Stereochemistry and Fate of Hydrogen Atoms in the Diol-Dehydratase-Catalyzed Dehydration of *meso*-Butane-2,3-diol

by **Paolo Manitto**[a])*, **Giovanna Speranza**[a])*, **Gabriele Fontana**[a]), and **Antonietta Galli**[b])

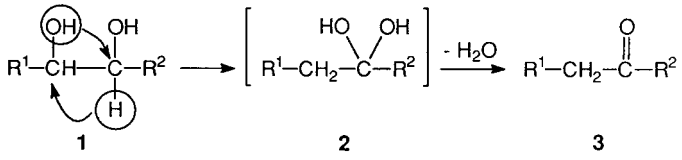
[a]) Dipartimento di Chimica Organica e Industriale, Università di Milano, e Centro di Studio sulle Sostanze Organiche Naturali, CNR, via Venezian 21, I-20133 Milano

[b]) Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università di Milano, via Celoria 2, I-20133 Milano

The transformation of *meso*-butane-2,3-diol into butan-2-ol by a strain of *Lactobacillus brevis* occurs through a diol-dehydratase-catalyzed conversion of the diol into butan-2-one, which is then reduced to the secondary alcohol by dehydrogenases. Experiments performed with deuterated *meso*-butane-2,3-diols showed that the dehydration reaction brings about an inversion of configuration at the (*R*)-configured C-atom of *meso*-butane-2,3-diol as a consequence of the substitution of the OH group by a H-atom; at the same time, the H-atom already bound to the (*R*) C-atom is retained in the resulting methylene group. The H-atom replacing the OH group was assessed to come from the medium since the H-atom at the (*S*)-configured C-atom was completely lost. By contrast, in the case of the conversion of (*RS*)-propane-1,2-diol into propan-1-ol under the same fermentation conditions, an extensive H-transfer (*ca.* 80%) from the primary-alcohol function to the adjacent C-atom was observed. This fact is taken as an indication of different modes in which the two substrates are processed by the enzyme (despite the same stereochemical outcome). A speculative hypothesis is presented to interpret such a dissimilarity.

1. Introduction. – About a dozen of adenosylcobalamin-dependent enzymatic rearrangements are known, all consisting of an interchange of a H-atom and a variable group between adjacent C-atoms [1][2]. Among them, the diol-dehydratase-catalyzed conversion of vicinal diols **1** into the corresponding aldehydes or ketones **3** with a $CH_2(\alpha)$ group *via* a geminal diol intermediate **2** [3][4] (*Scheme 1*) has attracted much

Scheme 1



1 **2** **3**

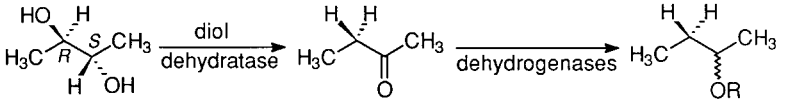
	R^1	R^2
a	H	H
b	CH_3	H
c	CH_2OH	H
d	CH_3	CH_3

attention for its stereochemical implications (*vide infra*) [4][5]. In addition, a lot of interest is devoted to the enzyme-controlled reaction mechanism, which continues to be subject of controversy and speculation [6][7].

(*RS*)-Propane-1,2-diol hydro-lyase (EC 4.2.1.28) [8] converting both enantiomers of propane-1,2-diol (**1b**) into propanal (**3b**) was first isolated in 1963 from *Klebsiella pneumoniae* ATCC 8724 (formerly *Aerobacter aerogenes*) [9] and shown to be present in other strains of *Klebsiella* ssp. as well as in bacteria of Enterobacteriaceae and Propionibacteriaceae [10]. A very similar enzyme, glycerol hydro-lyase (EC 4.2.1.30) was found to be produced by certain strains of *Klebsiella pneumoniae* [11] and by *Lactobacillus* ssp. [12] which were able to transform glycerol (**1c**) into 3-hydroxypropanal (**3c**).

In 1986, *Radler* and *Zorg* [13] purified and characterized a cobalamin-dependent dehydratase that showed a significant activity on *meso*-butane-2,3-diol (*meso*-**1d** = **4**). This enzyme was obtained from a strain of *Lactobacillus brevis* (B18) which furnished, when grown anaerobically in a medium containing *meso*-butane-2,3-diol, the highest amount of butan-2-ol among several strains of heterofermentative lactic-acid bacteria originally isolated from spoiled wines [14]. In fact, butan-2-ol (**6**) occurring in distillates [15] results from diol-dehydratase-catalyzed conversion of *meso*-butane-2,3-diol (**4**) into butan-2-one (**5**) followed by enzymatic reduction of the ketone to the secondary alcohol [16] (*Scheme 2*).

Scheme 2



4 **5** **6** R = H **7** R = PhNHCO

The three enzymes are distinguishable by separation and immunochemical techniques [17] and are different for their kinetic parameters [3b][13]. However, they have many features in common besides the prosthetic group, *e.g.*, the activity on the same substrates (**1a**–**d**), the irreversible inactivation during the reaction with diols other than ethanediol and propane-1,2-diols [18][19], and, presumably, the reaction mechanism following the C–Co bond homolysis of the adenosylcobalamin [2][7b].

Recently, we found that the conversion of *meso*-butane-2,3-diol (**4**) into butan-2-ol (**6**) by a strain of *Lactobacillus brevis* (LB19) of our collection occurred with complete discrimination between the two enantiomorphic-enantiotopic 1-hydroxyethyl groups [20]. It was shown that the (*R*)-MeCHOH group of **4** was transformed by the diol-dehydratase-catalyzed rearrangement into the $MeCH_2$ and the (*S*)-counterpart of the molecule into the MeCO group. This finding can be interpreted in the light of the three-point-attachment hypothesis [4][7a][21], as schematized in *Fig. 1* for (*R*)- and (*S*)-propane-1,2-diol, by assuming a complete preference for the (*R*)-binding mode when the *meso*-butane-2,3-diol molecule (Me in place of H_B in *Fig. 1,a*) interacts with the enzyme active site[1]).

