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

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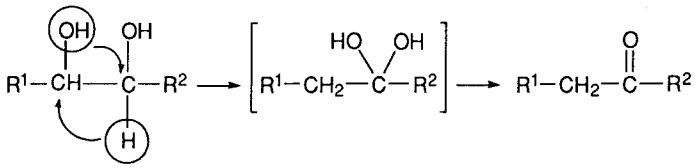
^{18}O-Substituted propane-1,2-diols and *meso*-butane-1,2-diols were synthesized and fed to growing cells of *Lactobacillus brevis*. Propan-1-ol and butan-2-ol, prepared from such diols through diol-dehydratase-catalyzed dehydration followed by intracellular reduction, were analyzed for their ^{18}O-content. For each propane-1,2-diol enantiomer, partial retention or complete loss of the isotope appeared to be related to the mode of substrate binding. Specific retention of the O-atom linked to the (*R*)-configured C-atom of *meso*-butane-1,2-diol indicates that the diol dehydratase handles this substrate like (*R*)-propane-1,2-diol.

1. Introduction. – Microbial diol dehydratases are coenzyme-B_{12}-dependent enzymes catalyzing the conversion of vicinal diols **1** to aldehydes or ketones **3** (*Scheme 1*) *via* a formal interchange of a OH group and the adjacent H-atom, followed by elimination of H_2O from the resulting geminal diols **2** [1]. Although the role of adenosylcobalamin as an initiator of a series of radical reactions is generally accepted, the exact mechanism of the enzyme-controlled 1,2-rearrangement remains to be clarified [2].

Scheme 1



1 **2** **3**

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

Four diol dehydratases have so far been described, namely (*RS*)-propane-1,2-diol hydro-lyase (EC 4.2.1.28) isolated from *Klebsiella pneumoniae* ATCC 8724 (formerly *Aerobacter aerogenes*) [3], glycerol hydro-lyase (EC 4.2.1.30) obtained from *K. pneumoniae* ATCC 25955 [4], a dehydratase produced by a strain of *Lactobacillus brevis* that shows marked activity on *meso*-butane-2,3-diol (*meso*-**1d**) [5], and a *K. oxytoca* ATCC 8724 diol dehydratase purified from an overexpressing *Escherichia coli* JM109 [6]. All of them appear to be active on the same substrates (**1a**–**d**), but differ in their kinetic parameters [1][5][7] and can be considered specifically induced isozymes [1b].

Results of extensive stereochemical studies on the dehydration of the diols **1a**, (*R*)-**1b**, (*S*)-**1b**, and **1c** [8] have led to the assumption that, at a single active site of the enzyme, the substrate molecule can be accommodated in two different binding modes, both of which demand the same three points of attachment [9], as depicted in *Fig. 1* for each enantiomer of propane-1,2-diol (**1b**). Each binding mode controls the specific migration of one of the two diastereotopic H-atoms at C(1) of the propanediol molecule [8][9][11b]. Such an atom is transferred to a H·-acceptor/donor (a transiently modified coenzyme or a site of the apoenzyme) and then back to the C(2) of propionaldehyde **3b** with inversion of the configuration at this center [2][10][11b].

Analogously, the opposite behavior of (*R*)- and (*S*)-propane-1,2-diol (**1b**) with respect to the alternative fate (retention or loss) of their O-atoms has been interpreted in terms of a dual mode of accommodation for substrates at the active site in conjunction with only one of the two possible enantiospecific H_2O eliminations from the aldehyde hydrate **2** (*Fig. 1*) [8][12]. It can be noted that the transformation of glycerol (**1c**) into 3-hydroxypropionaldehyde (**3c**) showed cryptostereochemical features and fate of H- and O-atoms[1]) in agreement with an exclusive [8] or a strongly prevalent (> 90%) [13] (*R*)-binding in the substrate-enzyme interaction (see *Fig. 1*).

Recently, with a strain of *Lactobacillus brevis* capable of converting *meso*-butane-2,3-diol (*meso*-**1d** ≡ **4**) into (*R*)-butan-2-ol (**6**; *ca.* 70% ee), we showed that the diol-dehydratase-catalyzed formation of butan-2-one (**5**) from **4** occurs with complete discrimination between the two enantiomorphic-enantiotopic 1-hydroxyethyl moieties of the substrate molecule [11a], and leads to the replacement of the OH group by a H-atom (from the medium) with inversion of configuration [11b] (*Scheme 2*).

The apparent resemblance of *meso*-butane-2,3-diol (**4**) and (*R*)-propane-1,2-diol (*Fig. 1, a*) in the stereochemical outcome of the biocatalyzed dehydration reaction (but not in the fate of the mobile H-atom) prompted us to extend the comparison between the two substrates to the fate of the O-atoms. The results reported here were obtained using the same LB19 strain of *L. brevis* as in previous investigations [11].

