

and absolute rate constants for hydrogen atom abstraction from arylalkyl, deuterated arylalkyl, and heteroatom donors.<sup>9</sup>

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**Supplementary Material Available:** Detailed kinetic data are provided in Tables IV-VIII of the microfilm edition (10 pages). Ordering information is given on any current masthead page.

## Preparation of Optically Active 1,2-Diols and $\alpha$ -Hydroxy Ketones Using Glycerol Dehydrogenase as Catalyst: Limits to Enzyme-Catalyzed Synthesis due to Noncompetitive and Mixed Inhibition by Product

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Glycerol dehydrogenase (GDH, EC 1.1.1.6, from *Enterobacter aerogenes* or *Cellulomonas sp.*) catalyzes the interconversion of analogues of glycerol and dihydroxyacetone. Its substrate specificity is quite different from that of horse liver alcohol dehydrogenase (HLADH), yeast alcohol dehydrogenase, lactate dehydrogenase, and other alcohol dehydrogenases used in enzyme-catalyzed organic synthesis and is thus a useful new enzymic catalyst for the synthesis of enantiomerically enriched and isotopically labeled organic molecules. This paper illustrates synthetic applications of GDH as a reduction catalyst by the enantioselective reduction of 1-hydroxy-2-propanone and 1-hydroxy-2-butanone to the corresponding *R* 1,2-diols (ee = 95-98%). (*R*)-1,2-Butanediol-2-*d*<sub>1</sub> was prepared by using formate-*d*<sub>1</sub> as the ultimate reducing agent. Comparison of (*R*)-1,2-butanediol prepared by reduction of 1-hydroxy-2-butanone enzymatically and with actively fermenting bakers' yeast indicated that yield and enantiomeric purity were similar by the two procedures. Reactions proceeding in the direction of substrate oxidation usually suffer from slow rates and incomplete conversions due to product inhibition. The kinetic consequences of product inhibition (competitive, noncompetitive, and mixed) for practical synthetic applications of GDH, HLADH, and other oxidoreductases are analyzed. In general, product inhibition seems the most serious limitation to the use of these enzymes as oxidation catalysts in organic synthesis.

The NAD(P)(H)-dependent oxidoreductases are useful in the synthesis of chiral synthons. Horse liver alcohol dehydrogenase (HLADH)<sup>1,2</sup> has been most thoroughly explored; lactate dehydrogenase and L-leucine dehydrogenase have also been used.<sup>3,4</sup> The practicality of these systems for organic synthesis has increased with the recent development of effective procedures for enzyme immobilization and nicotinamide cofactor regeneration.<sup>5-7</sup> This manuscript describes the use of another commercially available oxidoreductase, glycerol dehydrogenase (GDH, EC 1.1.1.6), to reduce  $\alpha$ -hydroxy ketones to chiral 1,2-diols. Previous studies have shown that chiral 1,2-diols having

**Table I. Relative Rates of Reduction of  $\alpha$ -Hydroxy Ketones by GDH/NADH<sup>a</sup>**

substrate	relative rate <sup>b</sup>		$K_m$ , mM	
	<i>C.s.</i>	<i>E.a.</i>	<i>C.s.</i>	<i>E.a.</i>
dihydroxyacetone	100	100	0.3	0.5
( <i>R</i> )-2,3-dihydroxypropanal	270			
( <i>S</i> )-2,3-dihydroxypropanal	130			
1-hydroxy-2-propanone <sup>c</sup>	30	20		0.04
1-hydroxy-2-butanone <sup>c</sup>	15	20		
2-oxopropanal	13			
2-hydroxycyclobutanone	290			
2-hydroxycyclopentanone	140		10	
2-hydroxycyclohexanone	14			
3-hydroxy-2-butanone	45		3	4

<sup>a</sup> Abbreviations: *C.s.*, *Cellulomonas sp.*; *E.a.*, *Enterobacter aerogenes*. Reactions were conducted at pH 7.6, 25 °C. Details of reaction conditions are given in the Experimental Section. The products of these reactions were not isolated, nor were their structures explicitly established. Compounds which did not react under these conditions include 1,2-cyclohexanedione, 2,3-butanedione, 2-propanone, 1-chloro-2-propanone, acetaldehyde, 2-hydroxy-4,4-dimethylcyclohexanone, and 4-hydroxy-3-hexanone. <sup>b</sup> The concentration of substrate in each experiment was 100 mM unless indicated otherwise. <sup>c</sup> The concentration of the substrate was 0.2 mM in these determinations of relative rate. Lower rates were observed at higher concentrations of substrate (100 mM).

the same absolute configuration obtained here are obtained by reduction of  $\alpha$ -hydroxy ketones using fermenting bakers' yeast.<sup>8,9</sup> We compare these enzymatic and ferment-

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tative methods and conclude that they generate products in similar yields and enantiomeric purities. GDH can also be used to prepare chiral  $\alpha$ -hydroxy ketones by oxidizing *cis*-1,2-diols to chiral  $\alpha$ -hydroxy ketones or by resolving a racemic mixture of  $\alpha$ -hydroxy ketones kinetically by enantioselective reduction of one enantiomer. Chemical syntheses of chiral  $\alpha$ -hydroxy esters,<sup>10</sup> chiral dihydroxycycloalkanones,<sup>11</sup> and chiral  $\alpha$ -hydroxy aldehydes<sup>12</sup> are available, but there are no general methods to synthesize chiral  $\alpha$ -hydroxy ketones.

Glycerol dehydrogenase is a nicotinamide cofactor-dependent oxidoreductase which catalyzes the interconversion of glycerol and dihydroxyacetone. Several sources of oxidoreductases having this activity are known, including *Enterobacter aerogenes*,<sup>13-16</sup> *Cellulomonas sp.* (the enzymes from these two sources are commercially available and are used in this study), *Erwinia aroideae*,<sup>16</sup> and *Klebsiella aerogenes*.<sup>17</sup> GDH from both *Cellulomonas* and *Enterobacter* is relatively inexpensive (\$40/1000 U and \$600/1000 U, respectively; 1 U = 1  $\mu\text{mol min}^{-1}$ ; 700 U of activity converts ca. 1 mol of substrate per day to product). GDH is stable and easily immobilized. The enzyme is used in several assay procedures.<sup>18</sup> Because GDH contains autooxidizable thiols, reactions using it are conducted under an inert atmosphere. The antioxidants dithiothreitol and mercaptoethanol activate the enzyme at low concentrations and deactivate the enzyme at higher concentrations;<sup>19</sup> these antioxidants should be avoided or used sparingly in preparative-scale reactions.

## Results

**Substrate Specificity of GDH.** Table I gives relative rates of reductions of several substrates catalyzed by GDH; Table II gives relative rates of oxidation. Initial concentrations of substrates in both sets of experiments were 100 mM. These relative rates were obtained by observing the reduction of NAD or oxidation of NADH; products were identified only in cases where preparative quantities (>1 g) were synthesized. Identifications of compounds that react very slowly as substrates should be considered tentative: experimental errors due to small quantities of rapidly reacting impurities become important in these

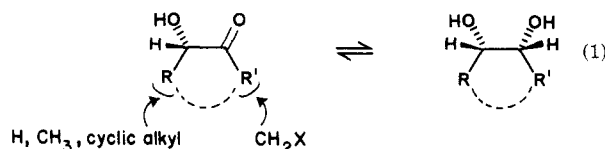
Table II. Relative Rates of Oxidation by GDH/NAD<sup>a</sup>

substrate	relative rate <sup>b</sup>		$K_m$ , mM	
	C.s.	E.a.	C.s.	E.a.
glycerol	100	100	9	9
1,2-propanediol <sup>c</sup>	60	40	0.10	0.09
( <i>R</i> )-1,2-propanediol <sup>c</sup>	70	45	0.07	0.09
( <i>S</i> )-1,2-propanediol <sup>c</sup>	0	0		
1,2-butanediol <sup>c</sup>	60	45	0.15	0.15
( <i>R</i> )-1,2-butanediol <sup>c</sup>	60	40	0.09	0.09
1,2-hexanediol	45			
1,2-octanediol (45 mM)	12			
1,2-ethanediol	13			
1,3-propanediol	39			
1,4-butanediol	2			
2,3-butanediol	21	30	33	20
3-mercapto-1,2-propanediol	33			
3-chloro-1,2-propanediol	22			
3-methoxy-1,2-propanediol	10			
3-(methylthio)-1,2-propanediol	30			
L- $\alpha$ -glycerophosphate	0			
DL- $\alpha$ -glycerophosphate	11			
( <i>R</i> )-1-amino-2-propanol	4			
( <i>S</i> )-1-amino-2-propanol	0.5			
<i>cis</i> -1,2-cyclopentanediol	33	80	14	18
<i>cis</i> -1,2-cyclohexanediol	6		51	
<i>trans</i> -1,2-cyclohexanediol	0			
2-hydroxycyclohexanone	14			
<i>cis</i> -1,2-cycloheptanediol	3			
<i>cis</i> -1,2-cyclooctanediol	0			
D-galactose	2			

<sup>a</sup> Abbreviations: C.s.: *Cellulomonas sp.* E.a.: *Enterobacter aerogenes*. Reactions were conducted at pH 9.0. Details of reaction conditions are given in the Experimental Section. Compounds with a relative rate of 0 (using GDH from *Cellulomonas*) include 2-amino-1-propanol, ethanol, 2-propanol, 3,3-dimethyl-1,2-propanediol, 2-phenyl-1,2-ethanediol, *cis,trans*-1,3-cyclopentanediol, *cis,trans*-1,3-cyclohexanediol, cyclohexanedimethanol, *myo*-inositol, ribose, glucose, mannose, 6-deoxygalactose, erythritol, mannitol, and sorbitol. <sup>b</sup> The concentration of the substrate was 100 mM, unless indicated otherwise. <sup>c</sup> The concentration of the substrate was 1 mM for the determination of the relative rate. Lower rates were observed at higher concentrations of substrate (100 mM).

types of experiments. GDH from both *Cellulomonas sp.* and *Enterobacter aerogenes* were examined; similar substrate specificities and values of  $K_m$  were observed for both enzymes. The two enzymes have different specific activities and costs: *Cellulomonas*, 90 U/mg, \$0.04/U; *Enterobacter*, 5 U/mg, \$0.6/U.<sup>20</sup> For most synthetic applications, the *Cellulomonas* species is superior because of its higher specific activity and lower cost.

The data in these tables are not sufficiently systematic to define all of the structural features required for activity of an  $\alpha$ -hydroxy ketone or 1,2-diol as a substrate, but their most useful implication is that for ketones (i.e.,  $R' \neq H$  in eq 1) the group R is restricted to H,  $\text{CH}_3$ , or a cyclic alkyl



connected to  $R'$ . The group  $R'$  can be a number of simple alkyl groups ( $\text{CH}_2\text{OH}$ ,  $\text{CH}_2\text{SH}$ ,  $\text{CH}_2\text{OCH}_3$ ,  $(\text{CH}_2)_n$ ). Certain of the substances listed in the tables that appear not to react may deactivate the enzyme (chloroacetone, cyclohexane-1,2-dione) and should probably be discounted

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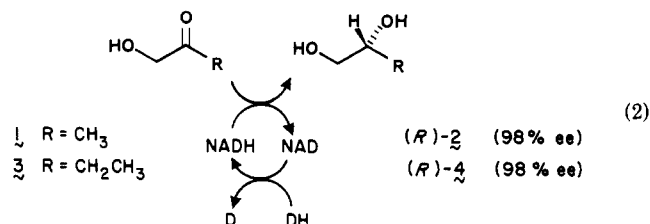
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(18) Assays utilizing glycerol dehydrogenase include serum aldolase determination (Sugiura, M.; Hirano, K.; Yoshimura, H.; Sugiura, M.; Shimizu, H. *Chem. Pharm. Bull.* **1976**, 24, 2647-2650), glycerol in serum and beverages (Hinsch, W.; Sundaram, P. V. *Clin. Chim. Acta* **1980**, 104, 87-94), and triglyceride determination (Abdulrahman, S. A.; Christian, G. D. *Anal. Chim. Acta* **1979**, 106, 226-231).