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[1]) It can be noted that a >90% preference for the (*R*)-binding mode was reported to occur in the enzymatic dehydration of glycerol [23].

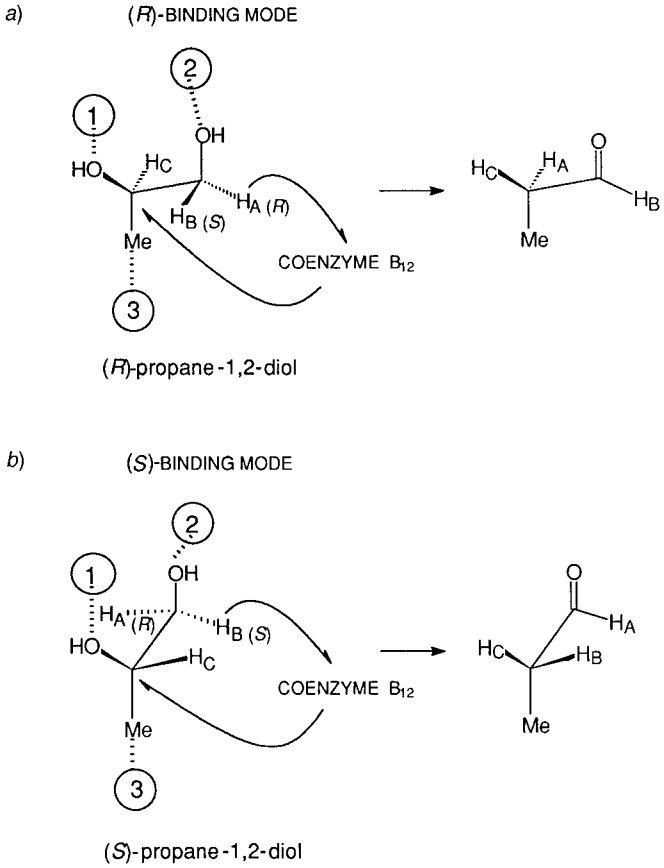

Fig. 1. *Schematic picture of the binding of propane-1,2-diol at the active site of diol dehydratase according to a three-point attachment hypothesis* [4][7a][21]. Protein-substrate binding sites are represented by circled numbers. $H_{A(R)} = H_{A,\,pro\text{-}R}$, $H_{B(S)} = H_{B,\,pro\text{-}S}$

In principle, *meso*-butane-2,3-diol could also have been handled by the enzyme as an (*S*)-propane-1,2-diol molecule in which a Me group replaces the (*pro-R*) H-atom (*i.e.*, H_A in *Fig. 1,b*) since the (*RS*)-propane-1,2-diol dehydratase has been shown to remove the (*pro-R*) H-atom of the primary-alcohol function in the (*R*)-enantiomeric form of propane-1,2-diol and the (*pro-S*) one in the (*S*)-enantiomer [5][21][22]. The finding that both the optically active butanediols behave as purely competitive inhibitors [19] is understandable in the light of the above stereochemical demand for H-abstraction.

The apparent resemblance of (*R*)-propane-1,2-diol and *meso*-butane-2,3-diol in their interaction with the biocatalyst prompted us to extend the comparison between these two substrates even to another cryptostereochemical aspect, namely the formal substitution of the OH group by a H-atom at the (*R*) C-atom, as well as to the fate of the mobile H-atom (which is linked to the (*S*) C-atom in **4**). This investigation, whose

results are reported here, was performed using the LB19 strain of *L. brevis* of our collection as the source of the diol dehydratase.

2. Results and Discussion. – 2.1 *Stereochemistry of the Substitution at the (*R*) C-Atom of* meso*-Butane-2,3-diol in the Diol-Dehydratase Reaction*. In an earlier study [16], we found that *Lactobacilli* reduce butan-2-one (**5**) to an enantiomer mixture of butan-2-ol (**6**) whose composition depends on the strain and the concentration of the substrate (the ketone or its precursor, *i.e.*, *meso*-butane-2,3-diol (**4**)). The most abundant stereoisomer of the secondary alcohol and its enantiomeric excess can be accurately determined by bidimensional chiral gas chromatography [15]. Thus, it appeared that the unequivocal relationship between the two enantiotopic H-atoms of butan-2-one (**5**) and those of *meso*-butane-2,3-diol (**4**) (or of the medium) could be established by ^{1}H(or ^{2}H)-NMR spectroscopy, provided a certain assignment of chemical shift was made for each of the diastereotopic protons in butan-2-ol (**6**) or in a suitable derivative, *e.g.*, **7**.

When analyzed by ^{1}H-NMR spectroscopy (300 MHz, C_6D_6), 1-methylpropyl phenylcarbamate (**7**, see *Fig. 2*) showed two separate *m* centered at δ 1.31 and 1.44. The assignments of the former resonance to the *anti*-H[2]) and of the latter to the *syn*-H were established by a comparison of the ^{1}H-NMR spectrum of **7** with those of its monodeuterated analogs, *i.e.* **8** (*syn*-D) and **9** (*anti*-D) (*Fig. 2*). These analogs were prepared by $LiAlD_4$ reduction of the epoxides of *cis*- and *trans*-but-2-ene, respectively, followed by treatment of the resulting alcohols with phenyl isocyanate.

