for a summary of these events with propane-1,2-diol as substrate).

We,[4] and Finke and coworkers,[5,6] have described model systems in support of the pathway of Scheme 1 in which an alkylcobalt complex, containing a 1,2-diol moiety (initially masked as a cyclic carbonate in the studies of ref. 5), is activated either by photolysis (*e.g.* Scheme 2) or thermolysis (after deprotection of the cyclic carbonate). A weakness of our model system is the need for photolytic activation (*i.e.* conditions *not* employed by the enzyme), its restriction to acidic pH (the enzyme's pH optimum[5] is 10), and the use of cobaloxime rather than cobalamin as the cobalt carrier. We now report experiments which overcome two of these objections and lay the groundwork to overcome the third.

It is well established that alkylcobalt complexes can be activated for homolysis of their cobalt–carbon σ-bond by steric destabilisation (see, for example, studies with neopentylcobalamin[7]). We have therefore synthesised 4,5-dihydroxy-2,2-dimethylpentyl(pyridine)cobaloxime (**1a**), which should be much more thermally labile than the previously studied[4] 4,5-dihydroxypentyl(pyridine)cobaloxime (**2a**). Furthermore, the primary radical (**9**) derived from homolysis of the Co–C bond of (**1a**) cannot undergo β-elimination to yield an alkene, which is an unwanted side reaction in experiments with (**2a**) and the corresponding cobalamin (**2b**).[8] The β-elimination,



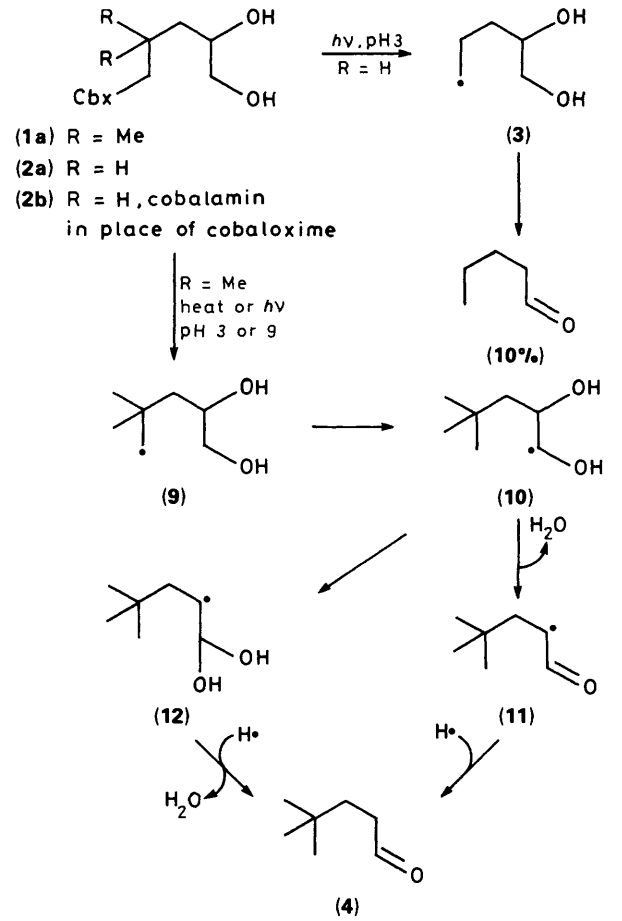
Scheme 1. Protein-bound free radicals in the conversion of propane-1,2-diol into propanal by AdoCbl-dependent diol dehydrase (AdoCbl = adenosylcobalamin).

leading to 4,5-dihydroxypent-1-ene, is the major pathway under all reaction conditions investigated with (**2a**) and (**2b**), and *only* with (**2a**) is there sufficient competing 1,5-hydrogen shift within radical (**3**) to lead to detectable aldehyde product (pentanal).[4]

