(C13a), 140.8 (C13b), 136.4 (C7a), 133.0 (C3a), 130.8 (C6), 129.7 (C10b), 125.2 (C4), 124.3 (C5), 122.0 (C13d), 121.4 (C3b), 118.4 (C7), 113.3 (C13c), 110.5 (C3), 110.6 (C10a), 94.2 (C10), 45.3 (NMe_2); HRFABMS obsd m/z = 365.0839 ($C_{20}H_{14}N_4S$ + Na requires 365.0837), 343.1039 ($C_{20}H_{15}N_4S$ requires 343.1019).

Single-Crystal X-ray Diffraction Analysis of Stellettamine (15). Preliminary diffraction photographs of stellettamine (15) displayed monoclinic symmetry, and accurate cell constants of a = 13.812 (3) Å, b = 12.638 (4) Å, c = 11.987 (3) Å, and β = 106.57 (2)° were determined from 20 diffractometer measured 2θ values. Systematic extinctions and density considerations were uniquely consistent with space group $P2_1/c$ with one molecule of composition $C_{20}H_{14}N_4S{\cdot}CHCl_3$ in the asymmetric unit. A total of 2879 reflections with $2\theta \leq 116°$ were collected on a computer controlled four-circle diffractometer using CuKα radiation and θ:2θ scans at approximately -15 °C. After Lorentz, polarization, background, and analytical absorption corrections, 2736 (95%) were judged observed ($|F_o| \geq 3\sigma|F_o|$). The structure was solved and refined routinely using the SHELXS system of programs. In the final model the non-hydrogen atoms are anisotropic, the hydrogens are isotropic and riding the appropriate heavy atom, and the final discrepancy index is R = 0.056. Additional X-ray parameters are available and are described in the supplementary material. A drawing of the final X-ray model of stellettamine (15) is given in Figure 1.

Isolation of Kuanoniamine D (9). A dark purple encrusting tunicate, tentatively identified as a species of *Cystodyes*, was collected from patch reefs (-2 m) near the airport at Pohnpei in April 1989. The freeze-dried tunicate (20 g) was extracted successively with hexane (200 mL) and 2:1 $MeOH/CH_2Cl_2$ (3 × 200 mL). The purple extract was chromatographed on a Sephadex LH-20 column using MeOH as eluant to yield two red fractions. The first fraction was concentrated to a gum, washed with $CHCl_3$ (2 × 5 mL), and redissolved in MeOH (2 mL) at 45 °C. After the solution had been allowed to stand at room temperature for 1 h, a purple solid precipitated. The solid (36 mg) was dissolved in water, and the solution was basified with 1% NaOH solution to pH 9. The yellow precipitate was extracted with $CHCl_3$ (2 × 20 mL) and chromatographed on reversed-phase HPLC (20:1 $MeOH/H_2O$) to yield kuanoniamine D (9, 14.4 mg, 0.07% dry wt) and dercitamide (13 ≡ 8, kuanoniamine C, 7.9 mg, 0.04% dry wt), both of which possess spectral data that are identical to literature values.[3,4] The second red fraction was dissolved in warm MeOH (2 mL) and the solution was cooled to obtain debromoshermilamine (17 mg, 0.085% dry wt) as a purple precipitate.[14]

Metal Binding Studies. ^1H NMR spectra were recorded using a Bruker 200-MHz spectrometer at 25 °C, using 0.01 M solutions in 5:1 MeOH-d_4/$CDCl_3$. Fluorescence studies were performed on a Farrand MK2 spectrofluorometer at 25 °C and analytical- or spectral-grade solvents were used. In all fluorescence experiments to determine the binding ratios and stability constants, the pH was adjusted to 8.5, the excitation wavelength was set at 350 nm, and the observation wavelength was 524 nm for $CoCl_2$ and 508 nm for $Cu(OAc)_2$. The binding ratios to Co^{2+} and Cu^{2+} were measured using the method of continuous variation[8] with a total concentration of ligand and metal ion of 10^{-5} M. The x_{max} values, the fraction of the total ligand concentration that gives the maximum formation of complex, of 0.66 and 0.65 for the complexes of kuanoniamine D (9) with $CoCl_2$ and $Cu(OAc)_2$, respectively, were obtained by curve fitting, using the least squares method, and the binding ratios (n) were calculated according to the equation $n = x_{max}/(1 - x_{max})$.[9] The stability constants (β_2) were calculated by plotting the observed relative fluorescence intensity (F_{obs}) against the ratio of the total concentration of metal ion to the total concentration of ligand (T_M/T_L). The value of F_{obs} was arbitrarily set at 1000 for [L] = 10^{-5} M so that [L] = $100*F_{obs}$. The values of log β_2 were calculated as 10.1 ± 0.4 for $Cu(OAc)_2$ (24 data points) and 10.4 ± 0.3 for $CoCl_2$ (27 data points).[10,11]