(2*R*,3*S*)-(1,1,1,2-$^2H_4$)butane-2,3-diol (**12**) was then synthesized as shown in *Scheme 3* from (*S*)-2-(benzyloxy)(1-^{2}H)propanal (**11**) by a procedure we previously described [20]. After fermentation of compound **12** by *Lactobacillus brevis* LB19 under anaerobic conditions, an optically active butan-2-ol was isolated whose (*R*)/(*S*) ratio was found to be 68(± 2) : 32(± 2) by means of chiral gas chromatography. Inspection of the ^{2}H-NMR spectrum (C_6D_6) of the product resulting from treatment of this butan-2-ol mixture with phenyl isocyanate revealed the presence of broad *s*s at δ 0.79 (CD_3), 1.44 (*syn*-D), and 1.31 (*anti*-D) in an intensity ratio 3 : 0.7 : 0.3. The fact that the ratios between the abundances of the (*R*)- and (*S*)-isomers in butan-2-ol and between the *syn*-D and *anti*-D resonance intensities in the corresponding 1-methylpropyl phenylcarbamate were practically identical, clearly indicated the existence of only two deuterated species in each mixture examined, *i.e.*, **13**/**14** in the alcohol and **15**/**16** in its *O*-carbamoyl derivative (*Scheme 3*).

By inference, one can conclude that in the diol-dehydratase-catalyzed conversion of *meso*-butane-2,3-diol into butan-2-one, *the H-atom at the (*R*) C-atom is retained, and the replacement of the OH group occurs with inversion of configuration*. It must be pointed out that the same outcome was observed in the case of the action of (*RS*)-propane-1,2-diol hydro-lyase from *Klebsiella pneumoniae* on both (*R*)- and (*S*)-propane-1,2-diols [4][5][21] (see *Fig. 1*).

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[2]) Throughout this paper, the methylene H-atoms of 1-methylpropyl phenylcarbamate will be called *syn*-H (or *syn*-D) and *anti*-H (or *anti*-D) according to their position relative to the (phenylcarbamoyl)oxy group in a staggered conformation having the two Me groups in *anti*-periplanar orientation (as in formula **7** of *Fig. 2*).

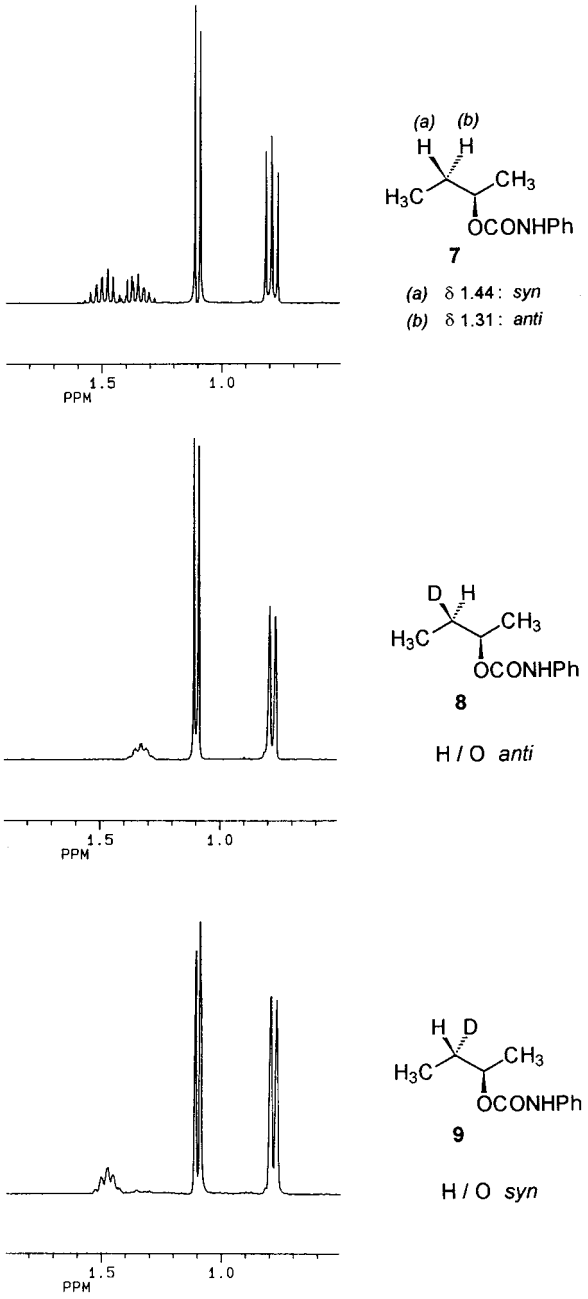
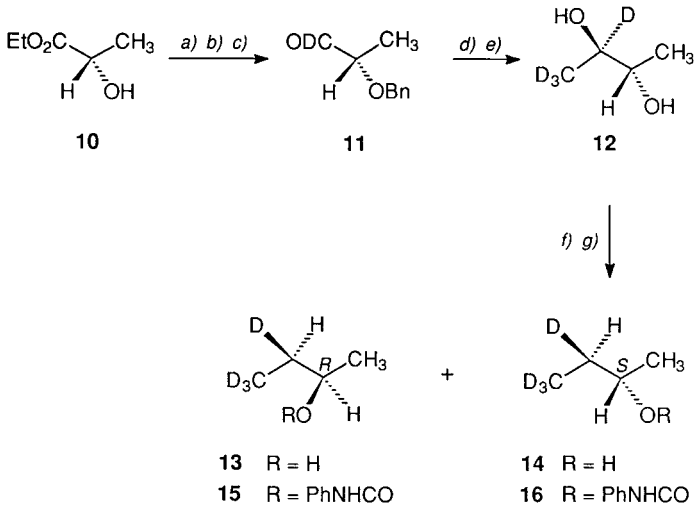