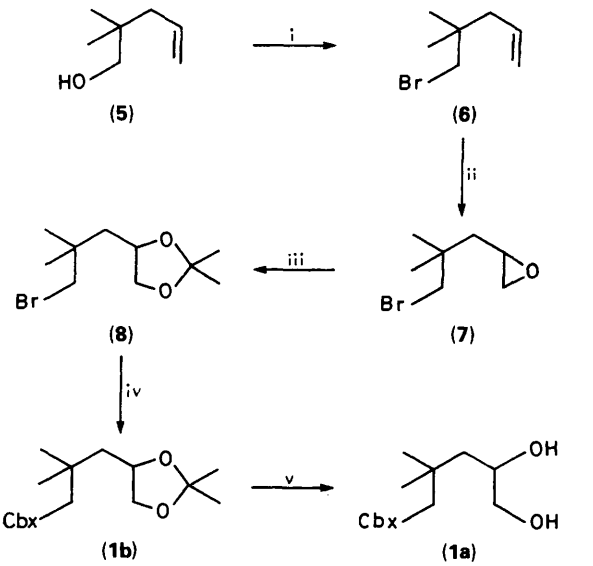
Cobaloxime (**1a**) and the expected product of its thermolyis [4,4-dimethylpentanal (**4**)] were synthesised as shown in Schemes 3 and 4, respectively. Thus, 2,2-dimethylpent-4-en-1-ol[9] (**5**) (1.16 M in acetonitrile) was reacted with triphenylphosphine (1 mol equiv.) and tetrabromomethane[10] (1 mol equiv.) (−30 °C, 10 min → room temp. → boil under reflux for 8 h) to yield 5-bromo-4,4-dimethylpent-1-ene (**6**) (90%, after purification by silica chromatography: elution with petrol).† Bromoalkene (**6**) (1 M in dichloromethane) was epoxidised with 3-chloroperbenzoic acid (1.1 mol equiv., 16 h, room temp.) to afford 2-(3-bromo-2,2-dimethylpropyl)oxirane (**7**)† (73% after silica chromatography: elution with diethyl ether–petrol, 1:8). The conversion[11] of epoxide (**7**) into acetal (**8**) required considerable experimentation to achieve optimisation. Eventually, it was found that exposure of the epoxide (1.3 M in acetone at −75 °C) to boron trifluoride (0.25 mol equiv., introduced as its etherate) for 5 h and quenching with aqueous sodium hydroxide gave 4-(3-bromo-2,2-dimethylpropyl)-2,2-dimethyl-1,3-dioxolan (**8**)† (64%, after silica chromatography: elution with diethyl ether–petrol, 1:8). Reaction of bromide (**8**) with cob(I)aloxime, prepared from bromo(pyridine)cobaloxime,‡ gave 4,5-di-*O*-isopropylidene-4,5-dihydroxy-2,2-dimethylpentyl(pyridine) cobaloxime (**1b**)† (35%, after silica chromatography: elution with dichloromethane–methanol–pyridine, 96:3:1). This was hydrolysed to yellow crystalline (**1a**)† (82%, after silica chromatography: elution with dichloromethane–methanol–pyridine 89:10:1) by exposing a 1 M solution in ethanol–water (1:0.15 v/v) to hydrogen chloride (3 mol equiv.) for 5 h, 20 °C. The conversion of bromoalkene (**6**) into a reference sample of

† New compounds were chromatographically homogeneous and gave analytical and spectroscopic data in accord with their assigned structures.

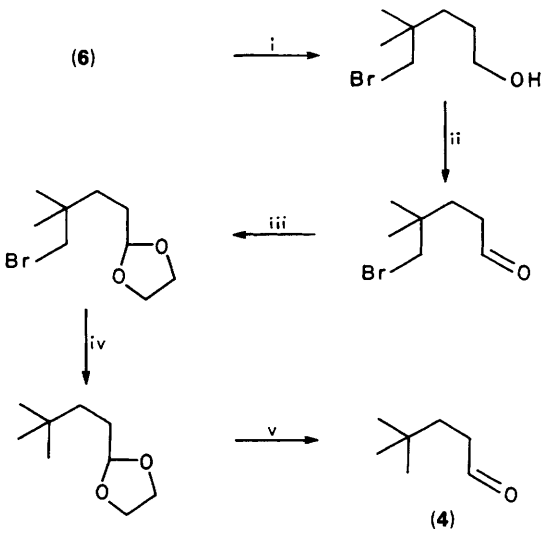
‡ A stirred suspension of bromo(pyridine)cobaloxime (0.52 g, 1.2 mmol) in ethanol (40 ml) at room temp. was thoroughly degassed with nitrogen in a Schlenk tube and reduced with sodium borohydride [0.13 g, 3.4 mmol, added as a partial solution in ethanol (2 ml)]. After 30 min, bromide (**8**) (0.16 g, 0.63 mmol) was added and the reaction was stirred for 16 h at room temp. in darkness. Air was bubbled through the orange solution for 30 min before water (100 ml) was added. The product cobaloxime (**1b**) was extracted into ethyl acetate. Removal of the solvent yielded crude cobaloxime, which was purified by silica chromatography: elution with dichloromethane–methanol–pyridine (96:3:1). The orange cobaloxime product was subjected to high vacuum to remove all traces of pyridine; 0.12 g (35%).[12]