2. Results. – 2.1. *Synthesis of ^{18}O-Substituted Propanediols and Butanediols.* A 52 : 48 mixture of (*R*)-(2-^{18}O)- and (*S*)-(1-^{18}O)propane-1,2-diol (**11** and **12**, resp.), was prepared by perchloric acid-catalyzed oxirane ring opening of a commercial sample of (*S*)-2-methyloxirane (**10**) in $H_2{}^{18}O$/MeCN (97 atom-% ^{18}O) [14] (*Scheme 3,I*). The

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[1]) *Arigoni* states (in [8a] p. 396) that the modest ^{18}O retention (44%) observed in the case of *in vitro* enzymatic dehydration of [2-^{18}O]glycerol *'reflects the difficulty of trapping the original form of the released aldehyde in a quantitative manner prior to its rapid nonenzymic exchange with the medium'.*

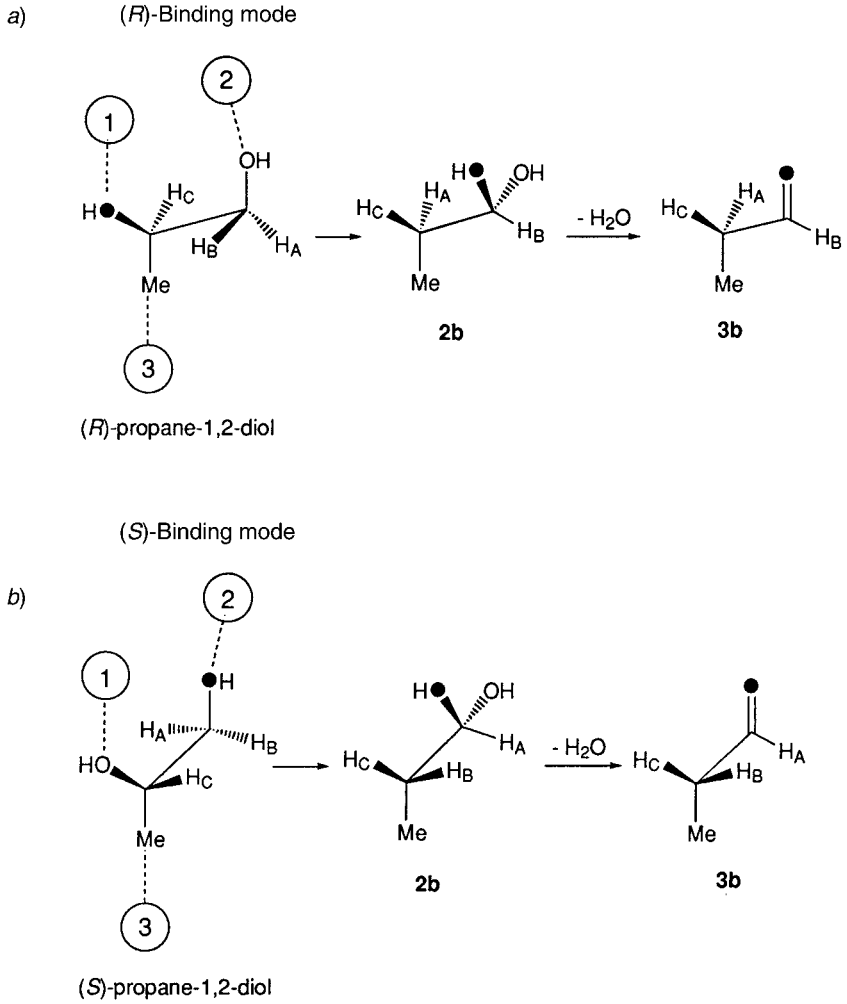
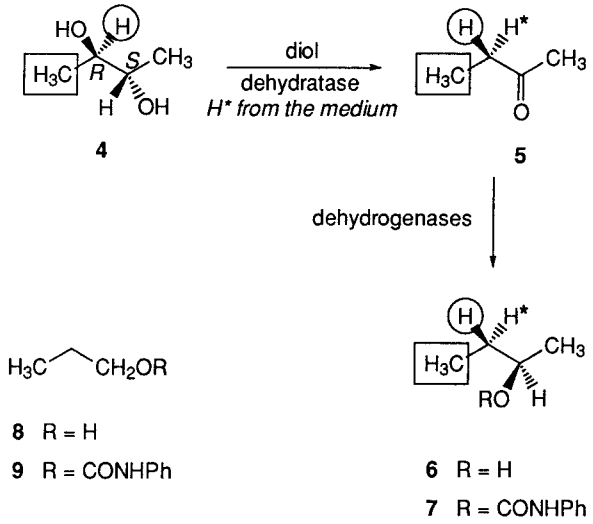


Fig. 1. *Schematic picture of the binding of propane-1,2-diol at the active site of diol dehydratase and fate of the H- and O-atoms during the conversion to propionaldehyde* [12] (H_A, H_B, H_C, and ●: isotopically labeled H- and O-atoms). The stereospecificity of OH migration from C(2) to C(1) and of H_2O elimination are related but arbitrary.

enantiomer ratio for **11/12** was determined by recording the ^{1}H-NMR spectrum of the mixture in (D_6)acetone in the presence of [Eu(tfc)$_3$] [15], while the ratio between molecules bearing a ^{18}O-atom at C(2) and C(1), together with their common ^{18}O enrichment (81.3%), was calculated from the relative intensities of MS peaks at *m/z* 47/45 and 63/61 (coming from the typical 'glycol split' represented in *Scheme 3,II*) [16]. The two ratios were practically identical, thus confirming the complete regiospecificity of the ^{18}O distribution in each enantiomer, as expected on the basis of mechanistic considerations (S_N2 solvolysis) [17].