Acknowledgment. We thank Dr. Jay S. Siegel for helpful discussions. The tunicate was tentatively identified by Dr. Chris Ireland. Research at Cornell was supported by a grant from the New York State Sea Grant Program and at Scripps by grants from the National Cancer Institute (CA 49084 and CA 50750).

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, and interatomic distances and angles (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(14) Rudi, A.; Kashman, Y. *J. Org. Chem.* **1989**, *54*, 5331-5337.

A *Pseudomonas* sp. Alcohol Dehydrogenase with Broad Substrate Specificity and Unusual Stereospecificity for Organic Synthesis

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Received September 17, 1991

A new alcohol dehydrogenase from *Pseudomonas* sp. strain PED has been isolated and characterized. The enzyme exhibits a broad substrate specificity, accepting aromatic, cyclic, and aliphatic compounds as substrates. The K_m values were determined as 525 μM for NAD and 75 μM for 2-propanol with a specific activity of 36 U/mg. The kinetic mechanism is ordered bi-bi with the cofactor binding first and releasing last. The enzyme transfers the *pro-R* hydride of NADH to the *si* face of carbonyl compounds to yield (*R*) alcohols. Synthetic-scale reductions of a number of representative compounds were carried out in high enantiomeric excess with in situ regeneration of NADH using 2-propanol as the hydride source and the same enzyme as catalyst.

Introduction

Alcohol dehydrogenases are now well-established catalysts in organic synthesis.[1] Enzymes from horse liver,[2] yeast,[2a] and *Thermoanaerobium brockii*[3] have been utilized extensively. Although the alcohol dehydrogenases isolated

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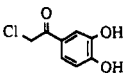
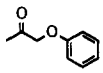
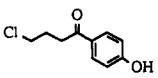
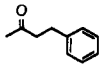
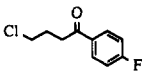
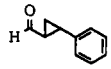
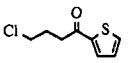
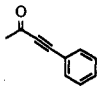
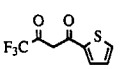
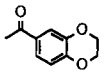
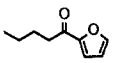
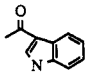
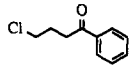
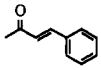
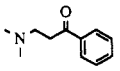
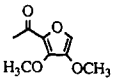
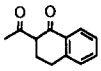
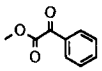
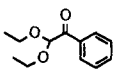
Table I. Relative Rate of Reduction of Aromatic Ketones with PED Alcohol Dehydrogenase

compound	rel rate[a]	compound	rel rate[a]	compound	rel rate[a]
	1[b]		<0.1		7
	7		0		6
	1.8		0		7
	<1		0		<0.1
	34		0		0
	<1		<0.1		0
	4		0		0
	8		0.6		0
	4		4		0
	<0.1				

[a] Rates were determined by assaying with 10 mM ketone, 0.45 mM NADH, and purified PED alcohol dehydrogenase and monitoring the change in absorbance at 340 nm. [b] 2.1 U/mg enzyme.

thus far reduce a variety of aldehydes and ketones, they are still limited to the reduction of a small number of carbonyl compounds. Another disadvantage of these redox enzymes is the necessary nicotinamide cofactor. However, stoichiometric use of the expensive cofactors has been alleviated by a number of in situ regeneration methods which are now routine.[4] In our continuing interest in alcohol dehydrogenases, we have isolated a new NADH-requiring alcohol dehydrogenase from *Pseudomonas* sp. which accepts a wide range of aliphatic, cyclic, and aromatic ketones as substrates with very high enantioselectivity. This enzyme has a broader substrate specificity than the one discovered in this laboratory previously (*Pseudomonas* sp. strain SBD6, PADH),[5] although both enzymes possess unique stereospecificity (i.e., the *pro-R* hydride is transferred from NADH to the *si* face of the carbonyl group) different from other known alcohol dehydrogenases.

