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Evidence for Enantiomorphic-Enantiotopic Group Discrimination in Diol Dehydratase-Catalyzed Dehydration of *meso*-2,3-Butanediol

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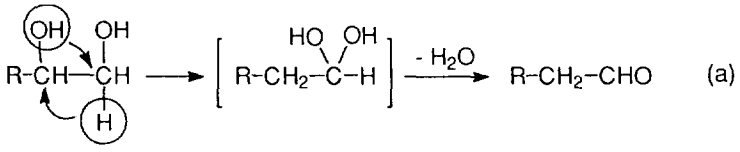
Abstract : The conversion of *meso*-2,3-butanediol (1) into 2-butanol (3) by *Lactobacillus brevis*, *via* diol dehydratase-catalyzed reaction to 2-butanone (2), was shown to occur with complete discrimination between the two enantiomorphic-enantiotopic 1-hydroxyethyl groups of 1.

Diol dehydratase [(*RS*)-1,2-propanediol hydro-lyase: EC 4.2.1.28] is an adenosylcobalamin-dependent enzyme which catalyzes the conversion of 1,2-propanediol (both enantiomers) and 1,2-ethanediol into propionaldehyde and acetaldehyde, respectively [Eq.(a)].[1, 2]



(a)

First isolated from *Klebsiella pneumoniae* ATCC 8724 (formerly known as *Aerobacter aerogenes*) grown anaerobically in glycerol media,[3] it was then characterized[4] and shown to be present in other strains of *Klebsiella* sp. as well as in bacteria of Enterobacteriaceae and Propionibacteriaceae.[5]

Extensive studies of the stereochemistry of the vicinal interchange rearrangement outlined in Eq.(a),[6] together with evidence for the homolytic cleavage of the Co-C bond of the coenzyme as an essential early event in the enzymatic reaction,[1] have led to a number of mechanistic proposals, involving highly reactive radicals.[2] To account for the stereochemical course of the reaction (Fig. 1) it is generally assumed that at the active site of the enzyme the substrate can be accommodated in two binding modes, each for one of the 1,2-propanediol enantiomers and both demanding the same points of attachment (complexes I and II).[6a]

Of the several vicinal diols, which have been shown to function as substrates (and inactivators) for the diol dehydratase,[4b, 7] *meso*-2,3-butanediol (**1**)[7c] is the only compound other than 1,2-alkanediols.[8] Both pathways (a) and (b) of Fig. 1 can be taken, *a priori*, by *meso*-2,3-butanediol (**1**), since both the enantiomorphic groupings (CH_3-CHOH-), each corresponding to that of (*R*)- or (*S*)-1,2-propanediol, are present in its molecule. Experiments based on product characterization from specifically labeled *meso*-2,3-butanediol (**1**) have so far been precluded by a substrate inactivation of the holoenzyme which was found to be rapid and irreversible.[7c]

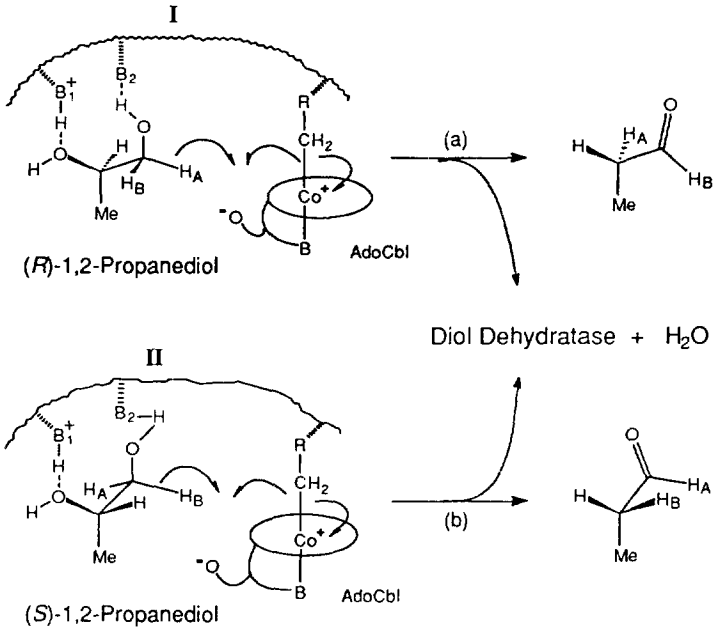
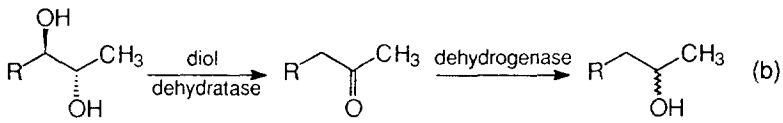