Fig. 2. *^{1}H-NMR Spectra* (300 MHz, C_6D_6, 303 K) *of 1-methylpropyl phenylcarbamate* (**7**; one enantiomer being represented for clarity) *and of its (2-$^2H_1$)-derivatives* **8** *and* **9**

Scheme 3

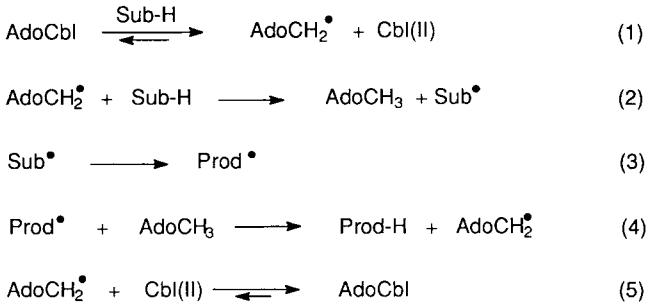


a) $PhCH_2Br$, Ag_2O. *b*) $LiAlD_4$, Et_2O. *c*) *Swern* oxidation. *d*) [$TiCl(^iPrO)_3$], CD_3Li, Et_2O, $-78°$, then addition of **11**, $0° \rightarrow$r.t. *e*) H_2, 10% Pd/C, EtOH, r.t. *f*) *Lactobacillus brevis*, 1 week. *g*) PhNCO, Et_2O.

2.2. *Fate of the H-Atom Linked to the (*S*) C-Atom of* meso*-Butane-2,3-diol*. In the case of the above mentioned enzyme, good evidence has established that the homolytic cleavage of the Co–C bond of the adenosylcob(III)alamin (AdoCbl) to give cob(II)alamin and the 5′-deoxy-5′-adenosyl radical ($AdoCH_2^{\bullet}$) is an early event in the enzymatic process [7b][24]. In addition, it was shown that the overall dehydration of propanediols occurs without appreciable exchange of the C-linked H-atoms with the solvent [25]. On the other hand, the H-migration did not appear to be intramolecular, since it was shown that a T-atom from C(1) of propane-1,2-diol is transferred to C(2) of the acetaldehyde derived from unlabeled ethylene glycol in experiments performed with different mixtures of the two diols [26][27]. The participation of $AdoCH_2^{\bullet}$ as a H-carrier having three equivalent H-atoms in the intermediate form ($AdoCH_3$), and the sequence of reactions of *Scheme 4* were postulated [27], despite the unusually large k_H/k_T isotope effects (up to 125) that were observed for the last H(T)-transfer step (*Step 4*), *i.e.*, when T-atom is transferred from the enzyme-bound [5′-^{3}H]adenosylcobalamin to the product-related radical to give acetaldehyde or propanal [28]. More recently, the participation of a second H-transfer site of the enzyme has been suggested as an explanation of the very high k_H/k_T value [24]. However, the actual mechanism has not yet been convincingly established.

Taking these facts into account, the retention of the mobile H-atom of *meso*-butane-2,3-diol (**4**) was tested by feeding *L. brevis* LB19 with a mixture of all the three (2,3-$^2H_2$)-stereoisomers of **1d** (the optically active stereoisomers being unaffected by the diol dehydratase) [19]. This mixture was prepared by $LiAlD_4$ reduction of butane-2,3-dione (= diacetyl) and consisted of 64% of the *meso*-form and 36% of the *rac*-form (dideuterated molecules 98 ± 1% by MS). Butan-2-ol resulting from fermentation ((R)/(*S*) *ca.* 75 : 25) was found to contain 1 D-atom per molecule by MS measure-

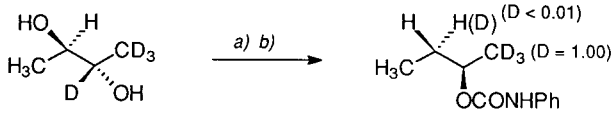
Scheme 4[a])



[a]) AdoCbl = adenosylcob(III)alamin; $AdoCH_3$ = 5′-deoxyadenosine; Cbl(II) = cob(II)alamin; Sub-H = substrate; Sub• = substrate-derived radical; Prod-H = product; Prod• = product-related radical.

ments; in addition, the ^{2}H-NMR spectrum of its *O*-(phenylcarbamoyl) derivative exhibited the two signals at δ 1.44 (*syn*-D) and at δ 1.31 (*anti*-D) in the ratio 3 : 1. These findings are consistent with both a loss of the H-atom linked to the (*S*) C-atom of *meso*-butane-2,3-diol and the configuration inversion at the (*R*) C-atom established above (see *Scheme 3*). That the H-loss was practically complete was confirmed by the microbial transformation of (2*S*,3*R*)-(1,1,1,2-$^2H_4$)butane-2,3-diol (**17**) (*Scheme 5*), which in turn was synthesized by the same procedure as for compound **12** (*Scheme 3*) but starting from ethyl (*R*)-lactate. Only a signal at δ 1.09 (CD_3) was observed in the ^{2}H-NMR spectrum of the resulting 1-methylpropyl phenylcarbamate **18**, even at high concentration in $CHCl_3$.

Scheme 5



17 **18**

a) *Lactobacillus brevis*, 1 week. *b*) PhNCO, Et_2O.