Scheme 2



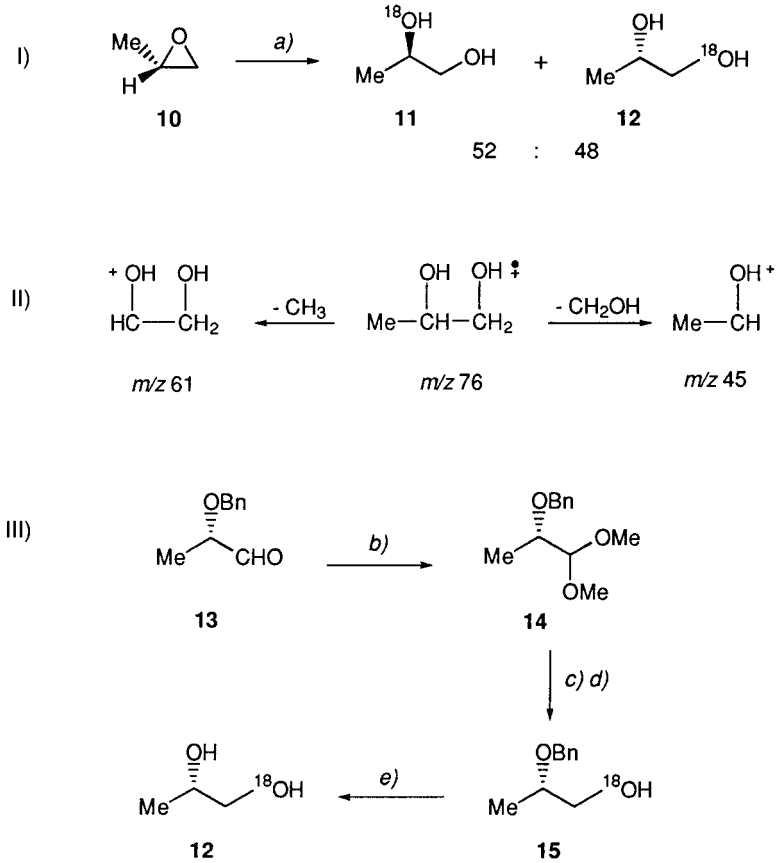
The preparation of each enantiomerically pure (1-^{18}O)propane-1,2-diol, was achieved as reported in *Scheme 3,III*, for **12**. No racemization was observed during the conversion of (*S*)-2-(benzyloxy)propanal (**13**) [18] to its dimethyl acetal **14**, followed by $H_2{}^{18}O$ hydrolysis, *in situ* reduction of the resulting ^{18}O-enriched aldehyde to give **15**, and debenzylation by hydrogenolysis.

The 1:1 mixture of (2*R*,3*S*)-(2-^{18}O)butane-2,3-diol (**17**) and (2*S*,3*R*)-(2-^{18}O)butane-2,3-diol (**18**) was prepared by hydrolysis of a commercial sample of racemic 2,3-dimethyloxirane (*rac*-**16**) under usual conditions (*Scheme 4*). The synthesis of enantiomerically pure **17** and **18** was centered on the transformation of optically active butane-2,3-diols (*e.g.*, **19**) into the corresponding 2,3-dimethyloxirane (*e.g.*, **16**) with retention of configuration at both stereogenic centers [19].

2.3. *Fermentation of ^{18}O-Enriched Diols by* Lactobacillus brevis *(LB19 strain)*. Supplying our strain (LB19) of *L. brevis* with samples of ^{18}O-enriched propane-1,2-diols and butane-2,3-diols, prepared as described above, furnished propan-1-ol (**8**) and butan-2-ol (**6**), respectively, of which the ^{18}O contents were revealed by mass-spectrometric analysis of the corresponding phenylcarbamates **9** and **7**, respectively. The values of ^{18}O retention are listed in the *Table*, together with those reported by *Arigoni* and co-workers [12] for similar experiments carried out *in vitro* with ^{18}O-labeled propane-1,2-diols.