Scheme 2. Some radical intermediates in the degradation of cobaloximes (**1a**) and (**2a**), and cobalamin (**2b**).



Scheme 3. *Reagents and conditions*: i, Ph_3P, CBr_4, −30 °C, 10 min → room temp. → reflux, 8 h; ii, 3-chloroperbenzoic acid, 16 h, room temp.; iii, BF_3, acetone, −75 °C, 5 h, then NaOH; iv, cob(I) aloxime; v, HCl, $EtOH/H_2O$, 5 h, 20 °C; see text.



Scheme 4. *Reagents and conditions*: i, disiamylborane, tetrahydrofuran (THF) (*cf.* ref. 13); ii, pyridinium dichromate, CH_2Cl_2; iii, ethane-1,2-diol/H^+ cat.; iv, Ph_3SnH/azoisobutyronitrile cat., benzene; v, aq. THF/Dowex 50W, H^+ form.

aldehyde (**4**)† followed standard reaction procedures (*cf.* Scheme 4).

Cobaloxime (**1a**) was subjected to four sets of degradative conditions: (i) thermolysis (100 °C, 7 h) in 0.1 M acetic acid (pH 3); (ii) thermolysis (100 °C, 7 h) in 0.01 M sodium tetraborate solution (pH 9); (iii) photolysis (20 °C, 20 min) at pH 3; (iv) photolysis (20 °C, 4.5 h) at pH 9. All experiments utilised a deoxygenated 2 mM solution of (**1a**) and reaction times were determined by TLC monitoring. The ranges of yields of aldehyde (**4**) were as follows [4 independent experiments, assay by determination of (**4**) by GLC and conversion into its 2,4-dinitrophenylhydrazone, which was analysed by UV-VIS. spectroscopy]: (i) 20—22%; (ii) 15—21%; (iii) 43—45%; (iv) 20—25%; the yield of the aldehyde was reduced to zero if air was admitted to reaction mixtures. The result for system (iii) is the best yield achieved in 'acidic photolysis' models.

The mechanism of formation of aldehyde (**4**) may be presumed to parallel that defined[4] for the generation of pentanal from (**2a**). Homolysis of the Co–C bond of (**1a**), induced either by photolysis or thermolysis, to give the 4,5-dihydroxy-2,2-dimethylpentyl radical (**9**), is followed by a 1,5-hydrogen shift to afford the 1,2-dihydroxy-4,4-dimethylpentyl radical (**10**). The latter is converted into the 1-formyl-3,3-dimethylbutyl radical (**11**) (by elimination of water at pH 3 or hydroxide at pH 9) or the 1-(dihydroxymethyl)-3,3-dimethylbutyl radical (**12**) (by 1,2-hydroxy shift). The various mechanistic possibilities have been fully discussed elsewhere.[4,8] Finally, radical (**11**) or (**12**) is neutralised, probably by abstraction of a hydrogen atom from dimethylglyoxime.

The model experiments described give further support to the 'bound radical' hypothesis for B_{12}-dependent diol dehydrase. Arguments against other mechanisms[14—16] have been presented.[8,17,18]

We thank Drs S. Muresigye-Kibende and D. N. R. Rao for preliminary experiments on the preparation of compound (**8**).

Received, 26th October 1989; Com. 9/04626G

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