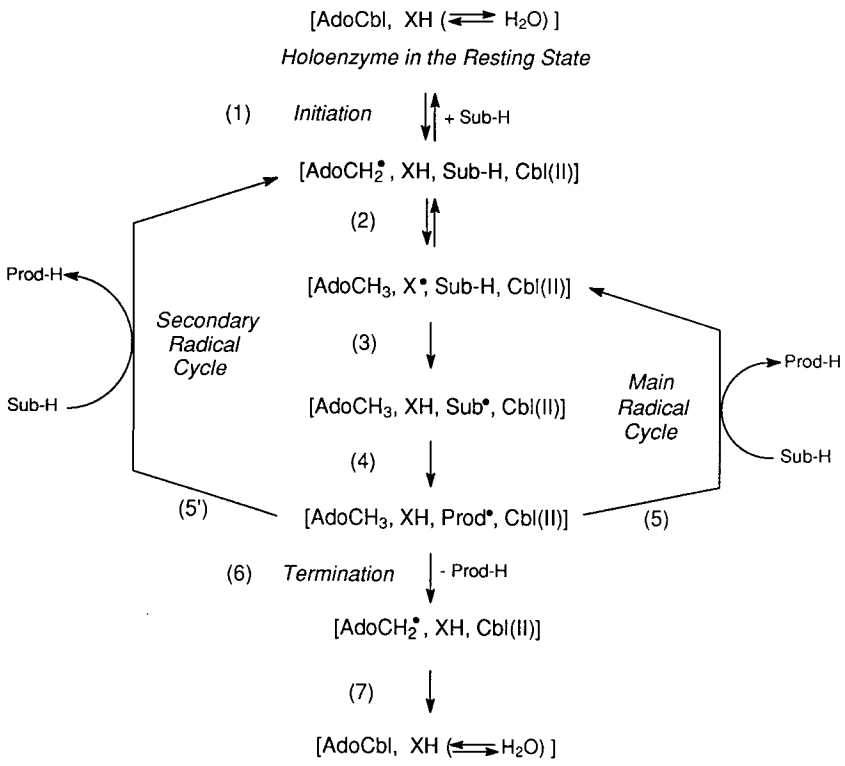
To rule out the possibility that the absence of D-atom at the 2-position of **18** was due to a rapid interconversion between *meso*-butane-2,3-diol and (*R*)-3-hydroxybutan-2-one (=(*R*)-acetoin), which might have occurred prior to the dehydration reaction [16], a fermentation experiment with **17** was interrupted at *ca.* 50% of the substrate conversion. No change of D-content and D-distribution in the recovered diol **17** was shown by ^{2}H-NMR analysis. It must also be noted that a H-exchange of butan-2-one with the medium, enzymatically or not, appears to be unlikely considering the D-retention previously observed in the conversion of compound **12** into butan-2-ol **13**/**14**.

Supplying our strain of *L. brevis* with (*RS*)-(1,1-$^2H_2$)propane-1,2-diol (prepared by $LiAlD_4$ reduction of ethyl lactate) resulted in the formation of propan-1-ol showing a considerable retention of the mobile D-atom, *i.e.*, a (2-^{2}H)/(1-^{2}H) ratio of 0.80 as estimated by ^{2}H-NMR analysis of the corresponding phenylcarbamate. In addition, when feeding experiments were carried out with equimolar mixtures of *meso*-butane-

2,3-diol and (*RS*)-propane-1,2-diol, one of these species having a D-atom in place of the labile H-atom, *ca.* 10% of the D-atom lost by propane-1,2-diol was found in the (*pro-S*) position at C(3) of butan-2-ol, whilst no D-transfer was observed from *meso*-butane-2,3-diol to propan-2-ol. Thus, it appears that the two diols are processed by the dehydratase in a rather different way, at least with regard to the H-transfer mechanism.

In our opinion, the loss of D-atom bound to the (*S*) C-atom of *meso*-butane-2,3-diol and the results of the above crossover experiments may be interpreted in terms of the general radical chain mechanism schematically represented in *Scheme 6*. This is an upgraded version of that proposed by *Finke* [7b] involving protein-bound radical intermediates with cob(II)alamin as a 'spectator' (bound-radical mechanism) [7a][29]. The main radical cycle is consistent with the large isotopic effects mentioned above [24][28][30] and is supported by a lot of analogies between diol dehydratase and two other adenosylcobalamin-dependent enzymes, namely ethanolamine ammonia lyase [31] and ribonucleotide reductase [32]. In fact, evidence has been provided for the participation of a Enz-XH site in the process catalyzed by both enzymes [33][32b] (XH being probably the thiol group of a cysteine residue) [31b][32a] and for the

Scheme 6. *Schematic Representation of the Putative Two-Cycle Radical Mechanism for Diol-Dehydratase-Catalyzed Transformations*[a]). See *Scheme 4* for abbreviations.



[a]) [– – –]: enzyme active site; $AdoCH_2^{\bullet}/AdoCH_3$: coenzyme H-carrier; $X^{\bullet}/XH$: protein H-carrier.

involvement of 5′-deoxyadenosine ($AdoCH_3$) in at most 1 turnover out of 1000 in the case of ribonucleotide reductase [7b]. The mechanism of *Scheme 6* encompasses an additional radical cycle in which the product-related radical abstracts a H-atom from $AdoCH_3$, while a second substrate molecule is entering the active site of the enzyme (as in the case of the main cycle)[3]). Finally, the reversibility of the H-transfer from Enz-XH to $AdoCH_2^{\bullet}$ and of the homolytic dissociation of AdoCbl (*Steps 2* and *1*, resp., in *Scheme 6*), may be responsible for the washout of T-atom from the coenzyme to solvent [7b][32b][33].

One can assume that in the course of both radical cycles, no significant H-exchange occurs between the Enz-XH group and the medium, while it is likely that it takes place when the holoenzyme is in the resting state. By the main cycle, a C-bound H-atom is transferred from the Enz-XH site to the adjacent C-atom of the same molecule, thus mimicking an intramolecular 1,2-shift. On the other hand, to account for the H-transfer from a substrate molecule to another one [26][27], we have introduced the secondary cycle. This demands the intermediacy of the vitamin-B_{12} coenzyme acting as a pool of enzyme-bound H-atoms in communication with the mobile H-atoms on different molecules of substrate and product.