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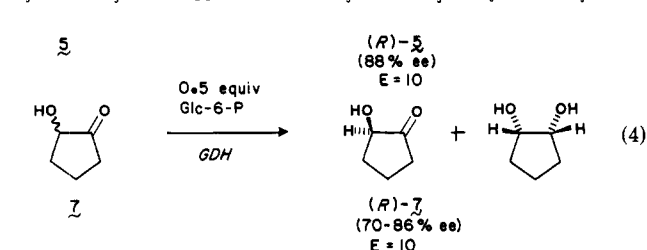
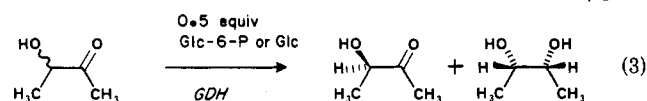
on this basis. The significant reactivity of 1,3-propanediol and 1,4-butanediol suggest that the two hydroxyl groups need not necessarily be adjacent, but the low reactivity of 1,3-cyclohexanediol and 1,3-cyclopentanediol indicate that there are additional, presently undefined constraints on acceptable geometry for these groups.

**Applications of GDH in Organic Synthesis. Stereoselective Reduction of Achiral  $\alpha$ -Hydroxy Ketones to Chiral 1,2-Diols.**<sup>21</sup> Reductions of  $\alpha$ -hydroxy ketones are straightforward. Cofactor regeneration was accomplished with formate and formate dehydrogenase (FDH) (eq 2; DH is the ultimate hydride donor used for in situ regeneration of NADH).<sup>4,22</sup> Conversion of 1-hydroxy-2-

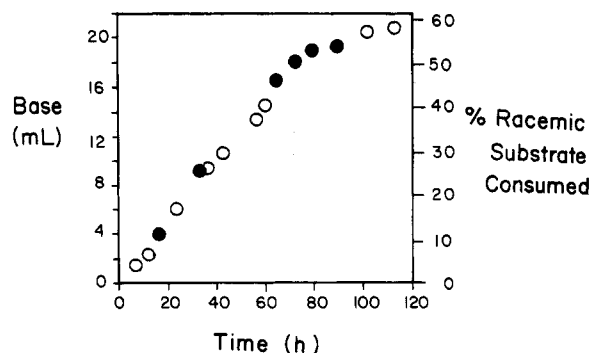


butanone to (*R*)-1,2-butanediol was carried out without difficulty on a scale generating 70 mmol of isolated product. Conversion of 1-hydroxy-2-propanone to (*R*)-1,2-propanediol was also straightforward. The ee for both diols was determined to be  $\geq 98\%$  by examination of the <sup>1</sup>H NMR spectra of the 1-*O*-tosyl-2-*O*-(-)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetyl (MTPA) derivatives.<sup>23</sup> Substitution of formic acid-*d*<sub>2</sub> for formate<sup>24</sup> generated (*R*)-1,2-butanediol-2-*d*<sub>1</sub> (98% *d*<sub>1</sub>). These reductions are straightforward at this scale and with the quantities of enzyme used here but are slow (complete reaction requires approximately 19 days).

**Kinetic Resolutions: Reduction of Racemic  $\alpha$ -Hydroxy Ketones.** Reduction of racemic  $\alpha$ -hydroxy ketones using 0.5 equiv of the ultimate hydride donor resulted in enantiomeric enrichment of the remaining unreduced  $\alpha$ -hydroxy ketone (eqs 3,4). Glucose-6-phosphate/glu-



ucose-6-phosphate dehydrogenase (G-6-PDH)<sup>25</sup> or glucose/glucose dehydrogenase (GlcDH)<sup>26,27</sup> were used to regenerate NADH in situ. The course of these reactions was followed by monitoring the quantity of KOH solution added at constant pH<sup>28</sup> to neutralize the 6-phosphogluconic



**Figure 1.** Quantity of 2.5 N KOH added to two reactors maintained at pH 7.6. The symbol (●) represents a reactor which contained 5, glucose-6-phosphate, GDH, G-6-PDH, and NAD. The symbol (○) represents a reactor which combined starting quantities and concentrations of 5, glucose, and NAD equal to those of the first reactor and approximately the same number of units of both GDH and GlcDH.

acid or gluconic acid generated by oxidation of the hydride donor; this procedure was more convenient than GLC or enzymatic assays. Figure 1 shows representative results. The ee of the reisolated  $\alpha$ -hydroxy ketones was determined from the <sup>1</sup>H NMR spectra of their (-)-MTPA esters.<sup>29</sup> The absolute configuration of enantiomerically enriched 5 was assigned as *R* on the basis of the sign of its rotation (-) at 589 nm.<sup>30</sup> Treatment of  $\alpha$ -hydroxycyclobutanone 8 using conditions similar to those in eq 4 resulted in reduction, but the recovered 8 was still racemic (ee = 0%).

Equations 3 and 4 give both observed values of ee and the values of the enantiomeric ratio, *E*, obtained using the formulas of Sih.<sup>31</sup> The value of *E* calculated from experimental data is very sensitive to the extent of conversion of the substrate. These extents of conversion were *not* measured independently here but are estimates based on the quantity of the reducing agent (DH, eq 2) added. As a result, values of *E* in these equations, and elsewhere in this paper, are approximate.

**Enantiotopic Stereoselective Oxidation of Meso-1,2-diols to  $\alpha$ -Hydroxy Ketones.** Enantioselective oxidation of *cis*-1,2-cyclohexanediol (9 mmol) was accomplished using diaphorase, methylene blue, and dioxygen to regenerate NAD (eq 5).<sup>32</sup> Reaction stopped spontaneously when 30% of the starting material had been consumed, apparently due to product inhibition. The absolute configuration at the chiral carbon of 2-hydroxycyclohexanone was not determined, but is assumed to be *S*. Oxidative resolution of 1,2-butanediol was carried out by using 2-oxoglutarate and glutamate dehydrogenase (GIDH) to regenerate NAD (eq 6). Reaction progress appeared to stop when  $\sim 30\%$  of the racemic substrate had been consumed.

**Reductions Using Actively Fermenting Yeast.** Bakers' yeast growing on glucose in an anaerobic envi-

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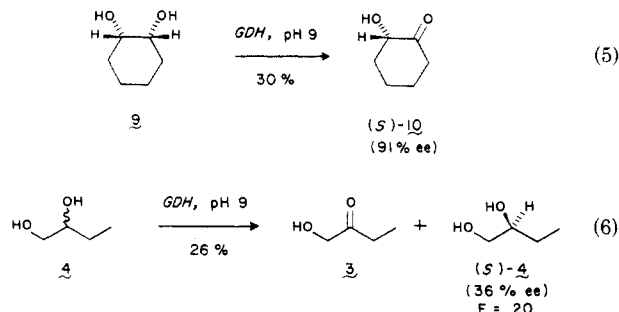
(28) The reduction of 7 was carried out at two values of pH: pH 5.5 and 6.5. The reaction at pH 5.5 produced (*R*)-2-hydroxycyclopentanone of 70% ee; the reaction at pH 6.5 produced (*R*)-2-hydroxycyclopentanone of 86% ee.

(29) (*R*)-5 could be inverted at the chiral center with the Mitsunobu reaction<sup>30</sup> (triphenylphosphine, diethyl azodicarboxylate, and (-)- $\alpha$ -MTPA acid) with some loss of enantiomeric purity (from 88% to 70%). The enantiomeric purity of the C-3 position of the product (3*S*)-3-*O*-(-)-MTPA-2-butanone was measured by <sup>1</sup>H NMR and found to be 70% ee. Similar treatment of racemic 5 provided 3-*O*-MTPA-2-butanone of 0% ee at the C-3 position; no diastereoselection in the reaction was observed (see Figure 6).

(30) Blom, R. H. *J. Am. Chem. Soc.* **1945**, *67*, 494.

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(32) Lee, L. G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 6999-7008.



ronment reduces ketones to alcohols.<sup>33</sup> The enzymes responsible for these reductions are probably in major part nicotinamide cofactor-dependent oxidoreductases, although the specific enzymes have not been identified. The advantage offered by reduction with yeast is simplicity: no enzymes need be isolated or immobilized and cofactor regeneration is accomplished by enzyme systems already present in the yeast. The disadvantage of yeast reductions relative to those using isolated enzymes is that they are intrinsically messy. The reactant and products are present in dilute solution in a medium containing yeast cells, nutrients, and other metabolites. Further, since the reactants or products in a ketone reduction using yeast may be metabolized, yields may not be high. Finally, several enzymes may be present in the yeast that are capable of reduction of the substrate. If these enzymes have different enantioselectivity, their combined action may lower enantioselectivity.

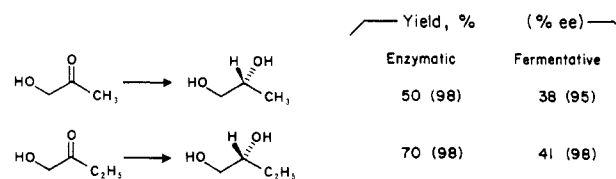
We were interested in comparing the practical efficacy of ketone reduction using cell-free enzymes and fermenting yeast. Scheme I summarizes experimental results.

The major conclusions from this limited comparison are that these enzymatic and fermentation reductions give products with the same absolute configuration and similar (high) enantiomeric excesses and that the yields are higher for the enzymatic reductions, the times required to accomplish the reduction longer, and the isolations simpler. The yeast reductions are operationally simpler to carry out.

**Preparations of Epoxides.** The chiral diols (*R*)-1,2-propanediol and (*R*)-1,2-butanediol-2-*d*<sub>1</sub> were converted into the corresponding epoxides in two steps:<sup>34</sup> initial treatment with hydrogen bromide in acetic acid provided the (*R*)-1-bromo-2-acetoxyalkanes; subsequent treatment with potassium pentoxide yielded the (*R*)-1,2-epoxyalkanes. The ee of the epoxides were determined by regioselective ring-opening with thiophenol, followed by preparation of the 2-*O*-MTPA esters and examination by <sup>1</sup>H NMR spectroscopy. The ee was ≥98% for both (*R*)-1,2-epoxypropane and (*R*)-1,2-epoxybutane-2-*d*<sub>1</sub>.

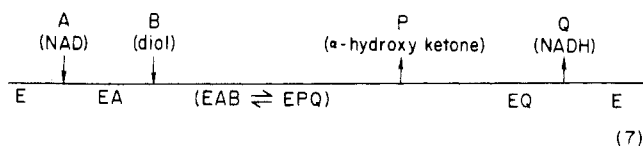
**Effect of Product Inhibition on Reaction Rates: Optimization of Reaction Conditions.** Preparative-scale oxidations of vicinal diols to α-hydroxy ketones using GDH with in situ cofactor regeneration are much more difficult to accomplish than are the reductive reactions: product inhibition seriously slows the rates of oxidative reactions and limits the final concentrations of products which can be obtained. This section analyzes the influence of product inhibition on the kinetics of reactions carried out under the constraints imposed by preparative conditions (espe-

**Scheme I. Comparison of Enzymatic and Fermentation Transformations**



cially the desirability of high concentrations, complete conversions, and short reaction times) and suggests strategies which minimize the undesirable consequences of product inhibition. This analysis indicates clearly, however, that product inhibition is a practical problem in dehydrogenase-catalyzed oxidations of alcohols to ketones that may, in fact, be considerably more serious than the problems posed by cofactor regeneration or enzyme cost and operating lifetime.