The commercially available alcohol dehydrogenases developed for organic synthesis usually follow Prelog's rule for acyclic compounds, i.e., hydride transfer from the cofactor occurs to the *re* face of the carbonyl to give an (*S*) alcohol.[6] *Pseudomonas* sp. strain PED alcohol dehydrogenase presented here has anti-Prelog specificity, producing (*R*) alcohols. Thus, this enzyme joins an emerging group of recently developed alcohol dehydrogenases,[5,7] which, together with Prelog-type alcohol dehydrogenases, will allow for the synthesis of both enantiomers of many useful compounds in high enantiomeric excess. We present here the characterization, specificity study, and synthetic applications of PED alcohol dehydrogenase.[8]

Results and Discussion

The new PED alcohol dehydrogenase was isolated by selection with (±)-1-phenyl-1,2-ethanediol as sole carbon and energy source.[9] The crude cell extract of *Pseudomonas* sp. strain PED contained nicotinamide cofactor dependent (NADH) alcohol dehydrogenase activity, while no nicotinamide adenine dinucleotide phosphate (NADPH) activity was observed. A 4-L culture volume produces about 17 g of wet cells containing 710 units of the enzyme. The PED alcohol dehydrogenase was further purified 257-fold with a combination of ammonium sulfate precipitation, anion exchange, gel filtration, and affinity chromatography (β-NAD-agarose) to yield 5 mg of the enzyme. The enzyme has K_m values of 525 and 75 μM for NAD and 2-propanol, respectively. The specific activity

(3) (a) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162. (b) Keinan, E.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 3474. (c) Drueckhammer, D. G.; Sadozai, S. K.; Wong, C.-H.; Roberts, S. M. *Enzyme Microb. Technol.* **1987**, *9*, 564. (d) Drueckhammer, D. G.; Barbas, C. F.; Nozaki, K.; Wong, C.-H.; Wood, C. Y.; Ciufolini, M. A. *J. Org. Chem.* **1988**, *53*, 1607.

(4) (a) Chenault, H. K.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1987**, *14*, 147. (b) Lee, L. G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 6999. (c) Wong, C.-H.; Drueckhammer, D. G.; Sweers, H. M. *J. Am. Chem. Soc.* **1985**, *107*, 4028.

(5) (a) Shen, G.-J.; Wang, Y. F.; Bradshaw, C.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1990**, 677. (b) Bradshaw, C. W.; Shen, G.-J.; Wong, C.-H. *Bioorg. Chem.* In press. (c) *Pseudomonas* sp. strain SBD6 is available from ATCC (ATCC 49688).

(6) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119.

(7) (a) Hummel, W. *Appl. Microb. Biotechnol.* **1990**, *34*, 15. (b) Bradshaw, C. W.; Hummel, W.; Wong, C.-H. *J. Org. Chem.*, following paper in this issue.

(8) *Pseudomonas* sp. strain PED has been deposited in the American Type Culture Collection (ATCC).

(9) For detailed information on the isolation and characterization of *Pseudomonas* sp. strain PED and purification of PED alcohol dehydrogenase, see the supplementary material.

Table II. Relative Rates of Reduction of Aliphatic and Cyclic Ketones with PED Alcohol Dehydrogenase

compound	rel rate[a]	compound	rel rate[a]	compound	rel rate[a]
	100		8		6
	6		6		4
	5		7		2
	5		0		<0.1
	3		32		0
	2		336		6
	1		135		3.4
	11		9		0
	11		0		0
	5		0		0
	0		22		2
	5		0		0
	5		6		0
	<0.1		6		
	0		2		0

[a] Relative rates were determined as described in Table I.

is 36 U/mg with respect to 2-propanol under saturating NAD conditions.

The kinetic mechanism of purified PED alcohol dehydrogenase was established with initial velocity patterns and product inhibition studies.[10] Lineweaver–Burke plots of the initial velocity for the oxidation of 2-propanol at different constant concentrations of NAD were intersecting. Product inhibition experiments for the oxidation of 2-propanol revealed noncompetitive inhibition with acetone and competitive inhibition with NADH versus NAD. These two products are both noncompetitive inhibitors of 2-propanol. These results fulfill criteria for an ordered bi-bi mechanism[10] where the binding order is NAD followed by 2-propanol. The release of products is acetone and then the reduced cofactor. The amounts of substrates used in these experiments were from 0.2 to 2.5 of the K_m concentrations.

The substrate specificity was explored with aromatic ketones as well as aldehydes (Table I). The variability of the side-chain ring structure is evident as compounds with pyridine, furan, and phenyl rings are all substrates. Bulky side chains like indole, thiochromanone, or substituted phenyl rings seem detrimental to enzyme activity.