It must be pointed out that, to prevent possible exchange of the carbonyl O-atom with the medium, *Arigoni* and co-workers [12] used a two-enzyme system consisting of the diol-dehydratase (really a cell-free extract of *K. pneumoniae* ATCC 8724) and yeast alcohol dehydrogenase (with NADH), the latter enzyme reducing the aldehyde released by the former. In our *in vivo* experiments, we exploited intracellular dehydrogenases that act on aldehydes and ketones [20]. In the cell, presumably, the competition between the enzymatic reduction of the diol dehydratase product and the exchange of its O-atom with the medium was in favor of the latter process [21], but not

Scheme 3



a) 5% $HClO_4$, $H_2{}^{18}O$/MeCN 1:4. *b*) $YbCl_3 \cdot 6H_2O$, $(MeO)_3CH$, MeOH. *c*) $H_2{}^{18}O$, *Amberlyst 15*, MeCN. *d*) $NaBH_4$, EtOH. e) H_2, 10% Pd/C, MeOH.

to such an extent to cause complete disappearance of the ^{18}O isotope originally present in the carbonyl group. The markedly smaller ^{18}O retention observed in butan-2-ol than in propan-1-ol, when similar retention could be expected considering the data summarized in *Fig. 1* [12] and the (*R*)-binding mode for *meso*-butane-2,3-diol [11] (*Entry 5 vs. Entries 1* and *3* in the *Table*), may be due to a longer survival in the cell of the ketone before reduction, as a consequence of a lower dehydrogenase activity on it than on the aldehyde[2]).

Note that (^{18}O)butan-2-one [23], when administered to a growing culture of *L. brevis*, was found to be converted into butan-2-ol with complete loss of the isotopic nuclide.

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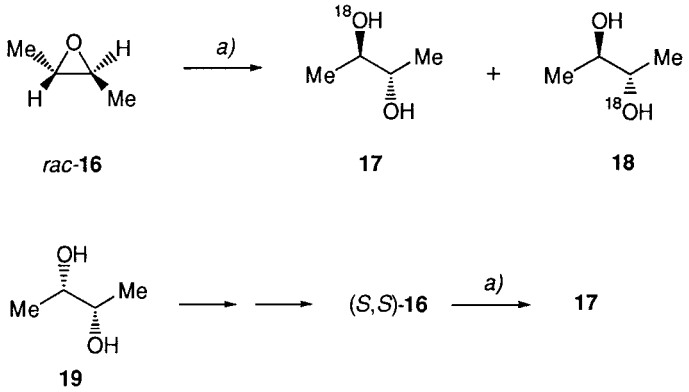
[2]) A higher rate of O exchange in butan-2-one than in propanal seems less probable [22].

Scheme 4



a) 5% $HClO_4$, $H_2{}^{18}O$/MeCN 1:4

Table. *^{18}O Content* [%] *in Diols and in Their Products of Transformation by* L. brevis, i.e., *Propan-1-ol* (**8**) *and Butan-2-ol* (**6**)

Entry	Substrates	^{18}O Content [%][a]		^{18}O Retention [%][b]	
		Reactant	Product		
1	(*S*)-MeCHOH-CH_2●H (**12**)	87.8	32.2	36.7 ± 0.5	(88 ± 7)[c]
2	(*R*)-MeCHOH-CH_2●H (*ent*-**12**)	91.2	nil	nil	(8 ± 4)[c]
3	(*R*)-MeCH●H–CH_2OH (**11**)/(*S*)-MeCHOH–CH_2●H (**12**) 52:48	81.3	39.8	48.9 ± 0.6	
4	(*RS*)-MeCH●H–CH_2OH				(43 ± 4)[c]
5	(*R*)-MeCH●H–CHOH–Me (**17**)	88.7	12.6	14.2 ± 0.5	
6	(*S*)-MeCHOH–CH●H–Me (**18**)	89.6	nil	nil	
7	**17**/**18** 1:1	88.4	7.2	8.1 ± 0.5	

[a]) Determined by MS analysis of the reactants (diols) and of the phenylcarbamates of the products (*i.e.*, **7** or **9**); average values of five spectra recorded for each sample. [b]) An experimental error of ±0.4% was assumed for MS data. [c]) Data in parentheses refer to experiments performed with *K. pneumoniae* cell-free extracts containing alcohol dehydrogenase [12]; error bars based on the published statement of ±0.5% precision in MS measurements [12].

3. Discussion. – Several mechanisms have been suggested [2] for the OH migration, which represents the key step in the formal 1,2-rearrangement catalyzed by diol dehydratases (*Scheme 1*). It is now generally accepted [2f] that such a migration occurs between the two H-abstraction reactions involved in the coenzyme and/or enzyme-mediated H-transfer process (steps **1** and **3** of *Fig. 2, a*). Considerable efforts have been expended in trying to reconcile plausible mechanistic hypotheses with the ^{18}O-labeling results obtained in the study of the enzymatic dehydration of propane-1,2-diols (*Fig. 1*). Some proposals have focused on Co-assisted OH migration [24]; however, in two critical and constructive reviews [2b,e], *Finke* has shown that the involvement of Co in the 1,2-rearrangement step (step 2 of *Fig. 2, a*) is not only unnecessary but also very improbable.

In the light of the above considerations, two alternative mechanisms have been proposed, both invoking protein-bound radicals as intermediates with Co as a 'spectator' ('*Bound Radical Mechanism*') [2b,e].