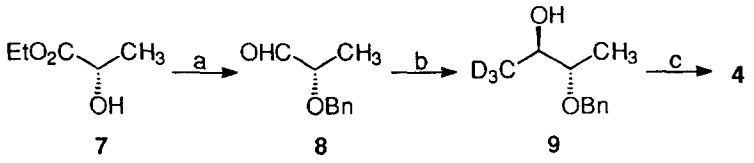
Figure 1. Proposed minimal mechanism for the diol dehydratase reaction (*cf.* ref. 2a. For stereochemical details see also ref. 6). The two binding modes that precede coenzyme homolysis and hydrogen abstraction are illustrated as complexes **I** and **II** (AdoCbl = adenosylcobalamin; B_1, B_2 = basic centers).

In the course of our studies on the origin of 2-butanol in wines and distillates[9] we found that a strain of *Lactobacillus brevis* (LB 19) from our collection was able to produce 2-butanol (**3**) in satisfactory yields through diol dehydratase-catalyzed conversion of compound (**1**) into 2-butanone (**2**),[10] followed by enzymatic reduction of the ketone to the secondary alcohol [Eq. (b)].[11] This prompted us to investigate the action of the diol dehydratase on *meso*-2,3-butanediol utilizing growing cultures of *Lactobacillus brevis* LB 19.



1 : R = CH_3 **2** : R = CH_3 **3** : R = CH_3
4 : R = CD_3 **5** : R = CD_3 **6** : R = CD_3

We report here experimental evidence that the enzyme distinguishes between the two stereogenic centers of (**1**). The (2*R*,3*S*)-[1,1,1-$^2H_3$]-2,3-butanediol (**4**) to be fed to *L. brevis* LB19 was prepared according to Scheme 1. The intermediate **9** was obtained as the predominant diastereoisomer (*anti-syn* ratio *ca.* 9 : 1 as determined by GC analysis)[13] and purified by flash chromatography before it was converted into chemically pure **4**[14] by hydrogenolysis.



Scheme 1. Synthesis of **4** : a) 3 steps, 56%, see ref. 12; b) $ClTi(O\text{-}iPr)_3$, CD_3Li, diethyl ether, -78°C, then addition of **8**, 0°C → r.t., 75%, see ref. 13; c) H_2, Pd/C (10%), EtOH, r.t., 1 atm, 98%.

After the microorganism was inoculated and grown anaerobically in a synthetic medium containing **4**, 2-butanol was isolated from the broth and transformed into its phenylcarbamoyl derivative.[15] A comparison of the ^{1}H-NMR spectrum of this compound[15] with that of a sample of non-deuterated 2-butyl phenylcarbamate (Fig. 2) revealed the selective presence of deuterium atoms at the 4-position in the molecule of the former (as in **10**) and, by inference, of the diol dehydratase product (**5**) giving rise to the deuterated 2-butanol (**6**) [Eq. (b)]. In fact, the proton signals of both the methyl groups were present in the ^{1}H-NMR spectrum of **10**, their intensity ratio being 92 to 8 in favor of CH_3-CH(O-)- (and the inverted ratio was found for the corresponding signals in the ^{2}H-NMR). Considering the purity in specific isotopic labeling of the metabolized *meso*-2,3-butanediol (**4**),[14] the deuterium distribution observed in the 2-butyl phenylcarbamate (**10**) appears to be consistent with a complete discrimination between enantiomorphic-enantiotopic groups in the diol dehydratase reaction. Thus, the two 1-hydroxyethyl moieties of **1** are distinguished and treated differently, the (*R*)-CH_3CHOH- group being converted into CH_3CH_2- and the (*S*)-counterpart into CH_3CO-. On the basis of the model system represented in Fig. 1, this fact is in agreement with a choice of the enzyme in favor of the pathway (a) starting from a complex of the type **I** (where a methyl group replaces H_B) (Fig. 1).