When the substrate is propane-1,2-diol, every escape route of those represented as *Steps 5*, *5′*, and *6* in *Scheme 6* can be exploited by the product-related radical, that is the *main cycle* giving rise to an 'apparently intramolecular' H-transfer or the *secondary cycle* causing 'intermolecular' H-transfer (with temporary incorporation of the mobile H-atom into the 'vitamin-B_{12}-coenzyme pool') or the *termination* path by which the H-atom removed from the substrate, and stored in the Enz-XH site, will be exchanged with the medium before a new turnover starts.

If the product-related radical arises from *meso*-butane-2,3-diol, only *termination steps* should be allowed. The reason might be a steric hindrance to the binding of a second substrate molecule, while the active site is occupied by reacting *meso*-butane-2,3-diol. This means that the mobile H-atom of *meso*-butane-2,3-diol is lost in the medium even in the presence of propane-1,2-diol as an accompanying substrate for the enzymatic transformation. According to the above assumptions, the observed D-transfer from (*RS*)-(1,1-$^2H_2$)propane-1,2-diol to the *meso*-butane-2,3-diol-derived product (*i.e.,* butan-2-ol) can be explained as due to isotopic labeling of the adenosyl H-carrier by the former diol molecules processed through the *secondary cycle.*

3. Concluding Remarks. – The stereochemistry and the fate of H-atoms in the transformation of *meso*-butane-2,3-diol into butan-2-one by action of the diol dehydratase occurring in *L. brevis* are summarized in *Scheme 7.* In addition, the loss, the intermolecular, and the (postulated) apparently intramolecular transfer of the mobile H-atom in diol molecules can be explained on the basis of the reactions depicted in *Scheme 6* (which have been formulated in the spirit of a working hypothesis).

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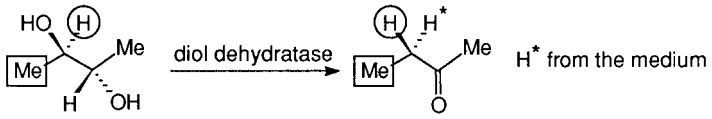
[3]) In this respect, it has been suggested that the enzyme might have at least *two* substrate-binding sites [7b].

Scheme 7. *Cryptostereochemical Features of the Dehydration of* meso-*Butane-2,3-diol by* Lactobacillus brevis



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Experimental Part

General. TLC: silica gel *60 F_{254}* precoated aluminum sheets (*Merck*); detection either by UV or spraying a ceric sulfate/ammonium molybdate soln., followed by heating to *ca.* 150°; eluent, hexane/Et_2O 7:3. Flash chromatography (FC): silica gel 40–63 μm (*Merck*). Anal. GC: *Dani 3800* gas chromatograph; home-made 2 m × 2 mm i.d. glass column, 20% *Carbowax 20M* on *Chromosorb W*, 60–80 mesh; injector, 210°; detector, 220°; *Conditions A*, 4 min at 60°, then to 150° at 10°/min, and 8 min at 150°; *Conditions B*, isothermal analysis at 200°; t_R in min. For chiral GC, see [15]. GC/MS: *Hewlett-Packard-CG-5972* instrument coupled with a *HP-5890* MS detector, *Ultra-1* (25 m × 0.2 mm i.d.) column; temp. 50°. M.p.: *Büchi-530* melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer-241* polarimeter; 1-dm cell. NMR Spectra: *Bruker-AC-300* spectrometer at 300.13 (^{1}H), 46.07 (^{2}H), and 75.47 MHz (^{13}C); δ in ppm *vs.* solvent as internal ref.: δ(H/D) 7.25, δ(C) 77.00 for C(H)DCl_3; δ(H/D) 7.15 for C_6(H)D_6; δ(H/D) 2.50 for (C(H)D_3)$_2$SO; *J* in Hz; ^{13}C multiplicities from DEPT spectra.

1-Methylpropyl Phenylcarbamate (**7**) *and 1-Propyl Phenylcarbamate.* To a soln. of commercial butan-2-ol (**6**; 0.2 ml, 2.2 mmol) in dry Et_2O (3 ml), excess of phenyl isocyanate was added and the mixture heated under reflux and N_2 until completion (GC analysis). Usual workup and recrystallization from hexane gave pure **7**. Following the same procedure, 1-propyl phenylcarbamate was prepared from the corresponding alcohol.

Data of **7**: R_f 0.34. M.p. 63–64°. ^{1}H-NMR (C_6D_6): 0.79 (*t*, *J* = 7.3, Me(3)); 1.09 (*d*, *J* = 6.8, Me–C(1)); 1.31 (*m*, H–C(2)); 1.44 (*m*, H–C(2)); 4.88 (*sext.* *J* = 6.3, H–C(1)); 6.01 (br. *s*, NH); 6.81 (*t*, *J* = 7.9, 1 arom. H); 7.06 (*t*, *J* = 7.8, 2 arom. H); 7.29 (*d*, *J* = 8.0, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 9.52 (*q*); 19.62 (*q*); 28.75 (*t*); 73.18 (*d*); 118.49 (*d*); 123.15 (*d*); 128.96 (*d*); 138.09 (*s*); 153.38 (*s*).

Data of 1-Propyl Phenylcarbamate: R_f 0.39. M.p. 57°. ^{1}H-NMR (C_6D_6): 0.74 (*t*, *J* = 7.4, Me); 1.41 (*m*, 2 H–C(2)); 3.97 (*t*, *J* = 6.7, 2 H–C(1)); 6.23 (br. *s*, NH); 6.82 (*t*, *J* = 7.5, 1 arom. H); 7.07 (*t*, *J* = 7.8, 2 arom. H); 7.30 (*d*, *J* = 7.8, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 10.29 (*q*); 22.28 (*t*); 66.83 (*t*); 118.70 (*d*); 123.31 (*d*); 128.99 (*d*); 138.03 (*s*); 153.76 (*s*).