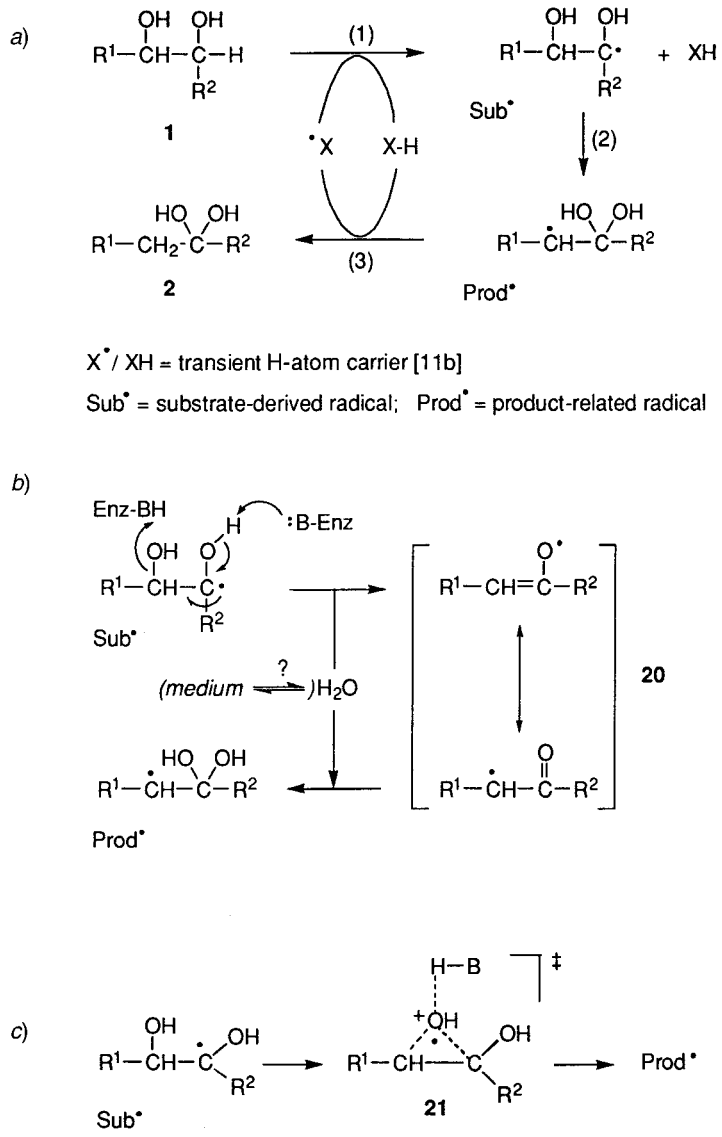


Fig. 2. a) *Minimal sequence of radical reactions involved in diol-dehydratase-catalyzed dehydration of vicinal glycols, and* b) *and* c) *putative mechanisms for the OH migration*

The radical anion ('*ketyl*') mechanism (*Fig. 2b*) [2f] is consistent with the behavior of 1,2-dihydroxyethyl radicals (Sub•), which are known to be 10^5 times more acidic than the corresponding alcohols and undergo rapid base- and acid-catalyzed β-OH cleavage, giving rise to a mesomeric radical (enoxy and 2-oxo radical as canonical forms) (*e.g.*, **20**) [2b][25]. An enantioselective backward addition of H_2O must be

considered to explain the formation of the 2,2-dihydroxy radical (Prod•) that is the precursor of the geminal diol intermediate **2** [2b, e].

On the other hand, *ab initio* molecular-orbital calculations have shown that the 1,2-shift of a OH group in the 1,2-dihydroxyethyl radical is greatly facilitated by *partial proton transfer* to the migrating O-atom [26] (*Fig. 2, c*), thus providing strong support for a strictly intramolecular OH migration in the course of the enzymatic process. Recently, based on the experimental evidence that, in the crystal structure of diol dehydratase, both OH groups of the substrate coordinate directly to a K^+ ion, a modification of the partial proton-transfer mechanism has been suggested, in which a K^+ ion takes the place of the partially transferred proton in **21** [27].

It can be noted that the OH-slithering mechanism (BH^+- or K^+-induced) (*Fig. 2, c*) brings about a complete retention of the migrating O-atom in the geminal-diol intermediate **2**, but this is not a necessary consequence of the fragmentation/recombination pathway (*Fig. 2, b*), in which a partial H_2O exchange between the enzyme-active site and the medium cannot be excluded.

Inspection of the *Table* reveals an agreement, with respect to ^{18}O retention, between the results of comparable experiments carried out by us and by *Arigoni* and co-workers [12], apart from the extent of ^{18}O retention. A plausible explanation of the lower ^{18}O retention observed with cells of *L. brevis* than with enzymatic preparations of *K. pneumoniae* has been given above. Thus, data from our ^{18}O-labeling experiments with propane-1,2-diols (*Table*) can be regarded as an *in vivo* validation of the stereospecificity of both the OH transfer and the H_2O elimination from the resulting 1,1-diol (*Fig. 1*).