The GDH-catalyzed oxidation of diols proceeds through an ordered bi-bi mechanism;<sup>19</sup> the order of addition is represented by eq 7.<sup>35</sup> The rate of reaction for an ordered



bi-bi mechanism in the absence of Q(NADH)<sup>36</sup> is given by eq 8<sup>35</sup> ( $V_{\max} = k_{\text{cat}}[\text{enzyme}]$ ). In this equation, the

$$\frac{v}{V_{\max}} = \frac{[\text{B}]/\left[ K_{\text{mB}} \left( 1 + \frac{K_{\text{ia}}}{[\text{A}]} \right) \left( 1 + \frac{K_{\text{mQ}}[\text{P}]}{K_{\text{iq}}K_{\text{mP}}} \right) + [\text{B}] \left( 1 + \frac{K_{\text{mA}}}{[\text{A}]} + \frac{[\text{P}]}{K_{\text{ip}}} \right) \right]}{\quad} \quad (8)$$

Michaelis constant for, e.g., Q, is given by  $K_{\text{mQ}}$  and the corresponding inhibition constant by  $K_{\text{iq}}$ . The product P (ketone) acts as a "mixed-type inhibitor" with respect to the substrate B (diol): the inhibition affects both  $K_{\text{mB}}$  and  $V_{\max}$ .<sup>35</sup> Equation 8 may be simplified to eq 11 if the concentration of A is saturating (eq 9; for dehydrogenases,  $[\text{A}] = [\text{NAD}] \geq 1 \text{ mM}$ ) and if the quantity  $(K_{\text{iq}}K_{\text{mP}}/K_{\text{mQ}})$  is expressed as a function of  $K_{\text{ip}}$  (eq 10). We define the

$$[\text{A}] \gg K_{\text{mA}}, K_{\text{ia}} \quad (9)$$

$$x = \frac{K_{\text{iq}}K_{\text{mP}}}{K_{\text{ip}}K_{\text{mQ}}} \quad (10a)$$

$$\frac{K_{\text{iq}}K_{\text{mP}}}{K_{\text{mQ}}} = xK_{\text{ip}} \quad (10b)$$

$$\frac{v}{V_{\max}} = \frac{[\text{B}]}{K_{\text{mB}} \left( 1 + \frac{[\text{P}]}{xK_{\text{ip}}} \right) + [\text{B}] \left( 1 + \frac{[\text{P}]}{K_{\text{ip}}} \right)} \quad (11)$$

extent of reaction as  $R$  (eq 12). We further assume that substrate is converted only to product and that the product exists only in the form of a single inhibitor I (eq 13).

(33) For reviews of microbial transformations, see: Sih, C. J.; Chen, C.-S. *Angew. Chem., Int. Ed. Engl.* 1984, 23, 570-578. Fischli, A. In "Modern Synthetic Methods"; Scheffold, R., Ed.; Salle and Sauerländer: Frankfurt, 1980; Vol. 2, pp 269-350. Sih, C. J.; Rosazza, J. P. In "Techniques of Chemistry"; Weissberger, A., Ed.; Wiley: New York, 1976; Vol. X, pp 69-106.

(34) Golding, B. T.; Hall, D. R.; Sakrikar, S. *J. Chem. Soc., Perkin Trans. 1* 1973, 1214-1220.

(35) For a discussion of the ordered bi-bi mechanism and an analysis of the kinetics, see: Segel, I. H. In "Enzyme Kinetics"; Wiley: New York, 1975; pp 561-590.

(36) In practice, Q(NADH) is maintained at a very low concentration by an efficient NAD regeneration system, e.g., 2-oxoglutarate/glutamate dehydrogenase.<sup>32</sup> (See also: Wong, C.-H.; McCurry, S. D.; Whitesides, G. M. *J. Am. Chem. Soc.* 1980, 102, 7938-7939.)

$$R = 1 - \left( \frac{[S]}{[S_0]} \right) \quad (12)$$

Equation 11 can then be rewritten as eq 14 (in this and subsequent equations describing mixed or noncompetitive inhibition,  $K_m$  is the Michaelis constant of the diol substrate and  $K_i$  is the inhibitor constant of the ketone product).

$$[I] = [S_0] - [S] \quad (13)$$

$$\frac{v}{V_{\max}} = \frac{(1-R)}{1 + \frac{K_m}{[S_0]} + R \left[ \frac{[S_0]}{K_i}(1-R) + \frac{K_m}{xK_i} - 1 \right]} \quad (14)$$

The value of  $x$  in eq 11 determines whether product inhibition is noncompetitive or mixed. When  $x$  is unity the inhibition is noncompetitive (only  $V_{\max}$  is affected). In practice,  $x$  is rarely unity and product inhibition is usually mixed (both  $K_m$  and  $V_{\max}$  are affected in eq 11). The value for  $x$  can be determined experimentally; for yeast and liver alcohol dehydrogenase it has been found to be 0.19 and 0.15, respectively.<sup>37</sup>

For comparison and for reference, the simpler equations analogous to eq 11 and 14 describing a reaction in which the product is a competitive inhibitor are eq 15 and 16.<sup>38,39</sup>

$$\frac{v}{V_{\max}} = \frac{1}{\left( \frac{K_m}{[S]} \right) \left( 1 + \frac{[I]}{K_i} \right) + 1} \quad (15)$$

$$\frac{v}{V_{\max}} = \frac{(1-R)}{K_m/[S_0] + 1 + R \left( \frac{K_m}{K_i} - 1 \right)} \quad (16)$$

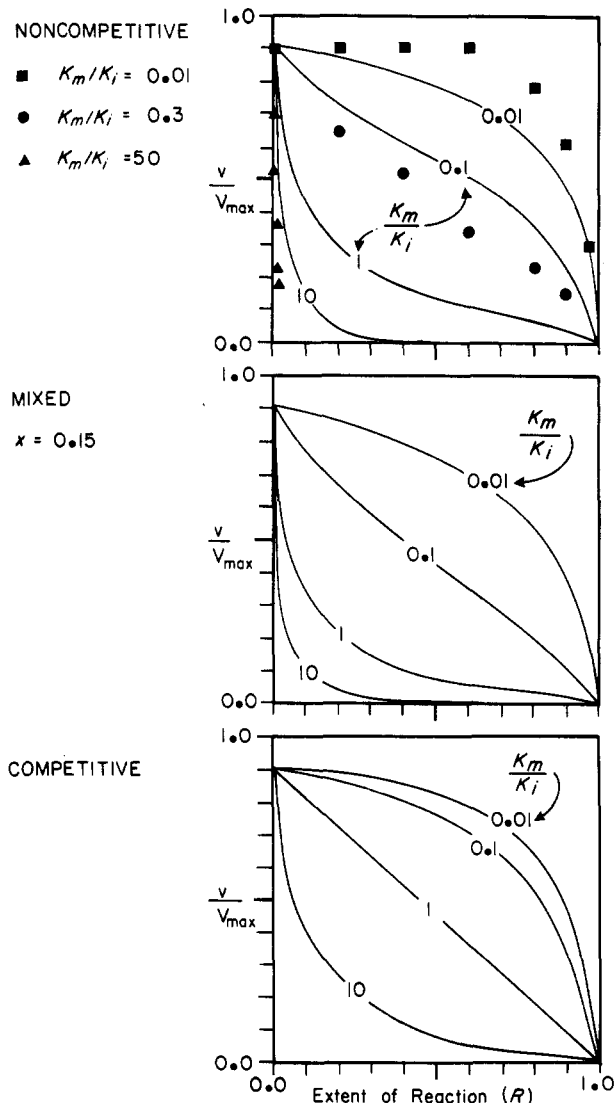
Arbitrarily setting the initial substrate concentration  $[S_0]$  as  $10 K_m$  (eq 17) allows eq 14 and 16 to be rewritten as eq 18 (for mixed inhibition) and 19 (for competitive inhibition). Figure 2 plots values of  $v/V_{\max}$  vs.  $R$  for non-

$$[S_0] = 10K_m \quad (17)$$

$$\frac{v}{V_{\max}} = \frac{(1-R)}{1.1 + R \left[ 10 \left( \frac{K_m}{K_i} \right) (1-R) + \left( \frac{K_m}{xK_i} \right) - 1 \right]} \quad (18)$$

$$\frac{v}{V_{\max}} = \frac{(1-R)}{1.1 + R \left( \frac{K_m}{K_i} \right)} \quad (19)$$

competitive (eq 18,  $x = 1$ ), mixed (eq 18,  $x = 0.15$ ) and competitive inhibition (eq 19); the values for the ratio



**Figure 2.** Rate of reaction,  $v$ , relative to the maximum rate of reaction  $V_{\max}$  as a function of extent of reaction  $R$  for reactions in which product is a noncompetitive (eq 18,  $x = 1$ ), mixed (eq 18,  $x = 0.15$ ), and competitive inhibitor (eq 19). The calculated curves are labeled with the ratio  $K_m/K_i$ . Experimental values are given by the symbols  $\blacksquare$ ,  $\bullet$ ,  $\blacktriangle$ . Kinetic parameters used were as follows: for reduction of 3-hydroxy-2-butanone to 2,3-butanediol,  $K_m = 2$  mM,  $K_i = 200$  mM,  $K_m/K_i = 0.01$ ; for oxidation of *cis*-1,2-cyclohexanedimethanol to (1*R*,6*S*)-8-oxabicyclo[4.3.0]nonan-7-one,  $K_m = 26$  mM,  $K_i = 80$  mM,  $K_m/K_i = 0.3$ ; for oxidation of glycerol to dihydroxyacetone,  $K_m = 15$  mM,  $K_i = 0.3$  mM,  $K_m/K_i = 50$ .

(37) Wratten, C. C.; Cleland, W. W. *Biochemistry* 1963, 2, 935-941.

(38) In this example, A(NAD) and Q(NADH) are competitive inhibitors and the competitive product inhibition represented in Figure 2 would be expected if  $[P]$  (ketone) = 0 and if the concentration of B (alcohol) was saturating. Other examples of the control of the ratio  $K_m/K_i$  in competitive systems are kinases; for derivations and discussion, see: Atkinson, D. E. In "The Enzymes"; Academic Press: New York, 1970; Vol. 1, pp 461-489.

(39) Using the number of units of enzyme available,  $U$ , and the desired extent of reaction,  $R$ , one can calculate the optimal volume required to complete the reaction from eq 27. For example, consider the HLADH-catalyzed oxidation of 100 mmol of *cis*-1,2-cyclohexanedimethanol to 95 mmol of the lactone ( $R = 0.95$ ) using 100 U ( $\mu$ mol product produced/min) of HLADH. The experimentally determined value of the ratio  $K_m/K_i$  is 0.3. The optimal value of the ratio  $[S_0]/K_i$  is then found to be 1.4 from eq 27. The experimental value of  $K_i$  is 0.08 M. We take, therefore, the value of  $[S_0]$  to be 0.11 M and the optimal volume of the reaction to be 0.96 L. The experimental value for the optimal volume is not known. The time  $t$  cannot be accurately determined unless the parameter  $x$  (eq 10) is known.

$K_m/K_i$  ranges from 0.01 to 10. Experimental points for three representative reactions involving NAD(H)-dependent dehydrogenases are plotted with the curves corresponding to noncompetitive inhibition. This particular juxtaposition of experimental and theoretical curves is an arbitrary one, since the value for  $x$  (eq 10) for these reactions is not known. In practice, however, if one is interested in qualitative estimations of rates and reaction times one can best approximate product inhibition of dehydrogenases by assuming simple noncompetitive inhibition if no specific value for  $x$  is available.

Figure 2 demonstrates that reactions with  $K_m/K_i \leq 1$  proceed rapidly to completion. For  $K_m/K_i > 1$ , however, it is difficult to achieve high rates and high conversions simultaneously. The consequences of incomplete reaction for GDH-catalyzed reactions are the following: For oxi-

dativ kinetic resolutions of diols, the diols are recovered in low enantiomeric excesses; for oxidative, enantioselective syntheses of  $\alpha$ -hydroxy ketones, low yields are obtained, although the enantiomeric purity of the  $\alpha$ -hydroxy ketone may be high.

The curves in Figure 2 represent the velocity of the reaction for different values of  $K_m/K_i$  at a fixed value of  $[S_0]/K_m = 10$ . In fact, this value of  $[S_0]$  is not necessarily the one which maximizes the rate of reaction or extent of conversion. We desired a method that would allow us to estimate the best value of  $[S_0]$  for known values of  $K_i$  and  $K_m$  and for a chosen extent of reaction  $R$ . We define "best" in this context to mean that value of  $[S_0]$  which minimizes the time required to achieve a given value of  $R$ .