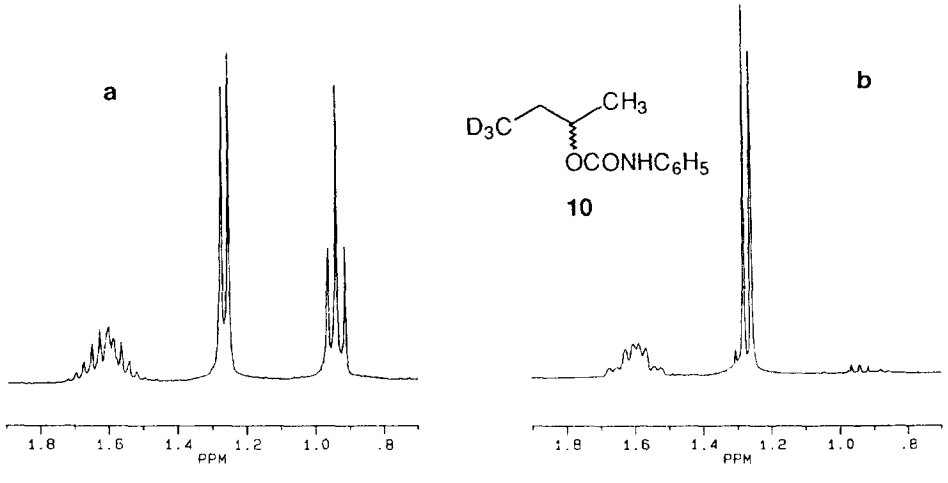


Figure 2. ^{1}H NMR spectra (300.133 MHz, $CDCl_3$, δ range: 0.8 - 1.9 ppm) of the phenylcarbamoyl derivative of 2-butanol: (a) from a commercial source and (b) from biotransformation of **4**.