In the case of *meso*-butane-2,3-diol (*meso*-**1d** ≡ **4**) the specific ^{18}O retention of the migrating oxygen, *i.e.*, that linked to the (*R*)-configured C-atom (*cf. Entry 5* in the *Table* and *Scheme 2*), is consistent with previous results [11], showing that diol dehydratase handles that substrate as a (*R*)-propane-1,2-diol molecule with Me in place of H_B in *Fig. 1, a*.

In conclusion, while our results provide further support for the hypothesis that the course of the diol-dehydratase-catalyzed reaction is determined by the initial binding mode of the substrate to the enzyme active site (*Fig. 1*), they do not allow a decision between mechanisms of the type *a* and *b* of *Fig. 2*; this, because an enzymatic H_2O exchange, in case it occurred, would be hidden by the extensive non-enzymatic ^{18}O exchange suffered by the carbonyl group in the released product prior to the reduction to an alcohol.

We gratefully acknowledge financial support from *MURST*. Thanks are due to Dr. *Enrico Caneva* and to *Sergio Crippa* (Dipartimento di Chimica Organica e Industriale, Milano) for recording NMR spectra.

Experimental Part

General. TLC: Silica gel *60* F_{254} precoated aluminum sheets (*Merck*); detection either by UV or spraying a ceric sulphate ammonium molybdate soln., followed by heating to *ca.* 150°. Flash chromatography (FC): silica gel 40–63 μm (*Merck*). GC: *Dani 3800* gas chromatograph; injector, 220°; detector, 220°, homemade 2 m × 2 mm i.d. glass columns; conditions *A*, 20% *Carbowax 20M* on *Chromosorb W*, 80–100 mesh, 4 min at 60°, then to 200° at 10°/min; conditions *B*, 5% *FFAP* on *Chromosorb* W, 80–100 mesh, isothermal analysis at 200°. NMR Spectra: *Bruker AC-300* spectrometer at 300.13 (^1H) and 75.47 MHz (^{13}C); δ in ppm *vs.* solvent as internal reference. EI-MS (*m/z* [%]): *VG 7070 EQ* mass spectrometer operating at 70 eV. GC/MS: *Dani 3800* gas

chromatograph coupled with *VG 7070 EQ*, *Carbowax 20M* (25 m × 0.2 mm i.d.) column (from *Mega*, Legnano, Italy); conditions, 5 min at 50°, then to 200° at 10°/min. (^{18}O)water (97 atom-% ^{18}O) was from *Isotec* (USA).

Fermentations. Lactobacillus brevis (LB 19 strain) was from our collection [20]. Fermentation experiments were carried out as described in [11b][20].

Fermentation Products. Butan-2-ol (**6**) and *propan-1-ol* (**8**) were isolated from the fermentation broth and treated with excess PhNCO as described in [11b]. The resulting phenylcarbamates **7** and **9**, resp., were analyzed for their ^{18}O content by EI-MS and ^{13}C-NMR.

Data of 1-Methylpropyl Phenylcarbamate (**7**): see [11b]. ^{13}C-NMR ($CDCl_3$): 73.142 (Me*C*H−^{18}O); 73.178 (Me*C*H−^{16}O). EI-MS: 193 (or 195) (43, M^+), 137 (or 139) (57), 120 (31), 93 (100).

Data of Propyl Phenylcarbamate (**9**): see [11b]. ^{13}C-NMR ($CDCl_3$): 66.785 (*C*H_2−^{18}O); 66.817 (*C*H_2−^{16}O). EI-MS: 179 (or 181) (55; M^+), 137 (or 139) (22), 120 (40), 93 (100).

*(*R*)-(2-^{18}O)Propane-1,2-diol* (**11**) *and (*S*)-(1-^{18}O)Propane-1,2-diol* (**12**). To a cold (0°) soln. of (−)-(*S*)-2-methyloxirane (=(*S*)-(−)-propylene oxide; *Aldrich*; 230 µl, 3.3 mmol; **10**) in MeCN (1 ml), (^{18}O)water (250 µl) and 70% $HClO_4$ (18 µl) were added. The mixture was stirred at 0° monitoring by GC (conditions *A*). After 2 h, the soln. was neutralized with sat. aq. $NaHCO_3$ and concentrated *in vacuo*. The residue was taken up in sat. aq. NaCl soln. (10 ml) and continuously extracted in a *Soxhlet* apparatus with AcOEt. The AcOEt extract was dried (Na_2SO_4), evaporated, and purified by bulb-to-bulb distillation to afford chemically pure propane-1,2-diol (170 mg, 66%) as shown by GC (conditions A) and ^{1}H-NMR analysis. The enantiomer ratio ((R)/(S) 52 : 48) was determined by ^{1}H-NMR in (D_6)acetone with [Eu(tfc)$_3$] as a shift reagent, and the ^{18}O specific incorporation by GC/MS (see text). ^{13}C-NMR ($CDCl_3$): 67.774 (*C*H_2−^{18}OH); 67.792 (*C*H_2−^{16}OH); 68.172 (*C*H−^{18}OH); 68.197 (*C*H−^{16}OH).