Defining velocity as a function of the concentration of inhibitor  $[I]$  (eq 20) and with the fact that  $K_m/[S_0]$  is the quotient of the two ratios  $K_m/K_i$  and  $[S_0]/K_i$  (eq 21), the equation for mixed inhibition (eq 14) can be rewritten as eq 22. Equation 22 was integrated and evaluated from

$$v = \frac{d[I]}{dt} = \frac{d([S_0]R)}{dt} \quad (20)$$

$$K_m/[S_0] = (K_m/K_i)([S_0]/K_i)^{-1} \quad (21)$$

$$dt = \frac{[S_0]}{V_{\max}}(1-R)^{-1} \left[ \frac{-[S_0]R^2}{K_i} + \left( \frac{[S_0]}{K_i} + \frac{K_m}{xK_i} - 1 \right)R + \frac{K_m}{K_i} \left( \frac{[S_0]}{K_i} \right)^{-1} + 1 \right] dR \quad (22)$$

$t = 0$  ( $R = 0$ ) to  $t = t$  to obtain eq 23. The expression for competitive inhibition (eq 16) was integrated by a similar procedure to obtain eq 24. (The validity of both integrations can be checked, if desired, by differentiation.)

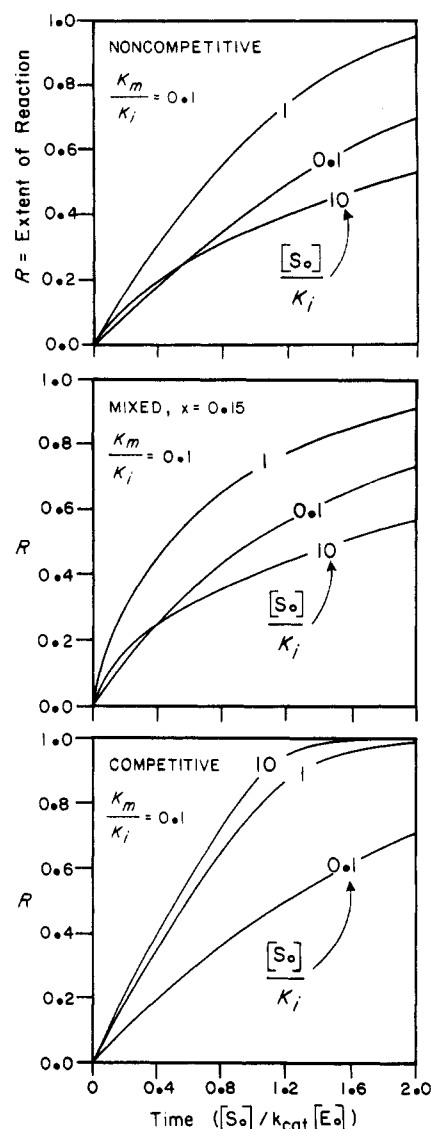
$$t = \frac{[S_0]}{V_{\max}} \left[ \frac{[S_0]}{2K_i} R^2 + \left( 1 - \frac{K_m}{xK_i} \right) R - \left( \frac{K_m}{xK_i} + \frac{K_m}{[S_0]} \right) \ln(1-R) \right] \quad (23)$$

$$t = \frac{[S_0]}{V_{\max}} \left[ \left( 1 - \frac{K_m}{K_i} \right) R - \left[ \frac{K_m}{K_i} + \left( \frac{K_m}{K_i} \right) \times \left( \frac{[S_0]}{K_i} \right)^{-1} \right] \ln(1-R) \right] \quad (24)$$

Figures 3 and 4 plot the extent of reaction  $R$  vs. time for noncompetitive (eq 23,  $x = 1$ ), mixed (eq 23,  $x = 0.15$ ), and competitive inhibition (eq 24) for different values of the ratio  $[S_0]/K_i$ . In interpreting these curves, it is helpful to remember that the time variables are  $[S_0]$  (since  $K_i$  and  $K_m$  are fixed by the enzyme being considered) and  $R$ . The abscissa in these figures is given in units (with dimension time) of the ratio  $[S_0]/V_{\max}$  (eq 25). Thus, numerical

$$\frac{[S_0]}{V_{\max}} = \frac{[S_0]}{k_{\text{cat}}[E_0]} = \frac{S_0}{k_{\text{cat}}E_0} \quad (25)$$

values along the abscissa depend on the ratio of the starting concentration of substrate to enzyme. The curves for different values of  $[S_0]/K_i$  can thus only be compared directly if the value of  $[S_0]/[E_0]$  is considered as a constant: that is, as the ratio  $[S_0]/K_i$  increases,  $[E_0]$  increases proportionately. Note, also, that the abscissa for Figure 3 ( $K_m/K_i = 0.1$ ) differs from that for Figure 4 by a factor of 100. That is, the reactions in Figure 3 are proceeding roughly 100 times faster than those in Figure 4; this dif-



**Figure 3.** Time (in units of  $[S_0]/V_{\max}$ ) as a function of extent of reaction  $R$  (eq 12) for reactions in which product is a noncompetitive (eq 23,  $x = 1$ ), mixed (eq 23,  $x = 0.15$ ), and competitive inhibitor (eq 24). The value of the ratio  $K_m/K_i$  is 0.1.

ference reflects, of course, the fact that for a given value of  $[S_0]/K_i$  and for a particular type of inhibition, the value of  $[S_0]/K_m$  in Figure 3 is greater than that in Figure 4 by  $10^2$ .

The curves in Figure 3 ( $K_m/K_i = 0.1$ ) represent an "easy" extreme from the vantage of practical catalysis: The substrate binds more tightly to the active site than the inhibitory product, and high values of  $R$  are achieved relatively rapidly. Note, however, that even for a constant ratio of starting concentrations of substrate to enzyme, the conversion achieved in a given time *decreases* as the substrate concentration increases beyond a certain point (from  $[S_0]/K_i = 1$  to  $[S_0]/K_i = 10$ ) for noncompetitive and mixed inhibition. This falloff in rate with conversion reflects the inhibitory effect of product. Enzyme is inhibited at a concentration of product determined by  $K_i$ , and an increase in  $[S_0]$  does not result in an increase in rate, only in an increase in the concentration of unreacted  $S_0$  (that is, in a decrease in  $R$ ) when inhibition occurs.

The curves in Figure 4 ( $K_m/K_i = 10$ ) represent a "difficult" extreme for preparative catalysis. The substrate binds *less* tightly than the inhibitory product, and rates (for a given quantity of enzyme) are much slower than for the corresponding case in Figure 3 in any event. Further,

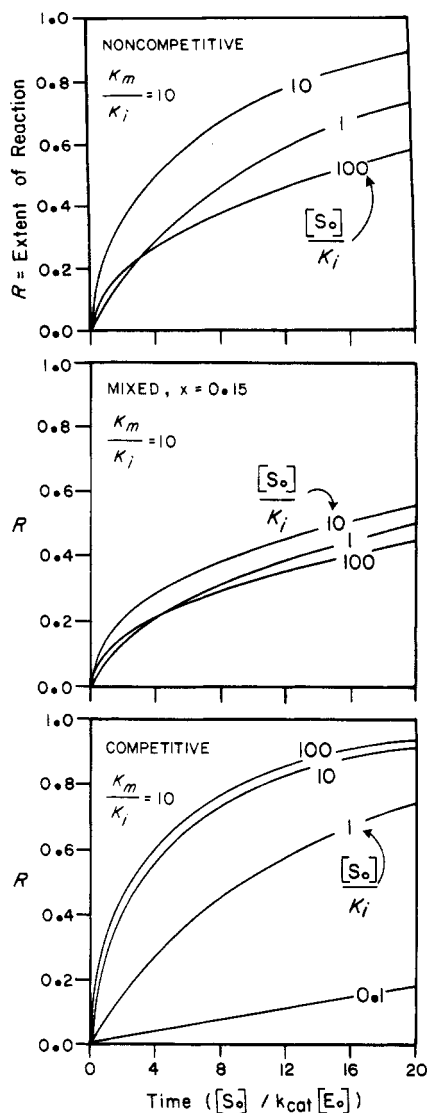


Figure 4. Same as Figure 3, except the value of the ratio  $K_m/K_i$  is 10.

for any values if  $[S_0]/K_i$  for mixed inhibition it is impractical to achieve high conversions: Product inhibition is severe even for moderate extents of conversion [ $R \approx 0.3$ ].

Of the three sets of experimental data plotted in Figure 2, one—the reduction of 3-hydroxy-2-butanone—is very straightforward:  $K_m/K_i = 0.01$ . Of the two oxidations, the HLADH-catalyzed oxidation of *cis*-1,2-cyclohexanedi-methanol to the corresponding lactone falls close to the “easy” kinetic extreme represented by Figure 3: The rate falls off approximately linearly with conversion. This type of reaction has been exploited extensively and successfully by Jones and co-workers for the preparation of 1–10-g quantities of products. Note, however, that a 90% conversion, the rate of catalysis is only 10–20% that at the start of the reaction. This falloff suggests difficulties in efforts to scale such reactions to high concentrations and large quantities, unless some other strategy is used to minimize product inhibition (such as continuous extraction of the product as it is formed.) The third experimental case in Figure 2—the oxidation of glycerol to dihydroxyacetone—is a “difficult” case of the type represented for mixed inhibition in Figure 4. Product inhibition is severe even at very low conversions, and it is not possible to achieve high conversions at any practical concentration of enzyme. We believe this type of product inhibition underlies the slow rates and low conversions characterizing the reactions summarized in eq 5 and 6.

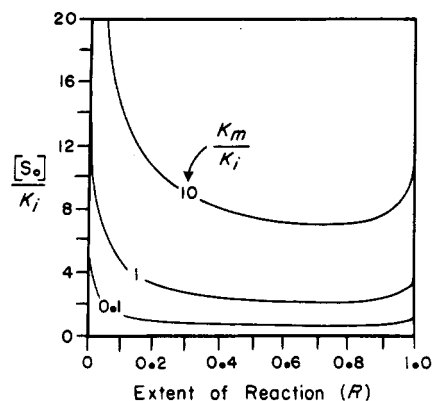


Figure 5. Values of the ratio  $[S_0]/K_i$ , which minimize the time required to achieve an extent of conversion  $R$  (eq 27). Note that for each value of  $K_m/K_i$ , the optimal value of  $[S_0]/K_i$  remains essentially constant between  $R = 0.2$  and  $0.9$ .

This analysis indicates that for reactions subject to noncompetitive and mixed inhibition by product, there will be an optimal value of the starting concentration of substrate  $[S_0]$ . At low values of  $[S_0]$ , the efficiency of the reaction will be low because the system will use dilute solutions and large volumes, and because rates may be low if  $[S_0]/K_m \gtrsim 10$ . At high values of  $[S_0]$ , rates and conversions are limited by product inhibition. We pose the following question: for a given ratio of starting concentrations of substrate and enzyme, what starting concentration of substrate minimizes the time required to achieve a selected extent of conversion? An alternative phrasing of this question which may be more relevant to practical synthesis is as follows: For given quantities of substrate and enzyme (and thus of the ratio  $S_0/E_0$ ), what volume of solution minimizes the time required to synthesize a given quantity of product? To solve this (these) problem(s), we note that the  $[S_0]/V_{\max}$  term in eq 23 is independent of volume (eq 25). Differentiating eq 23 with respect to  $[S_0]$  (eq 26) (or with respect to volume  $V$ ) and setting the derivative equal to zero yields eq 27. Figure

$$\frac{dt}{d[S_0]} = 0 = \frac{S_0}{K_{\text{cat}}E_0} \left[ \frac{R^2}{2K_i} + \frac{K_m}{[S_0]^2} \ln(1-R) \right] \quad (26)$$

$$\frac{[S_0]}{K_i} = \left[ \frac{-2K_m}{R^2K_i} \ln(1-R) \right]^{1/2} \quad (27)^{39}$$

5 plots eq 27 for representative values of  $K_m/K_i$ . These plots are most simply interpreted physically by considering  $K_i$  to be the same for all three lines. With this constraint, for higher values of  $K_m$  it is necessary to use higher  $[S_0]$  (and, proportionately,  $[E_0]$ ) to achieve a chosen conversion in the minimum time. For a given value of  $K_m/K_i$ , the optimal value of  $[S_0]/K_i$  remains roughly constant between  $R = 0.2$  and  $0.9$ . Note that  $R$  is not directly correlated with time in these plots (see Figures 3 and 4); estimates of time can be derived from eq 23, if desired.