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References and Notes

1. Reviews on the mechanism of cobalamin-dependent rearrangements: a) Zagalak, B.; Friedlich, W. Eds.; *Vitamin B_{12}, Proc. 3rd Eur. Symp. Vitamin B_{12} and Intrinsic Factor*; W. de Gruyter: Berlin, 1979; b) Golding, B. T.; Ramakrishna Rao, D. R. Adenosylcobalamin-dependent Enzymic Reactions. In *Enzyme Mechanisms*; Page, M. I.; Williams, A. Eds.; Royal Society of Chemistry: London, 1989; pp. 404-428; c) Rétey, J. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 355-361.
2. Leading papers on diol dehydratase model studies: a) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1-22; b) Wang, Y.; Finke, R. G. *Inorg. Chem.* **1989**, *28*, 983-986; c) Anderson, R. J.; Ashwell, S.; Dixon, R. M.; Golding, B. T. *J. Chem. Soc., Chem. Commun.* **1990**, 70-72.
3. Lee, H. A.; Abeles, R. A. *J. Biol. Chem.* **1963**, *238*, 2367-2373.
4. a) Jensen, F. R.; Neese, R. A. *Biochem. Biophys. Res. Commun.* **1975**, *62*, 816-821; b) Toraya, T.; Shirakashi, T.; Kosuga, T.; Fukui, S. *ibid.* **1976**, *69*, 475-480.
5. Toraya, T.; Kuno, S.; Fukui, S. *J. Bacteriol.* **1980**, *141*, 1439-1442.
6. a) Rétey, J.; Umani-Ronchi, A.; Arigoni, D. *Experientia* **1966**, *22*, 72-73; b) Zagalak, B.; Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. *J. Biol. Chem.* **1966**, *241*, 3028-3035; c) Rétey, J.; Umani-Ronchi, A.; Seibl, J.: Arigoni, D. *Experientia* **1966**, *22*, 502-503.
7. a) Eagar, R. G.; Bachovchin, W. W.; Richards, J. H. *Biochemistry* **1975**, *14*, 5523-5528; b) Bachovchin, W. W.; Moore, K. W.; Richards, J. H. *ibid.* **1978**, *17*, 2218-2224; c) Moore, K. W.; Richards, J. H. *Biochem. Biophys. Res. Commun.* **1979**, *87*, 1052-1057.
8. It must be noticed that (*R,R*)- and (*S,S*)-2,3-butanediol act as purely competitive inhibitors,[7c] a fact that is well understandable considering that they have a methyl group in the position occupied by the hydrogen abstracted in (*R*)- and (*S*)-1,2-propanediol, respectively (*i.e.* H_A and H_B in Fig.1).
9. Manitto, P.; Chialva, F.; Speranza, G.; Rinaldo, C. *J. Agric. Food Chem.* **1994**, *42*, 886-889.
10. Radler, F.; Zorg, J. *Am. J. Enol. Vitic.* **1986**, *37*, 206-210.
11. (*R*)-2-Butanol was found to be formed with variable *ee* in the range 50-90% depending on the concentration of the diol (**1**). Results to be published. No biotransformation of both the optically active 2,3-butanediols was observed in parallel experiments, *cf.* ref. 7c.
12. Takai, K.; Heathcock, C. H. *J. Org. Chem.* **1985**, *50*, 3247-3251.
13. Reetz, M. T.; Kesseler, K.; Schmidtberger, S.; Wenderoth, B.; Steinbach, R. *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 989-990; *Angew. Chem. Suppl.* **1983**, 1511-1526. (2*R*,3*S*)-[1,1,1-$^2H_3$]-3-(benzyloxy)-2-butanol (**9**), liquid, pure in GC and TLC; ^{1}H NMR (300 MHz, $CDCl_3$), δ 1.16 (d, 3H, J = 6.5 Hz, C(4)H_3), 2.13 (br s, 1H, OH), 3.48 (dq, 1H, J = 6.5, 3.4 Hz, 3-H), 3.89 (br s, 1H, 2-H), 4.50 (d, 1H, J = 11.8 Hz) and 4.62 (d, 1H, J = 11.8 Hz) (-CH_2Ph), 7.27-7.34 (m, 5H, aromatic protons); ^{13}C NMR (75 MHz, $CDCl_3$), δ 13.42 (C-4), 16.83 (7 lines, C-1), 68.97 (C-2), 70.58 ($\underline{C}H_2$Ph), 78.18 (C-3), 127.43, 128.23 and 138.50 (aromatic carbons); [1,1,1-$^2H_3$]-species abundance > 99% as indicated by the absence of the doublet due to C(1)H_3 (δ 1.13 in the spectrum of the non-deuterated compound).
14. **4** : Pure in GC (R_t 18.5 min, 20% Carbowax 20M, 60°C for 4 min, then to 150°C at 10°C/min, and at 150°C for 8 min); ^{1}H NMR (300 MHz, DMSO-d_6), intensity ratio between the signals of C$\underline{H}$ (δ 3.34, m) and those of C$\underline{H}_3$ (δ 1.03, d, J = 5.8 Hz) : 1.5. Despite the high optical purity of the (*S*)-(-)-ethyl lactate (**7**) (*ee* > 98%), the resulting compound **4** contained its (2*S*,3*R*)-[1,1,1-$^2H_3$]-isomer as an impurity (at least 8% considering the *ee* of **8** determined as described in ref. 12). This was presumably due to a partial racemization of the stereogenic center during the conversion of **7** into **8**.[12]
15. The sterilized synthetic medium[10] (300 mL) containing compound **4** (300 mg) was inoculated (1% inoculum prepared from lyophilized cells of *L. brevis* LB19) and kept at 30°C in anaerobic conditions for a week. The broth was then distilled and the distillate (20 ml) was extracted with diethyl ether. After concentration, the residue was treated with excess of an ethereal solution of phenyl isocyanate and compound **10** was isolated by preparative TLC (hexane-diethyl ether, 7 : 3) (40 mg) : m.p. 63.5 °C, from hexane; ^{1}H NMR (300 MHz, $CDCl_3$), δ 0.94 (t, 0.24H, J = 7.5 Hz, C(4)H_3), 1.26 (d, 2.76H, J = 6.6 Hz, C(1)H_3), 1.55 and 1.64 (2H, AB part of an ABX system, J_{AB} = 14.0 Hz, J_{AX} = J_{BX} = 6.5 Hz, C(3)H_2), 4.85 (app sextet, 1H, <J> = 6.5 Hz, H-2), 6.56 (br s, 1H, NH), 7.04 (app t, 1H, <J> = 7.3 Hz) and 7.26-7.39 (m, 4H) (aromatic H); FAB MS : *m/z* 219 $[M+Na]^+$, 197 $[M+H]^+$.

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