*(*S*)-(1-^{18}O)Propane-1,2-diol* (**12**). *(*S*)-2-(benzyloxy)propanal* (**13**; 1.3 g, 7.9 mmol), prepared from commercial ethyl (*S*)-lactate (98% ee) according to a published procedure [18], was dissolved in MeOH (19 ml). Subsequently $YbCl_3 \cdot 6\,H_2O$ (4.3 g, 11.1 mmol) and $(MeO)_3CH$ (14.2 ml, 130 mmol) were added, and the mixture was stirred at r.t., monitoring by TLC (hexane/acetone 9 : 1) and GC (conditions *B*). After 3 h, the mixture was poured into 90 ml of 5% aq. $NaHCO_3$ soln. and extracted several times with Et_2O. The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo* to give pure *(*S*)-2-benzyloxy-1,1-dimethoxypropane* (**14**; 1.6 g, 95%) [28], which was used in the next step without further purification.

(^{18}O)Water (0.5 ml) and *Amberlyst-15* (400 mg) were added to a soln. of **14** (410 mg, 1.95 mmol) in MeCN (4 ml), and the mixture was kept at 40° under stirring. After 30 h, the resin was filtered off, the filtrate cooled to 0°, diluted with EtOH (8 ml), and slowly treated with $NaBH_4$ (500 mg, 13.2 mmol). The mixture was allowed to warm to r.t. and stirred for additional 30 min. After concentration under reduced pressure, the residue was acidified with 1N HCl and continuously extracted with Et_2O. The org. layer was dried (Na_2SO_4), evaporated *in vacuo*, and purified by FC (hexane/AcOEt 5 : 1) to give pure **15** (274 mg, 83%) [18], which was hydrogenated over 10% Pd/C (200 mg) in MeOH (20 ml) at r.t. Usual workup and bulb-to-bulb distillation furnished **12** (112 mg, 89%). ^{13}C-NMR ($CDCl_3$): 67.774 (*C*H_2−^{18}OH); 67.792 (*C*H_2−^{16}OH); 68.197 (*C*H−^{16}OH). 87.8% Yield of the mono-^{18}O compound by GC/MS.

*(*R*)-(1-^{18}O)Propane-1,2-diol* (*ent*-**12**) was obtained in 67% total yield from commercial ethyl (*R*)-lactate by the same sequence of reactions described above for **12**. 91.2% of the mono-^{18}O compound by GC/MS and ^{13}C-NMR analysis.

*(2*R*,3*S*)-(2-^{18}O)Butane-2,3-diol* (**17**) *and (2*S*,3*R*)-(2-^{18}O)Butane-2,3-diol* (**18**). Commercially available *trans*-2,3-dimethyloxirane (*rac*-**16**; =*trans*-2,3-epoxybutane, *Aldrich*) was subjected to acid hydrolysis under the same conditions (5% $HClO_4$, $^{18}H_2O$/MeCN 1 : 4) described above for **11** and **12** from (−)-(*S*)-2-methyloxirane to give a 1 : 1 mixture **17/18** (87%), which was shown to be pure by GC analysis (conditions *A*). ^{13}C-NMR ($CDCl_3$): 70.779 (*C*H−^{18}OH); 70.803 (*C*H−^{16}OH). 88.4% of mono-^{18}O molecules by GC/MS.

*(2*R*,3*S*)-(2-^{18}O)Butane-2,3-diol* (**17**). Diastereoisomerically and enantiomerically pure *(2*S*,3*S*)-2,3-dimethyloxirane* (**16**), prepared from commercial *(2*S*,3*S*)-butane-2,3-diol* (**19**) according to the procedure in [19], was converted into **17** (79%) under the hydrolytic conditions described above; 88.7% of mono-^{18}O molecules by GC/MS and ^{13}C-NMR analysis. Analogously, compound **18** was obtained from commercial (2*R*,3*R*)-butane-2,3-diol.

REFERENCES

[1] a) 'Vitamin B_{12} – Proceedings of the Third European Symposium on Vitamin B_{12} and Intrinsic Factor', Eds. B. Zagalak and W. Friedlich, W. de Gruyter, Berlin, 1979; b) 'B_{12}', Ed. D. Dolphin, Wiley-Interscience, New York, 1982, Vols. I and II; c) T. Toraya, S. Fukui, in [1a], pp. 223–261; d) T. Toraya, in 'Metal Ions in

Biological Systems', Eds. H. Sigel and A. Sigel, Marcel Dekker, New York, 1994, Vol. 30, pp. 217–254; d) T. Toraya, in 'Chemistry and Biochemistry of B_{12}', Ed. R. Banerjee, John Wiley & Sons, New York, 1999, pp. 783–809.