## Discussion

Among the many commercially available oxidoreductases, only horse liver alcohol dehydrogenase (HLADH) has been thoroughly investigated for synthetic utility. The most useful applications of this enzyme have been to the enantioselective oxidation of 1,4-diols to lactones. This type of reaction seems only moderately influenced by product inhibition. The data in this paper suggest that glycerol dehydrogenase (GDH) should also be useful in chiral synthesis and that the most useful appli-

cations will lie in the enantioselective *reduction* of prochiral and racemic  $\alpha$ -hydroxy ketones. Product inhibition in oxidations with GDH is likely to be severe, and unless the product can be removed continuously as it is formed (by extraction, distillation, or further reaction) or unless  $K_i$  is small, reactions proceeding in the direction of oxidation will be slower and complete conversion more difficult than those run in the direction of reduction. Glycerol dehydrogenases from two sources are commercially available: *Cellulomonas* sp. and *Enterobacter aerogenes*. Both enzymes appear to be *R* specific: Both utilize (*R*)-1,2-propanediol but not (*S*)-1,2-propanediol. The enzymes have similar substrate specificities. GDH from *Cellulomonas* has a higher specific activity and is less expensive than that obtained from *Enterobacter* and is thus preferred for most applications. GDH accepts a variety of diols and hydroxy ketones as substrates. We found few compounds that were substrates for both glycerol dehydrogenase and horse liver alcohol dehydrogenase.<sup>40</sup> Ethanol, *cis*-cyclohexanedimethanol, and cyclohexanol were substrates for HLADH but not for GDH; *cis*-1,2-cyclohexanediol, glycerol, and glyceraldehyde were substrates for GDH but not for HLADH. The presence of vicinal diols in a substrate promotes activity with GDH and hinders activity with HLADH. The presence of more than three hydroxyl moieties apparently inhibits activity with GDH: Sugars were generally poor substrates. Bulky substituents  $\alpha$  to the ketone moiety were not tolerated, although 1,2-*n*-alkanediols were generally good substrates. The group  $R'$  in eq 1 is probably limited in size to  $\text{CH}_3$ , unless the substrate is cyclic: 3-Hydroxy-2-butanone is a good substrate whereas 4-hydroxy-3-hexanone is not.

Reductions catalyzed by GDH were successfully performed by using three different NADH regenerating systems: formate/formate dehydrogenase (FDH), glucose-6-phosphate/glucose-6-phosphate dehydrogenase (G-6-PDH), and glucose/glucose dehydrogenase (GlcDH). Formate/FDH is the least efficient because the enzyme has low specific activity and high cost (0.4 U/mg, \$0.8/U).<sup>41</sup> Formate and  $\text{CO}_2$  cause no problems in workup. Glucose-6-phosphate/G-6-PDH is very efficient (600 U/mg, \$0.02/U), but commercially available glucose-6-phosphate is prohibitively expensive (\$1500/mol) for large-scale reactions. This compound can, however, be easily synthesized on multikilogram scale.<sup>25</sup> Separation of product from 6-phosphogluconate may in some instances be inconvenient. Glucose/GlcDH is probably the most convenient system for laboratory-scale reactions: The enzyme is moderately expensive but has good specific activity (200 U/mg, \$0.2/U). Glucose is readily available. Gluconic acid is normally easily separated from product. In each method, one balances cost against efficiency; glucose/GlcDH is a good compromise. GDH successfully resolved racemic 3-hydroxy-2-butanone in 8-g quantities with 88% ee;<sup>42</sup> both

glucose/GlcDH and glucose-6-phosphate/G-6-PDH were used uneventfully for regeneration.

The most important result to emerge from this work for the use of enzymes as catalysts in practical organic synthesis is the analysis of the importance of noncompetitive and mixed product inhibition. In principle, the proper strategy for addressing *competitive* product inhibition is to increase the concentration of reactant. Since increasing the concentration of product in the reaction is generally helpful in synthesis, the desirability of increasing the reactant concentration is not necessarily a disadvantage (although limits in solubility of reactant, and denaturation of enzyme in solutions containing high concentrations of organic substances may limit the practicality of this strategy). The corresponding strategy for overcoming *noncompetitive and mixed* inhibition must be to limit the concentration of product: Simply increasing the concentration is not, in general, helpful and may, in fact, be clearly undesirable (by increasing the concentration of unconverted reactant remaining in the reaction mixture at the point at which product inhibition causes the level of enzymatic activity to fall to an impractically low value). In the latter instances, starting reactant concentration must be limited to values sufficiently low that high conversion to product is possible before the product concentration becomes sufficiently high to cause kinetically unacceptable product inhibition. For systems in which  $K_m/K_i \lesssim 1$ , the limitation of reactant concentration may be practical, howbeit at the inconvenience of manipulating dilute solutions of products and reactants. For systems in which  $K_m/K_i > 1$ , it is difficult to achieve high conversion of reactants to products at an acceptable rate, unless product can be removed continuously.

An important question concerning this work, as well as work directed toward synthetic applications of HLADH, is that of the circumstances in which NAD cofactor-requiring enzymatic oxidation-reduction reactions are preferable to enzyme-catalyzed, enantioselective hydrolysis as a method of preparing chiral alcohols and derivatives. Answering this general question—the question of the relative utility of cofactor-requiring and cofactor-independent reactions—is one important focus of current work in applied enzymology. No esterase has been tested for enantioselectivity in hydrolysis of esters of  $\alpha$ -hydroxy ketones, and reduction reactions have clear utility in certain reactions involving the introduction of isotopes. In enantioselective production of monoalcohols, 1,2-diols, and related substances, however, current research suggests that lipases or esterases provide the basis for more practical preparative methods than do GDH and HLADH.<sup>6,7,43-45</sup>

## Experimental Section

**General.** UV spectra were measured using a Perkin-Elmer Model 552 UV-vis spectrophotometer. GLC analyses were carried out by using a 5-ft 3% Carbowax column. Separations of enzyme-containing gels from suspension were accomplished by using a table-top clinical centrifuge. The pH in reaction solutions was maintained either by manual addition of 2.5 N KOH or by automatic addition with a LKB Model 12000 Varioperpex peristaltic pump, connected to a Chemtrix pH controller. Formate dehydrogenase was obtained from Boehringer Mannheim. Fleish-

(40) Glycerol is actually a substrate for HLADH but is a very poor one (1% the rate of oxidation of ethanol). Racemic 1,2-propanediol is a substrate for both GDH and HLADH. There is evidence that HLADH preferentially oxidizes the *S* enantiomer<sup>26</sup> in contrast to GDH, which is selective for the *R* enantiomer.

(41) Higher specific activity (48 U/mg) has been obtained experimentally. See: Shütte, H.; Flossdorf, J.; Sahm, H.; Kula, M.-R. *Eur. J. Biochem.* **1976**, *62*, 151-160.

(42) Other methods to obtain chiral **3** include asymmetric hydrogenation of 2,3-butanedione (3% ee) (see: Ohgo, Y.; Takeuchi, S.; Natori, Y.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2124-2135),  $\text{O}_2/\text{Cu}$  oxidation of (2*R*,3*R*)-butanediol (<5% ee) (see: ref 30), and a crude, nematode extract catalyzed condensation of acetaldehyde (see: Berl, S.; Bueding, E. *J. Biol. Chem.* **1951**, *191*, 401-418). The last reaction provided **3** only in small quantities and in dilute, aqueous solution. It is not known if racemization of 2-hydroxycyclobutanone occurs under the reaction conditions.

(43) Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250-7251.

(44) Wang, Y.-F.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1984**, *106*, 3695-3696. Sabbioni, G.; Shea, M. L.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 236-238. Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, Y.-F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405-2406.

(45) Cambou, B.; Klivanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687-2692.



mann's yeast (cake type) was obtained from the local supermarket. All other enzymes and biochemicals were obtained from Sigma. 1-Hydroxy-2-butanone was purchased from BASF and was purified by spinning-band distillation [58–59 °C (30 torr)]. Formic acid- $d_2$  was purchased from Stohler Isotope Chemicals. Organic chemicals were purchased from Aldrich. Water was doubly distilled, the second time through a Corning Model AG-1b glass still. Welding grade argon was used as the inert atmosphere.

**Enzymatic Assays.** Assays were performed by the literature procedures.<sup>46</sup> Units of activity are  $\mu\text{mol min}^{-1}$ . Units of GDH refer to activity with glycerol as substrate, unless otherwise indicated. Glucose dehydrogenase (GlcDH) was assayed by a procedure similar to glucose-6-phosphate dehydrogenase:<sup>46</sup> glucose-6-phosphate was replaced by glucose. Diaphorase (*Clostridium kluyveri*) was assayed in cuvettes containing 0.96 mL of pH 9.0 glycine buffer, 0.01 mL of NADH solution (10 mg/mL), and 0.01 mL of methylene blue (0.01 M). An initial rate of decrease in absorbance at 340 nm was measured. A solution of diaphorase (0.02 mL) was added and the final rate of decrease in absorbance was measured. The difference in initial and final rates was used to determine the activity of enzyme.

Several assays for glycerol dehydrogenase were used. The assays for the relative rates in Table I were performed in 1.5-mL cuvettes containing 0.86 mL of pH 7.6 Hepes buffer, 0.02 mL of NADH solution (5 mg/mL), and 0.10 mL of substrate solution (1 M). The reaction was initiated by the addition of 0.02 mL of GDH solution (1 mg/mL), and the absorption at 340 nm was measured. The relative rates found in Table II were obtained in 1.5-mL cuvettes containing 0.83 mL of pH 9.0 glycine buffer, 0.05 mL of NAD solution (20 mg/mL), and 0.10 mL of substrate solution (1 M). The reaction was initiated by the addition of 0.02 mL of GDH (1 mg/mL) and the absorbance measured at 340 nm.

**Quantitative Assays with GDH.** The success of enantiomeric resolutions catalyzed by GDH is dependent on the difference in the rate of reduction (or oxidation) of the two enantiomers. The assay used here depends on stoichiometric reaction of the substrate with NAD(H). The reaction, initiated by addition of GDH, was performed in a cuvette, and the change in absorbance at 340 nm was measured. After several minutes (10–30) the absorbance was constant; the difference between the initial and final absorbance was measured, and the quantity of substrate consumed was calculated. This method could be used both for oxidations with NAD and reductions with NADH. The oxidative assays were measured in 3-mL cuvettes containing 2.9 mL of pH 9.0 glycine buffer, 0.05 mL of a solution containing NAD (30 mg/mL), and 0.05 mL of substrate (0.01 M). The reaction was initiated by the addition of GDH (1 mg); after several minutes (5–20) the final absorbance was measured. The difference in the initial and final absorbances was used to determine the quantity of substrate consumed. The quantitative, reductive assays were similar, except pH 7.6 Hepes buffer and NADH (0.2 mM) were used. GDH oxidized half of a racemic solution of 1,2-propanediol and of 1,2-butanediol; solutions of the *R* enantiomers were completely oxidized. GDH reduced half of a racemic solution of 3-hydroxy-2-butanone and 2-hydroxycyclopentanone. Greater than 85% of a racemic solution of 2-hydroxycyclobutanone was reduced.

**Enzyme Immobilization.** Enzymes were immobilized with PAN-900 following a general procedure;<sup>47</sup> yields were in the range of 30–50%. Assays of immobilized enzymes were similar to those for soluble enzymes: To a 1-mL cuvette containing the assay solution was added 20  $\mu\text{L}$  of a buffer containing the gel in suspension. The cuvette was capped and inverted several times to mix the contents and the absorbance read at 340 nm. The solution was mixed every 30 s over a period of 5 min and the rate of change in absorbance monitored on a chart recorder.