*(1*RS,*2*RS*)-1-Methyl(2-$^2H_1$)propyl Phenylcarbamate* (**8**) *and (1*RS,*2*SR*)-1-Methyl(2-$^2H_1$)propyl Phenylcarbamate* (**9**). To a stirred suspension of $LiAlD_4$ (290 mg, 6.9 mmol) in dry Et_2O (15 ml), 500 mg (620 μl, 6.9 mmol) of *cis*-2,3-dimethyloxirane (= *cis*-2,3-epoxybutane, *Aldrich*) was added dropwise at r.t. under N_2. The mixture was heated under reflux for 2 h and then stirred at r.t. overnight. After cooling to 0°, the reaction was quenched by the sequential addition of H_2O (0.5 ml), 5N NaOH (1 ml), and H_2O (0.5 ml). The mixture was stirred for additional 2 h, the resulting white precipitate filtered off and washed with Et_2O, the aq. layer of the filtrate separated, and the org. phase dried (Na_2SO_4) and cautiously distilled using a *Vigreux* column. Fractions containing butan-2-ol (by GC analysis, *Conditions A*) were collected and treated with excess phenyl isocyanate as described above to give pure **8** (690 mg, 51% overall yield).

Following the same procedure, pure **9** was obtained from *trans*-2,3-dimethyloxirane (56% overall yield).

Data of **8**: ^{1}H-NMR (C_6D_6): δ as for **7**, except for the absence of the *m* at 1.44. ^{2}H-NMR (C_6H_6): 1.44 (*s*, ^{2}H–C(2)).

Data of **9**: ^{1}H-NMR (C_6D_6): δ as for **7**, except for the absence of the *m* at 1.31. ^{2}H-NMR (C_6H_6): 1.31 (*s*, ^{2}H–C(2)).

*(2*R,*3*S*)-(1,1,1,2-$^2H_4$)Butane-2,3-diol* (**12**). (*S*)-2-(Benzyloxy)(1-^{2}H) propanal (**11**) was prepared in 61% yield from commercial ethyl (*S*)-lactate (**10**; 98% e.e.) by a published procedure [34] using $LiAlD_4$ instead of $LiAlH_4$. An Et_2O soln. (*ca.* 40 ml) of [Ti(CD_3)(^{i}PrO)$_3$], prepared from [TiCl(^{i}PrO)$_3$] (1.9 ml, 8 mmol) and $CD_3Li \cdot LiI$ (0.5M in Et_2O, 17 ml) according to [35], was treated dropwise with a soln. of **11** (1.24 g, 7.5 mmol) in dry Et_2O (4 ml) at 0° under N_2, then allowed to warm to r.t., and stirred overnight. The mixture was cooled to 0°,

quenched by addition of 2N HCl, and extracted with Et_2O. The org. layer was washed with H_2O, dried (Na_2SO_4), and evaporated. FC (hexane/Et_2O 8:2 →6:4) gave (2*R*,3*S*)-*3-(benzyloxy)-(1,1,1,2-$^2H_4$)butan-2-ol* (950 mg, 69%) as a colorless oil. R_f 0.11. GC (*Conditions B*): t_R 14.7. ^{1}H-NMR ($CDCl_3$): 1.16 (*d*, *J* = 6.5, Me(4)); 2.28 (br. *s*, OH); 3.48 (*q*, *J* = 6.5, H–C(3)); 4.50, 4.62 (2*d*, each *J* = 11.8, PhCH_2); 7.26–7.35 (*m*, 5 arom. H). ^{2}H-NMR ($CHCl_3$): 1.13 (C^2H_3); 3.89 (^{2}H–C(2)).

(2*R*,3*S*)-3-(Benzyloxy)-(1,1,1,2-$^2H_4$)butan-2-ol (400 mg, 2.1 mmol) was hydrogenated over 10% Pd/C (270 mg) in EtOH (25 ml) at r.t. for 2 h (GC control). After removal of the catalyst and the solvent, bulb-to bulb distillation afforded **12** (190 mg, 96%). Colorless oil. B.p. 110°/4 Torr ('Kugelrohr'). GC (*Conditions A*): t_R 18.5. ^{2}H-NMR (DMSO): 1.03 (C^2H_3); 3.34 (^{2}H–C(2)); ratio 3:1.

(2S,3R)-(1,1,1,2-$^2H_4$)-Butane-2,3-diol (**17**). The same procedure as in the synthesis of **12** was used, starting from ethyl (*R*)-lactate. ^{2}H-NMR (DMSO): 1.03 (C^2H_3); 3.34 (^{2}H–C(2)); ratio 3:1.

(2,3-$^2H_2$)Butane-2,3-diols. The published procedure [36] was used with the following modification: the mixture (1 g of butane-2,3-dione (= diacetyl) and 0.4 g of $LiAlD_4$ in 20 ml of Et_2O) was quenched at 0° by the sequential addition of H_2O (0.5 ml), 4N NaOH (1 ml), and H_2O (0.5 ml). Usual workup and bulb-to-bulb distillation (100–105°/4 Torr) afforded (2,3-$^2H_2$)butane-2,3-diols (770 mg, 72%) as a 64:36 mixture of the *meso*- (t_R 18.5) and *rac*-diastereoisomer (t_R 17.6) by GC analysis (*Conditions A*). ($^2H_2$)-Species 98 ± 1% by GC/MS. ^{1}H-NMR ((D$_6$)DMSO): 0.98 (*s*, Me (*rac*)); 1.03 (*s*, Me (*meso*)); 4.24 (*s*, OH (*meso*)); 4.28 (*s*, OH (*rac*)). ^{2}H-NMR (DMSO): 3.34 (^{2}H–C(*meso*)); 3.39 (^{2}H–C(*rac*)).