[2] a) B. T. Golding, in [1b], Vol. I, pp. 543–582; b) R. G. Finke, D. A. Schiraldi, B. J. Mayer, *Coord. Chem. Rev.* **1984**, *54*, 1; c) B. T. Golding, D. R. R. Rao, in 'Enzyme Mechanisms', Eds. M. I. Page and A. Williams, Royal Society of Chemistry, London, 1989, pp. 404–428; d) B. T. Golding, *Chem. Brit.* **1990**, *26*, 950; e) R. G. Finke, in 'Molecular Mechanisms in Biorganic Processes', Eds. C. Bleasdale and B. T. Golding, Royal Society of Chemistry, London, 1990, pp. 244–280; f) B. T. Golding, W. Buckel, in 'Comprehensive Biological Catalysis', Ed. M. L. Sinnot, Academic Press, London, 1998, Vol. III, pp. 239–259.

[3] H. A. Lee, R. A. Abeles, *J. Biol. Chem.* **1963**, *238*, 2367.

[4] Z. Schneider, E. G. Larsen, G. Jacobson, B. C. Johnson, J. Pawelkiewicz, *J. Biol. Chem.* **1970**, *245*, 3388.

[5] F. Radler, J. Zorg, *Am. J. Enol. Vitic.* **1986**, *37*, 206.

[6] a) T. Toraya, K. Mori, *J. Biol. Chem.* **1999**, *274*, 3372; b) J. Masuda, T. Yamaguchi, T. Tobimatsu, T. Toraya, K. Suto, N. Shibata, Y. Morimoto, Y. Higuchi, N. Yasuoka, *Acta Crystallogr. Sect. D.* **1999**, *55*, 907; c) N. Shibata, J. Masuda, T. Tobimatsu, T. Toraya, K. Suto, Y. Morimoto, N. Yasuoka, *Structure* **1999**, *7*, 997.

[7] T. Toraya, T. Shirakashi, T. Kosuga, S. Fukui, *Biochem. Biophys. Res. Commun.* **1976**, *69*, 475; K. W. Moore, J. H. Richards, *Biochem. Biophys. Res. Commun.* **1979**, *87*, 1052.

[8] a) D. Arigoni, in [1a], pp. 389–410; b) J. Rétey, J. A. Robinson, 'Stereospecificity in Organic Chemistry and Enzymology', Verlag Chemie, Weinheim, 1982, pp. 185–207.

[9] J. Rétey, A. Umani-Ronchi, D. Arigoni, *Experientia* **1966**, *22*, 72.

[10] R. H. Abeles, in [1a], p. 371.

[11] a) G. Speranza, P. Manitto, G. Fontana, D. Monti, A. Galli, *Tetrahedron Lett.* **1996**, *37*, 4247; b) P. Manitto, G. Speranza, G. Fontana, A. Galli, *Helv. Chim. Acta* **1998**, *81*, 2005.

[12] J. Rétey, A. Umani-Ronchi, J. Seibl, D. Arigoni, *Experientia* **1966**, *22*, 502.

[13] W. W. Bachovchin, R. G. Eagar, K. W. Moore, J. H. Richards, *Biochemistry* **1977**, *16*, 1082.

[14] P. G. M. Wuts, R. D'Costa, W. Butler, *J. Org. Chem.* **1984**, *49*, 2582.

[15] L. M. Sweeting, D. C. Crans, G. Whitesides, *J. Org. Chem.* **1987**, *52*, 2273.

[16] F. A. Long, J. G. Pritchard, *J. Am. Chem. Soc.* **1956**, *78*, 2663.

[17] R. A. Wohl, *Chimia* **1974**, *28*, 1.

[18] K. Takai, C. H. Heathcock, *J. Org. Chem.* **1985**, *50*, 3247.

[19] V. Schurig, B. Koppenhoefer, W. Buerkle, *J. Org. Chem.* **1980**, *45*, 538.

[20] G. Speranza, S. Corti, G. Fontana, P. Manitto, A. Galli, M. Scarpellini, F. Chialva, *J. Agric. Food Chem.* **1997**, *45*, 3476.

[21] P. E. Soerensen, W. P. Jenks, *J. Am. Chem. Soc.* **1987**, *109*, 4675 and refs. cit. therein.

[22] M. Cohn, H. C. Urby, *J. Am. Chem. Soc.* **1938**, *60*, 679.

[23] B. T. Golding, A. K. Womg, *Angew. Chem., Int. Ed.* **1981**, *20*, 89.

[24] B. B. Wayland, S. L. Van Voorhees, K. L. del Rossi, *J. Am. Chem. Soc.* **1987**, *109*, 6513, and refs. cit. therein.

[25] B. C. Gilbert, R. O. C. Norman, P. S. Williams, *J. Chem. Soc., Perkin., Trans. 2* **1981**, 1401.

[26] D. M. Smith, B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **1999**, *121*, 5700.

[27] T. Toraya, K. Yoshizawa, M. Eda, T. Yamabe, *J. Biochem.* **1999**, *126*, 650.

[28] K. Kudo, Y. Hashimoto, M. Sukegawa, H. Masaki, K. Saigo, *J. Org. Chem.* **1993**, *58*, 579.

Received: October 31, 2000