**(*R*)- and (*S*)-1,2-Propanediol.** The *R* enantiomer was prepared from the zinc salt of D-lactate by LAH reduction of the methyl ester. D-Lactic acid was obtained by the reduction of pyruvate catalyzed by D-lactic dehydrogenase.<sup>22</sup> The *S* enantiomer

was prepared by LAH reduction of ethyl L-lactate.<sup>48</sup>

***cis*-1,2-Diols.** The *cis*-1,2-diols were prepared by oxidation of the corresponding olefins with *N*-morpholine *N*-oxide and catalytic quantities of osmium tetroxide according to a general procedure.<sup>49</sup> *cis*-1,2-Cyclopentanediol,<sup>50</sup> *cis*-1,2-cyclohexanediol,<sup>50</sup> and *cis*-1,2-cyclooctanediol<sup>51</sup> were prepared in 1:2 water-acetone. *cis*-1,2-Cycloheptanediol<sup>52</sup> and *cis*-*exo*-norbornanediol<sup>53</sup> were prepared in 10:3:1 *tert*-butyl alcohol-tetrahydrofuran-water. All the diols, except *cis*-1,2-cyclopentanediol, were crystalline solids at room temperature and melted within 1–2 °C of the reported temperature.

**$\alpha$ -Hydroxy ketones** were prepared by acyloin condensation of the corresponding (di)esters, using chlorotrimethylsilane as a trapping agent, according to a general procedure.<sup>54</sup> The intermediate 1,2-bis(trimethylsilyloxy)alkenes were hydrolyzed by one of two methods: adding to dry methanol<sup>54</sup> or refluxing in a mixture of 1 N HCl and ether.<sup>55</sup> Compounds prepared by the former method were 2-hydroxycyclobutanone (8) and 4-hydroxy-3-hexanone; compounds prepared by the latter method were 2-hydroxycyclopentanone (7) and 4,4-dimethyl-2-hydroxycyclopentanone.

**Enzymatic Preparation of (*R*)-1,2-Propanediol [(*R*)-2] by Reduction of 1-Hydroxy-2-propanone (1).** A 1-L, four-necked, round-bottomed flask was equipped with a pH electrode, argon inlet, outlet to a bubbler, and a magnetic stirring bar. Water (100 mL), ammonium formate (6.3 g, 100 mmol), 1-hydroxy-2-propanone (3.7 g, 50 mmol), and 2-mercaptoethanol (0.02 g, 0.25 mmol) were added. The pH was adjusted to 7.5 with 2 N KOH. GDH (44 U, *Enterobacter aerogenes*), FDH (9 U), and NAD (0.07 mmol) were added. The solution was maintained under an argon atmosphere in a pH range of 7.5–8.3. The progress of the reaction was monitored by GLC; after 9 days less than 2% of the starting material remained. The solution was decanted and centrifuged to separate the immobilized enzymes. The gel was washed with two 50-mL portions of pH 7.5 Hepes buffer, and the decanted buffer washes were combined. The recovered activities of the enzymes were 61% for GDH and 62% for FDH. The reaction solution and the washings were combined and extracted continuously with ether for 6 days. The ethereal solution was concentrated and distilled [bp 85–86 °C (10 torr)] through a short-path distillation head to yield a clear oil [(*R*)-2; 1.9 g, 25 mmol, 50%]. The <sup>1</sup>H NMR spectrum of this material was indistinguishable from that of authentic material.

**Enzymatic Preparation of (*R*)-1,2-Butanediol [(*R*)-4] by Reduction of 1-Hydroxy-2-butanone (3).<sup>56</sup>** A 1-L, four-necked, round-bottomed flask was equipped with a pH electrode, argon inlet, outlet to a bubbler, and a magnetic stirring bar. Water (150 mL), ammonium formate (12.6 g, 200 mmol), 1-hydroxy-2-butanone (8.8 g, 100 mmol), and 2-mercaptoethanol (0.04 g, 0.5 mmol) were added, and argon was bubbled through the solution for 1 h to remove dioxygen. The pH was adjusted to 7.4 with 2 N KOH. Immobilized GDH (88 U, *Enterobacter aerogenes*) and FDH (11 U) and NAD (0.13 mmol) were added. The reaction mixture was stirred under an argon atmosphere and maintained in a pH range of 7.0–7.8 by periodic addition of 2.5 N KOH. After 7 days, additional NAD (0.13 mmol) was added. The progress of the reaction was monitored by GLC; after 19 days no starting material remained. The mixture was decanted, and the immobilized enzymes were separated by centrifugation. The gel was washed with two 30-mL portions of pH 7.6 Hepes buffer. The recovered activities of the enzymes were 50% for GDH and 88% for FDH.

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(56) Another method to produce chiral 1,2-butanediol is based on lipase-catalyzed transesterification of racemic 1,2-butanediol; both enantiomers are obtained in high enantiomeric excess by using this procedure.<sup>45</sup>

(46) Bergmeyer, H. U. In "Methods of Enzymatic Analysis"; Verlag Chemie and Academic Press: Weinheim and New York, 1974. For specific assays, see pp 459 (G-6-PDH), 653 (GID), and 1555 (FDH) of this reference.

(47) Pollak, A.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* 1980, 102, 6324–6336.

The aqueous solution was continuously extracted with ether for 5 days. The ethereal solution was concentrated and distilled through a short-path distillation head [bp 80–81 °C (5 torr)] to yield a colorless oil [(*R*)-4; 6.3 g, 70 mmol, 70%]. The <sup>1</sup>H NMR of this material was indistinguishable from that of an authentic sample.

**Preparation of (*R*)-3-Hydroxy-2-butanone [(*R*)-5] by Reduction of Racemic 3-Hydroxy-2-butanone (5).** A 500-mL, round-bottomed flask was charged with 3-hydroxy-2-butanone (8.0 g, 91 mmol), glucose-6-phosphate (12.8 g, 45 mmol),<sup>25</sup> and water (80 mL). The pH was adjusted to 7.5 with KOH. NAD (0.1 g, 0.13 mmol) and immobilized GDH (90 U, *Cellulomonas sp.*) and G-6-PDH (85 U, *Leuconostoc mesenteroides*) were added. A pH electrode connected to a pH controller was added; the pH was maintained at 7.4–7.5 by automatic addition of a 2.5 N KOH solution, which was contained in a graduated cylinder. After 3 days of stirring under an argon atmosphere, no further change in pH was observed; 19 mL of KOH solution (0.52 equiv, 47 mmol) had been added. The immobilized enzymes were removed by centrifugation: The recovered yields were 130% for GDH and 100% for G-6-PDH. The aqueous solution was saturated with sodium chloride and continuously extracted with ethyl acetate for 2 days. The organic solution was concentrated (8.7 g) and distilled at 25 torr through a 30-cm Helmholtz column. Two fractions were collected: at 54–55 and 95–100 °C. The first fraction contained (*R*)-5 (1.6 g, 18 mmol, 20%;  $[\alpha]_D^{20}$  -40.76° (neat),  $[\alpha]_D^{20}$  -40.8°;  $d = 1.0$  g/mL). The second fraction contained 2,3-butanediol (6; 2.7 g, 30 mmol, 34%).

**Enzymatic Preparation of (*R*)-3-Hydroxy-2-butanone [(*R*)-5] Using Glucose Dehydrogenase to Regenerate NADH.** Racemic 3-hydroxy-2-butanone (5) (8.0 g, 91 mmol) was also resolved by using GDH; glucose (9.0 g, 50 mmol) and glucose dehydrogenase (GlcDH) were used to regenerate NADH. Immobilized GDH (60 U, *Cellulomonas sp.*) and GlcDH (63 U) were recovered in 133% and 51% yield, respectively. The aqueous solution was saturated with sodium chloride and extracted continuously with ethyl acetate for 7 days. The organic solution was concentrated (8.0 g) and distilled through a 30-cm Helmholtz column at 26 torr. Two fractions were collected: at 55–56 and 96–98 °C. The first fraction contained (*R*)-5 (1.7 g, 21%;  $[\alpha]_D^{20}$  -50.74° (neat),  $[\alpha]_D^{20}$  -50.8°;  $d = 1.0$  g/mL).<sup>57</sup> The second fraction contained 6 (3.2 g, 39%).

**Enzymatic Preparation of (*R*)-2-Hydroxycyclopentanone [(*R*)-7] by Reduction of Racemic 2-Hydroxycyclopentanone (7).** A 500-mL, round-bottomed flask was charged with 2-hydroxycyclopentanone (7; 6.0 g, 60 mmol), glucose-6-phosphate (9.6 g, 33 mmol), and water (150 mL). The pH was adjusted to 6.5<sup>58</sup> with KOH. NAD (0.1 g, 0.13 mmol) and immobilized GDH (60 U, *Cellulomonas sp.*) and G-6-PDH (200 U, *Leuconostoc mesenteroides*) were added. The pH was maintained at 6.3–6.5 by automatic addition of a 2.5 N KOH solution. After 26 h of stirring under an argon atmosphere, no further change in pH was observed; 6.0 mL of KOH solution (0.25 equiv, 15 mmol) had been added. The color of the mixture was brown; the color of the enzyme-containing gel was black. The immobilized enzymes were removed by centrifugation: The recovered yields were 90% for GDH and 38% for G-6-PDH. The aqueous solution was saturated with sodium chloride and continuously extracted with ethyl acetate for 36 h. The organic solution was concentrated (4.9 g) and distilled at 2 torr through a 10-cm Helmholtz column. Two fractions were collected: at 50–53 and 70–72 °C. The first fraction contained (*R*)-7 (1.0 g, 10 mmol, 16%). The second fraction

contained 1,2-cyclopentanediol (1.1 g, 11 mmol, 18%).

The reaction was repeated at pH 5.5 with the same quantities of enzymes and starting materials. After 3 days, 6.3 mL of 2.5 N KOH had been added. The products of the reaction were isolated by distillation as before: (*R*)-2-Hydroxycyclopentanone (1.3 g, 13 mmol, 22%) and 1,2-cyclopentanediol (1.4 g, 14 mmol, 23%) were obtained.

**Enzymatic Reduction of Racemic 2-Hydroxycyclobutanone (8).** A 500-mL, round-bottomed flask was charged with 2-hydroxycyclobutanone (8; 1.5 g, 17 mmol), glucose (1.7 g, 10 mmol), Hepes buffer (pH 7.6, 50 mL), NAD (0.1 g, 0.13 mmol), and immobilized GDH (40 U, *Cellulomonas sp.*) and GlcDH (36 U, *Bacillus cereus*). The pH was maintained at 7.2–7.4 by automatic addition of 2.5 N KOH solution. After 16 h of stirring under an argon atmosphere, no further change in pH was observed; 4.0 mL of KOH solution (0.59 equiv, 10 mmol) had been added. The immobilized enzymes were removed by centrifugation. The aqueous solution was saturated with sodium chloride and continuously extracted with ether for 24 h. The organic solution was concentrated to an oil (0.6 g); GLC showed the oil contained a 1:1 mixture of  $\alpha$ -hydroxy ketone 8 to 1,2-cyclobutanediol.

**Preparation of (*R*)-1,2-Butanediol [(*R*)-4] and (*R*)-1,2-Propanediol [(*R*)-2] by Reduction of 1-Hydroxy-2-butanone (3) and 1-Hydroxy-2-propanone (1) Using Fermenting Bakers' Yeast.<sup>59</sup>** A 1-L, round-bottomed flask was charged with sucrose (60 g, 0.3 mol) and water (0.5 L). Yeast (60 g) was suspended in water (60 mL), and the slurry was added to the stirred sugar solution. After 2 h, 1-hydroxy-2-butanone (3; 2.7 g, 31 mmol) was added to the bubbling mixture. After 24 h, no further bubbling was observed. The mixture was stirred for 3 days and filtered through Celite. The filtrate was concentrated and 20 mL of a 1:1 solution of ether–ethanol was added. The resulting white precipitate was removed by centrifugation and discarded. The supernatant was concentrated (2.6 g) and distilled through a short-path distillation head [bp 64–65 °C (2.5 torr)] as a colorless oil [(*R*)-4; 1.1 g, 13 mmol, 41%].