(RS)-(1,1-$^2H_2$)Propane-1,2-diol was prepared by $LiAlD_4$ reduction of (*RS*)-ethyl lactate [23]. GC (*Conditions B*): t_R 2.1. ($^2H_2$)-Species 98 ± 1% by GC/MS. ^{1}H-NMR ($CDCl_3$): 1.13 (*d*, *J* = 6.0, Me); 3.19 (br. *s*, OH); 3.87 (br. *q*, *J* = 6.0, H–C(2)); no signals in the range 3.20–3.70.

Fermentations. Lactobacillus brevis (LB 19 strain) was from our collection. The inoculum was prepared from freeze-dried cells as previously described [16]. Fermentation experiments (1% inoculum) were carried out at 30° for a week in a sterilized synthetic medium [16] under anaerobic conditions using (labeled) *meso*-butane-2,3-diol (1 g/l), propane-1,2-diol (1 g/l), or a 1:1 mixture of the two diols (1 g/l each).

Isolation of Fermentation Products. Cells of *Lactobacillus brevis* were separated by centrifuging, and the broth was distilled. Fractions containing butan-2-ol and/or propan-1-ol (GC, *Conditions A*, t_R 6.8 and 5.7, resp.) were collected and continuously extracted with Et_2O. This Et_2O extract, when necessary, was analyzed by chiral GC and/or GC/MS. To prepare the *O*-(phenylcarbamoyl) derivatives of butan-2-ol and propan-1-ol, the Et_2O extract was cautiously concentrated using a *Vigreux* column and the residue treated with excess phenyl isocyanate. After refluxing with stirring for 24 h, phenylurea was filtered off and washed with Et_2O and the combined Et_2O soln. evaporated. FC (hexane/Et_2O 8:2 →7:3) of the residue gave 1-methylpropyl and/or 1-propyl phenylcarbamate which were characterized by comparison with authentic samples prepared from commercial butan-2-ol and propan-1-ol.

Fermentation of (2R,3S)-(1,1,1,2-$^2H_4$)Butane-2,3-diol (**12**). The extracted butan-2-ol was shown (chiral GC) to be a (2*R*)/(2*S*) mixture (**13**/**14**) in the ratio 68(± 2):32(± 2). The 1-methyl-(2,3,3,3-$^2H_4$)propyl phenylcarbamate (**15**/**16**) was isolated in 38% yield. $[\alpha]_D^{25} = -7.6$ (*c* = 0.2, $CHCl_3$) ($[\alpha]_D^{25} = -20.2$ (*c* = 1.1, $CHCl_3$) for a reference sample prepared from enantiomerically pure (–)-(*R*)-butan-2-ol). ^{2}H-NMR (C_6H_6): 0.79 (C^2H_3(3)); 1.31 (^{2}H–C(2)); 1.44 (^{2}H–C(2)); ratio 3:0.3:0.7.

Fermentation of (2,3-$^2H_2$)Butane-2,3-diols. After fermentation of *meso*/*rac*-(2,3-$^2H_2$)butane-2,3-diol (64:36; prepared as described above), 1-methyl(2-$^2H_1$)propyl phenylcarbamate was isolated in 31% yield (rel. to the *meso*-isomer). ^{2}H-NMR (C_6H_6): 1.31 (^{2}H–C(2)); 1.44 (^{2}H–C(2)); ratio 0.25:0.75. ($^2H_1$) Species > 98% by GC/MS of the corresponding alcohol.

Fermentation of (2S,3R)-(1,1,1,2-$^2H_4$)Butane-2,3-diol (**17**). The 1-($^2H_3$)methylpropyl phenylcarbamate (**18**) was obtained in 35% yield. ^{2}H-NMR (C_6H_6): 1.09 (C^2H_3(1)).

Fermentation of (RS)-(1,1-$^2H_2$)Propane-1,2-diol. The (1,2-$^2H_2$)propyl phenylcarbamate was obtained in 47% yield. ^{2}H-NMR (C_6H_6): 1.41 (^{2}H–C(2)); 3.97 (^{2}H–C(1)); ratio 0.80:1.0.

Fermentation of meso-*Butane-2,3-diol/(RS)-(1,1-$^2H_2$)Propane-1,2-diol 1:1.* The (1,2-$^2H_2$)Propyl phenylcarbamate was obtained in 35% yield. ^{2}H-NMR (C_6H_6): 1.41 (^{2}H–C(2)); 3.97 (^{2}H–C(1)); ratio 0.72:1.0.

The 1-methyl(2-$^2H_1$)propyl phenylcarbamate was obtained in 15% yield. ^{2}H-NMR (C_6H_6): 1.31 (^{2}H–C(2)); 1.44 (^{2}H–C(2)); ratio 0.63:0.37.

The ^{2}H-NMR of the 1:1 mixture of the above carbamates showed an intensity ratio 1:0.03 when referred to the signals at 3.97 and at 1.31 and 1.44, the last two being taken together.

*Fermentation of (2,3-$^2H_4$)Butane-2,3-diols and (RS)-Propane-1,2-diol (*meso-*(2,3-$^2H_2$)Butanediol/Propanediol 1:1).* The 1-Methyl(2-$^2H_1$)propyl phenylcarbamate was obtained in 21% yield. ^{2}H-NMR (C_6H_6): 1.31 (^{2}H–C(2)); 1.44 (^{2}H–C(2)); ratio 0.22:0.78.

No D-atom was observed in the ^{2}H-NMR of 1-propyl phenylcarbamate which was obtained in 34% yield.

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