A similar procedure was used to reduce 1-hydroxy-2-propanone (1; 6.4 g, 86 mmol). The yield of (*R*)-1,2-propanediol [(*R*)-2] was 38% (2.5 g, 33 mmol).

**Preparation of (*S*)-2-Hydroxycyclohexanone by Oxidation of 1,2-Cyclohexanediol.** A 500-mL, round-bottomed flask was charged with 1,2-cyclohexanediol (1 g, 8.6 mmol), NAD (0.1 g, 0.13 mmol), methylene blue (0.035 g, 0.09 mmol), pH 9.0 glycine buffer (80 mL), and immobilized GDH (*Cellulomonas sp.*, 145 U) and diaphorase (140 U). The reaction mixture was stirred while open to the atmosphere; the color was dark blue. The reaction progress was followed by GLC analysis. After 3 days, the reaction appeared to be 30% complete and no further progress was observed. The immobilized enzymes were separated by centrifugation. The recovered activities of the enzymes were 83% for GDH and 58% for diaphorase. The aqueous solution was extracted with two 200-mL portions of methylene chloride. The combined organic extracts were concentrated, redissolved in ether (20 mL), filtered through charcoal and Celite, and concentrated to a colorless oil (0.2 g), which crystallized upon standing:  $[\alpha]_D^{20}$  -0.07 ( $c$  0.53, CHCl<sub>3</sub>),  $[\alpha]_D^{20}$  -13.3°.

**Enzymatic Preparation of (*S*)-1,2-Butanediol [(*S*)-4] by Oxidation of Racemic 1,2-Butanediol [(±)-4].** To a deoxygenated solution of sodium 2-oxoglutarate (5.0 g, 30 mmol), 1,2-butanediol (4, 4.5 g, 50 mmol), and 2-mercaptoethanol (0.008 g, 0.1 mmol) in water (1 L) were added immobilized GDH (65 U, *Enterobacter*), GIDH (34 U) and NAD (0.1 g, 0.13 mmol). The pH was adjusted with NH<sub>4</sub>OH to 8.5 and maintained in the pH range 8.3–8.5 by the addition of 2.5 N KOH. After 5 days, additional NAD (0.1 g, 0.13 mmol) was added. The reaction progress was followed by GLC; after 11 days no further reaction was observed. The gel was allowed to settle and the solution decanted through a stainless steel cannula under a positive pressure of argon. The recovered enzyme activities were 30% for GDH and 18% for GIDH. The aqueous solution was extracted continuously with ether for 3 days. The ethereal solution was concentrated (3.8 g) and distilled through a short-path distillation head at 4.2 torr. Two fractions were collected: at 25 and 67–70 °C. The first

(57) The physical properties of chiral 5 were quite different from those of racemic 5; racemic 5 solidifies as the dimer at room temperature, whereas chiral 5 remains a mobile, colorless oil even at 0 °C. The absolute rotation of (*R*)-3-hydroxy-2-butanone [(*R*)-5] is  $[\alpha]_D^{20}$  -58°. This value is extrapolated from the rotation of material found to be of 88% ee based on <sup>1</sup>H NMR data of the (–)-MTPA ester:  $[\alpha]_D^{20}$  (-50°)/(0.88)(1.0 g/mL)(1 dm) = -58°. Previous workers<sup>42</sup> found a higher absolute rotation of (*R*)-5; however, the authors themselves questioned the validity of the rotation since the concentration was determined by colorimetric methods; i.e., the sample was not weighed.

(58) The  $\alpha$ -hydroxy ketone was unusually unstable in aqueous solution, particularly at basic pH; 7 dissolved in buffered solutions at pH 7–9 gradually darkened to a black color. Decomposition was minimized by maintaining the pH at 6–7.

fraction contained 1-hydroxy-2-butanone (**3**; 0.71 g, 8 mmol, 16%); the second fraction contained (*S*)-1,2-butanediol [(*S*)-**4**; 1.95 g, 22 mmol].

**Preparation of (*R*)-1,2-Butanediol-2-*d*<sub>1</sub> [(*R*)-4-2-*d*<sub>1</sub>].** The preparation of (*R*)-1,2-butanediol-2-*d*<sub>1</sub> [(*R*)-4-2-*d*<sub>1</sub>] was similar to the enzymatic preparation of (*R*)-1,2-butanediol [(*R*)-**4**] except that ammonium formate was replaced with formic acid-*d*<sub>2</sub> (neutralized with NH<sub>4</sub>OH). The substrate, 1-hydroxy-2-butanone (**3**; 4.4 g, 50 mmol) was reduced to (*R*)-1,2-butanediol-2-*d*<sub>1</sub> [(*R*)-4-2-*d*<sub>1</sub>; bp 62–63 °C (2.4 mmHg); 1.9 g, 21 mmol, 42%] by using GDH (20 U, *Enterobacter*, 100% recovery at the conclusion of the reaction), FDH (5 U, 83% recovery), and NAD (0.16 mmol) over a period of 22 days. The deuterium incorporation was determined by GC-MS and found to be 98%, based on *m/e* 60 (CH<sub>3</sub>CH<sub>2</sub>CH(D) = OH<sup>+</sup>) <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.68 (d, 1 H, *J* = 10.8 Hz), 3.46 (d, 1 H, *J* = 10.8 Hz), 2.05 (br s, 1 H), 1.90 (br s, 1 H), 1.49 (m, 1 H, *J* = 7.4 Hz), 0.98 (t, 3 H, *J* = 7.4 Hz).

**Preparation of 1-*O*-Tosyl-1,2-propanediol and 1-*O*-Tosyl-1,2-butanediol.** The tosylation of 1,2-propanediol and 1,2-butanediol was performed according to the literature procedure.<sup>8</sup> The reaction product was contaminated with small amounts of ditosylate and with the secondary tosylate. The desired 1-*O*-tosyl derivatives were isolated by preparative TLC or medium-pressure column chromatography; for both, silica gel was used as the stationary phase and methylene chloride or 3:2 hexane-ethyl acetate as the mobile phase. <sup>1</sup>H NMR spectral data of these compounds were in agreement with the literature values.<sup>8</sup>

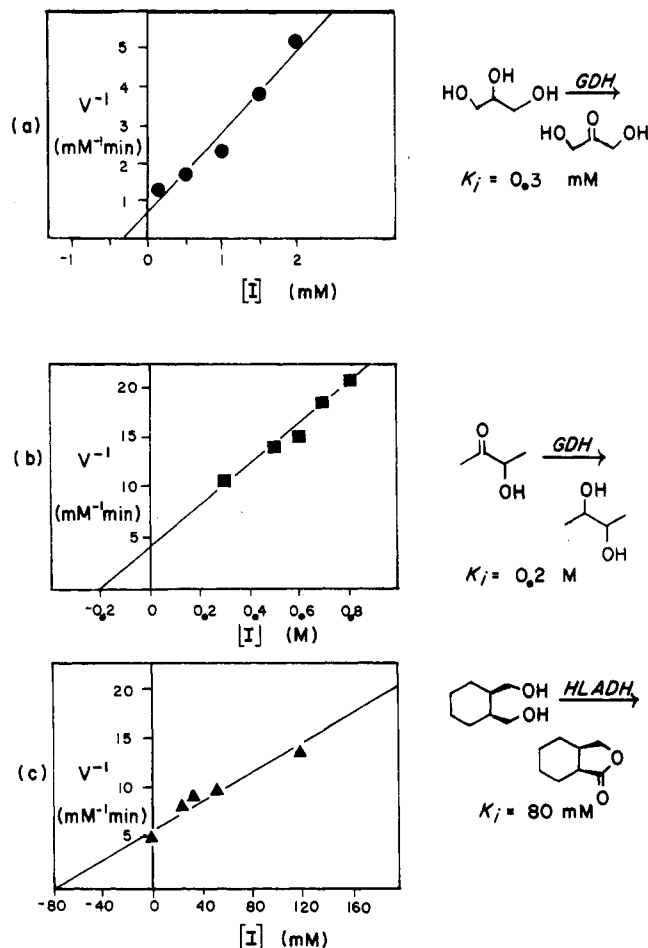
**Preparation of (*R*)-2-Acetoxy-1-bromopropane and (*R*)-2-Acetoxy-1-bromobutane.**<sup>34</sup> A 50-mL, round-bottomed flask equipped with a magnetic stirring bar was charged with (*R*)-1,2-butanediol [(*R*)-**4**; 1.1 g, 14 mmol; prepared enzymatically] and cooled in ice. HBr in acetic acid (31%, 43 mmol, 11 mL) was added over a period of 5 min. The solution was stirred at room temperature for 30 min. Water (40 mL) was added and the solution neutralized with NaHCO<sub>3</sub> (12 g) and extracted with three 50-mL portions of ether. The combined organic extracts were dried (MgSO<sub>4</sub>) and concentrated (2.0 g). The crude (*R*)-1-bromo-2-acetoxypropane was distilled through a short-path distillation head [bp 75–80 °C (40 torr)] as a colorless oil (1.6 g, 8.9 mmol, 63%). <sup>1</sup>H NMR spectral data were in agreement with the literature values.<sup>34</sup>

(*R*)-1,2-Butanediol-2-*d*<sub>1</sub> [(*R*)-4-2-*d*<sub>1</sub>; 1.7 g, 19 mmol] was converted to (*R*)-1-bromo-2-acetoxybutane-2-*d*<sub>1</sub> by a similar procedure. The product (2.5 g, 13 mmol) was obtained in 69% yield [bp 75–80 °C (20 torr)]; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  3.49 (d, 1 H, *J* = 5.3 Hz), 3.46 (d, 1 H, *J* = 5.3 Hz), 2.10 (s, 3 H), 1.76 (m, 2 H), 0.93 (t, 3 H, *J* = 7.6 Hz).

**(*R*)-1,2-Epoxypropane and (*R*)-1,2-Epoxybutane.**<sup>34</sup> A 50-mL, round-bottomed flask equipped with a magnetic stirring bar was charged with (*R*)-1-bromo-2-acetoxypropane (1.6 g, 8.9 mmol) and 1-pentanol (2 mL). A solution of potassium pentafluoride in 1-pentanol (prepared by reaction of 0.35 g of potassium metal with 10 mL of pentanol) was added to the stirred solution via stainless steel cannula under a positive pressure of argon over a period of 5 min. A white precipitate (KBr) formed immediately. The mixture, containing (*R*)-1,2-epoxypropane, was distilled through a 15-cm Vigreux column (32–35 °C) as a colorless oil (0.25 g, 4.3 mmol, 48%). <sup>1</sup>H NMR spectral data were in agreement with the literature values.<sup>34</sup>

(*R*)-1-Bromo-2-acetoxybutane-2-*d*<sub>1</sub> was converted to (*R*)-1,2-epoxybutane-2-*d*<sub>1</sub> by a similar procedure. The product [bp 65–70 °C; 0.41 g, 5.6 mmol] was obtained in 44% yield: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  2.74 (d, 1 H, *J* = 4.6 Hz), 2.48 (d, 1 H, *J* = 5.4 Hz), 1.58 (q, 2, *J* = 7.6 Hz), 1.01 (t, 3 H, *J* = 7.6 Hz).

**Preparation of 1-(Phenylthio)-2-propanol and (*R*)-1-(Phenylthio)-2-butanol-2-*d*<sub>1</sub>.** A 5-mL test tube was charged with (*R*)-1,2-epoxypropane (0.08 g, 1.4 mmol), thiophenol (0.29 g, 2.6 mmol), and methanol (0.5 mL). After 12 h, the product was isolated by chromatography on silica gel (5 g): The column was eluted initially with hexane to remove thiophenol, followed by methylene chloride. 1-(Phenylthio)-2-propanol was obtained as a colorless oil (0.05 g, 0.3 mmol, 21%): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.4–7.2 (m, 5 H), 3.82 (m, 1 H), 3.12 (dd, 1 H, *J* = 13.8, 3.8 Hz), 2.84 (dd, 1 H, *J* = 14.0, 8.6 Hz), 2.41 (s, 1 H), 1.27 (d, 3 H, *J* = 5.9 Hz). A similar procedure was used to obtain (*R*)-1-(phenylthio)-2-butanol-2-*d*<sub>1</sub>: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.4–7.2 (m, 5 H), 3.16



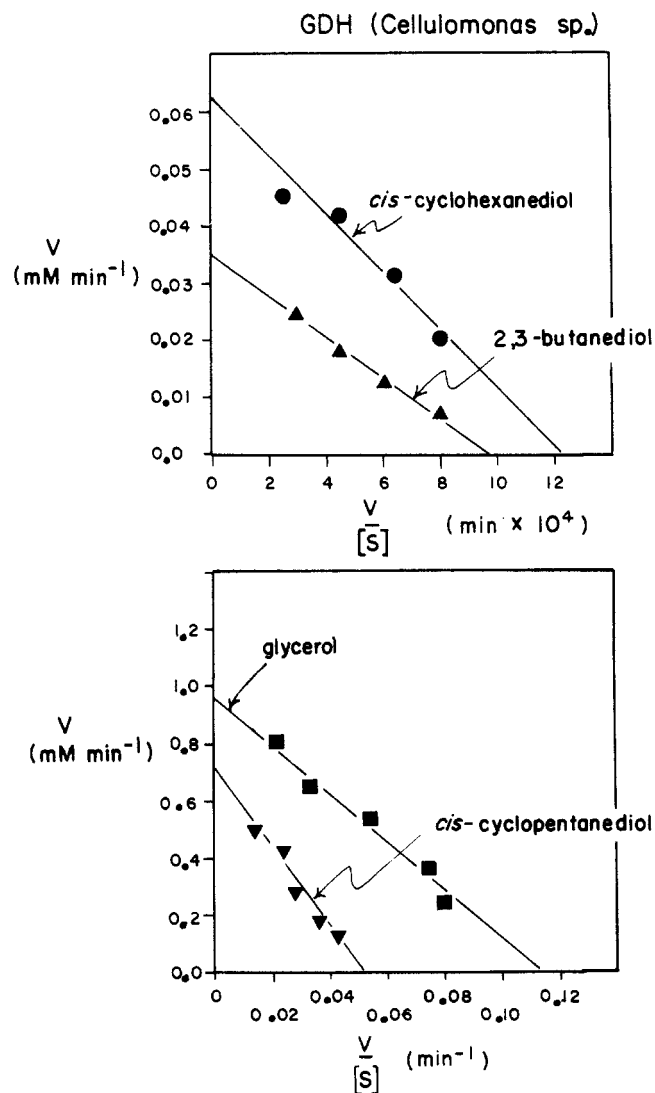
**Figure 6.** Dixon plots used in determinations of  $K_i$ . (a) GDH-catalyzed oxidation of glycerol (*Cellulomonas* sp.). (b) GDH-catalyzed reduction of 3-hydroxy-2-butanone (*Cellulomonas* sp.). (c) HLADH-catalyzed oxidations of *cis*-1,2-cyclohexanedimethanol.

(d, 1 H, *J* = 13.5 Hz), 2.84 (d, 1 H, *J* = 13.5 Hz), 2.39 (s, 1 H), 1.57, (m, 2 H), 0.97 (t, 3 H, *J* = 7.4 Hz).

**Mitsunobu Reaction Using (*R*)-3-Hydroxy-2-butanone [(*R*)-**5**] and ( $\pm$ )-1-Hydroxy-2-butanone (**5**).**<sup>60</sup> A 25-mL, round-bottomed flask was charged with (*R*)-3-hydroxy-2-butanone [(*R*)-**5**; 0.05 g, 0.57 mmol], triphenylphosphine (0.15 g, 0.57 mmol), (–)-MTPA acid (0.13 g, 0.57 mmol), and THF (5 mL). Diethyl azodicarboxylate (DEAD, 0.57 mmol) in THF (5 mL) was added via syringe. The yellow color of the DEAD solution disappeared immediately upon addition. After 4 h, the solution was concentrated. Ether (20 mL) was added and the white precipitate removed by filtration. The ethereal solution was concentrated to an oil (0.41 g). Purification by preparative TLC on silica gel (to remove residual triphenyl phosphine oxide) using 1:1 methylene chloride-petroleum ether provided 3-*O*-MTPA-2-butanone as an oil (90 mg, 0.27 mmol, 54%). Racemic **3** was converted to the (–)-*O*-MTPA ester by a similar procedure.

**Racemization of (*R*)-3-Hydroxy-2-butanone (**5**).** (*R*)-3-Hydroxy-2-butanone (**5**) was found to be stable to 1 N HCl but to rapidly racemize in 1 N NaOH. The rate of racemization was measured by monitoring the decrease in the polarimeter reading of a solution of **5** dissolved in 1 N NaOH. Chiral 3-hydroxy-2-butanone [(*R*)-**5**; 0.11 g, 1.3 mmol, 80% ee] was dissolved in 1 mL of water. Aliquots of the solution were diluted in 1 mL of distilled water, 1 mL of 1 N HCl, and 1 mL of 1 N NaOH. The optical rotation of the diluted solutions were measured over time. The neutral and acidic solutions showed constant rotations of  $-0.65^\circ$  and  $-0.70^\circ$ . After 30 h, the acidic solution had a rotation of  $-0.69^\circ$ . The rotation of the basic solution decreased rapidly; a measurement was taken every 30 s. A first-order plot of the loss of

(60) Mitsunobu, O.; Eguchi, M. *Bull. Chem. Soc. Jpn.* 1971, 44, 3427–3430.



**Figure 7.** Eadie-Hofstee plots used in the determination of values of  $K_m$  for GDH catalyzed oxidations (*Cellulomonas sp.*).

rotation of (*R*)-5 indicated that the half-life of the racemization was 6.3 min.

**Preparation of (-)-MTPA Esters.** The (-)-MTPA esters were prepared according to a general procedure<sup>23</sup> using 2 equiv of (-)-MTPA-Cl per equiv of alcohol. <sup>1</sup>H NMR spectral data are given as supplementary material.

**$K_m$  Values for Substrates for GDH and HLADH.** The values of  $K_m$  of substrates in GDH-catalyzed oxidations were determined in glycine buffer (pH 9.0) with  $(\text{NH}_4)_2\text{SO}_4$  (10 mM) and NAD (3 mM). The concentration of *cis*-1,2-cyclohexanediol was varied from 25 to 500 mM, of 2,3-butanediol and *cis*-cyclopentanediol from 3 to 40 mM, and of 1,2-propanediol and 1,2-butanediol from 0.3 to 0.4 mM. The  $K_m$  values for GDH-catalyzed reductions were determined in Hepes buffer (pH 7.6) with  $(\text{NH}_4)_2\text{SO}_4$  (10 mM) and NADH (0.2 mM). The concentration of 2-hydroxycyclopentanone was varied from 2.5 to 5 mM, of 3-hydroxy-2-butanone from 0.4 to 20 mM, of dihydroxyacetone from 0.05 to 0.8 mM, and of 1-hydroxy-2-propanone from 0.03 to 0.20 mM. The value of  $K_m$  of *cis*-cyclohexanedimethanol with HLADH was determined in glycine buffer (pH 9) with NAD (3 mM). The concentration of the substrate was varied from 6–40 mM. A

representative Eadie-Hofstee plot is shown in Figure 6; the remainder of the plots are given as supplementary material.

**Determination of Values of  $K_i$ .** Product inhibition constants ( $K_i$ ) for three reactions were determined: GDH-catalyzed oxidation of glycerol, GDH-catalyzed reduction of 3-hydroxy-2-butanone, and horse liver alcohol dehydrogenase (HLADH) catalyzed oxidation of *cis*-1,2-cyclohexanedimethanol. The inhibition for each reaction was assumed to be noncompetitive for these substrates, and the method of Dixon<sup>61,62</sup> was used to determine each value of  $K_i$ . For the oxidation of glycerol at pH 9 the glycerol concentration was maintained at 0.1 M and the dihydroxyacetone concentration varied between 0.2 and 2 mM. For the reduction of 3-hydroxy-2-butanone at pH 7.6, the substrate was maintained at 10 mM and the 2,3-butanediol concentration varied between 0.3 and 0.8 M. For HLADH catalyzed oxidation of *cis*-1,2-cyclohexanedimethanol at pH 9.0, the substrate concentration was maintained at 40 mM and the lactone<sup>63</sup> concentration varied between 20 and 60 mM. Plots of  $1/V$  vs.  $[I]$  are given in Figure 7.

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**Registry No.** 1, 116-09-6; (*R*)-2, 4254-14-2; 3, 5077-67-8; (*R*)-4, 40348-66-1; (*S*)-4, 73522-17-5; ( $\pm$ )-4, 26171-83-5; (*R*)-4-2-*d*<sub>1</sub>, 99441-00-6; ( $\pm$ )-5, 52217-02-4; (*R*)-5, 53584-56-8; ( $\pm$ )-7, 99440-98-9; (*R*)-7, 99493-88-6; ( $\pm$ )-8, 99440-99-0; GDH, 9028-14-2; HLADH, 9031-72-5; (*S*)-2-hydroxycyclohexanone, 53439-93-3; 1,2-cyclohexanediol, 931-17-9; (*R*)-1-bromo-2-acetoxypropane, 99457-42-8; (*R*)-1-bromo-2-acetoxybutane-2-*d*<sub>1</sub>, 99441-01-7; (*R*)-1,2-epoxypropane, 15448-47-2; (*R*)-1,2-epoxybutane-2-*d*<sub>1</sub>, 99441-02-8; 1-(phenylthio)-2-propanol, 937-56-4; (*R*)-1-(phenylthio)-2-butanediol-2-*d*<sub>1</sub>, 99441-03-9; 3-*O*-MTPA-2-butanone, 99441-04-0; dihydroxyacetone, 96-26-4; (*R*)-2,3-dihydroxypropanal, 453-17-8; (*S*)-2,3-dihydroxypropanal, 497-09-6; 1-hydroxy-2-butanone, 5077-67-8; 2-oxopropanal, 78-98-8; 2-hydroxycyclobutanone, 17082-63-2; 2-hydroxycyclopentanone, 473-84-7; 2-hydroxycyclohexanone, 533-60-8; glycerol, 56-81-5; 1,2-propanediol, 57-55-6; (*R*)-1,2-propanediol, 4254-14-2; (*S*)-1,2-propanediol, 4254-15-3; 1,2-hexanediol, 6920-22-5; 1,2-octanediol, 1117-86-8; 1,2-ethanediol, 107-21-1; 1,3-propanediol, 504-63-2; 1,4-butanediol, 110-63-4; 2,3-butanediol, 513-85-9; 3-mercapto-1,2-propanediol, 96-27-5; 3-chloro-1,2-propanediol, 96-24-2; 3-methoxy-1,2-propanediol, 623-39-2; 3-(methylthio)-1,2-propanediol, 22551-26-4; (L)- $\alpha$ -glycerophosphate, 5746-57-6; (DL)- $\alpha$ -glycerophosphate, 1509-81-5; (*R*)-1-amino-2-propanol, 2799-16-8; (*S*)-1-amino-2-propanol, 2799-17-9; *cis*-1,2-cyclopentanediol, 5057-98-7; *cis*-1,2-cyclohexanediol, 1792-81-0; *trans*-1,2-cyclohexanediol, 1460-57-7; *cis*-1,2-cycloheptanediol, 42114-72-7; *cis*-1,2-cyclooctanediol, 27607-33-6; D-galactose, 59-23-4; *cis*-1,2-cyclohexanedimethanol, 15753-50-1.

**Supplementary Material Available:** <sup>1</sup>H NMR data for MTPA esters and Eadie-Hofstee plots used in the determination of  $K_m$  values (8 pages). Ordering information is given on any current masthead page.

(61) Product inhibition for the natural substrates of both GDH and HLADH are noncompetitive for the alcohol vs. the ketone (or aldehyde).<sup>37</sup>

(62) Dixon, M. *Biochem. J.* 1953, 55, 170–171.

(63) The product of the oxidation, (+)-(1*R*,6*S*)-*cis*-8-oxabicyclo-[4.3.0]nonan-7-one, was obtained from a large-scale HLADH-catalyzed oxidation of *cis*-1,2-cyclohexanedimethanol.<sup>1</sup>