(10) Cleland, W. W. *Techniques of Chemistry* (Invest. Rates Mech React., 4th ed., Pt 1), Bernasconi, C. F., Ed.; Wiley: New York, 1986; pp 791–870. The kinetic mechanism of PED is represented as the following:

Scheme I

The position of the carbonyl group with respect to the aromatic ring is not fixed. 4-Phenyl-2-butanone is a good substrate as well as acetophenone. The versatility of this enzyme is further exemplified by the aliphatic and cyclic ketone substrates (Table II). *T. brockii* alcohol dehydrogenase and PADH generally require that one of the ketone side chains is a methyl group. Although substrates with one methyl side chain are typically better for PED alcohol dehydrogenase, this is an unnecessary requirement (Tables I and II). Under assay conditions, the rate of reaction for some substrates could be greatly enhanced by the addition of 0.25 M ammonium sulfate or 5% methanol. For the oxidation of 2-propanol, increases of 100% and 60% were seen with ammonium sulfate and methanol, respectively. Other salts or organic solvents tested did not induce this phenomena. The substrate specificity of PED alcohol dehydrogenase overlaps with PADH and *T. brockii*. PED alcohol dehydrogenase accepts a wide range of functional groups attached to the aliphatic ketones. Of interest is the ability of the enzyme to reduce terminal alkynyl ketones, albeit at a slow rate. The alkynyl ketones were synthesized according to Scheme I.[11]

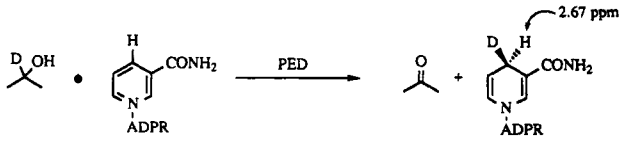
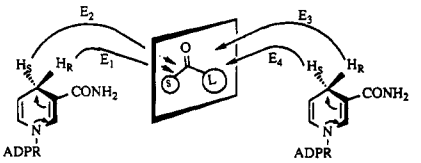


Figure 1. PED alcohol dehydrogenase catalyzed reduction of NAD. ADPR, adenosine diphosphate ribose.



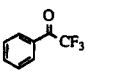
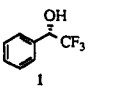
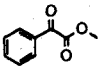
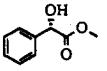
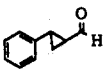
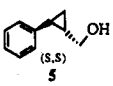
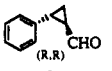
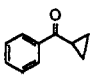
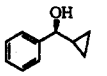
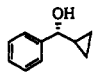
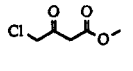
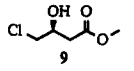
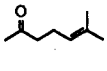
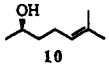
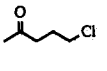
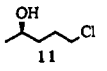
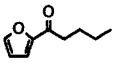
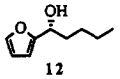
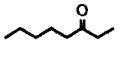
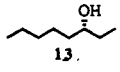
E_1: pro-R/*si* face, *Pseudomonas sp.* PED and PADH, *Lactobacillus kefir* (see ref 7) alcohol dehydrogenase
E_2: pro-S/*si* face, *Mucor javanicus* alcohol dehydrogenase
E_3: pro-R/*re* face, yeast, horse liver and *Thermoanaerobium brockii* alcohol dehydrogenases
E_4: pro-S/*re* face, unknown

Figure 2. Stereospecificity of alcohol dehydrogenases where s is a small group and L is a large group.

The stereochemical outcome of alcohol dehydrogenase catalyzed reactions has been described by Prelog's rule.[6] The synthetically useful and commercially available alcohol dehydrogenases follow this rule with few exceptions.[12] However, the purified PED alcohol dehydrogenase transfers the hydride to the carbonyl group in anti-Prelog fashion to give (*R*) alcohols. The stereochemistry with respect to the cofactor was determined by the enzyme-catalyzed transfer of deuteride from 2-propanol-d_8 to NAD followed by NMR analysis as illustrated in Figure 1. The diastereotopic protons at C4 of NADH differ by 0.1 ppm, 2.77 ppm for the *pro-R* hydrogen, and 2.67 ppm for the *pro-S* hydrogen. Therefore, the deuterium-labeled NAD(D)H will show a single peak representative of the stereochemistry of hydride transfer with respect to the cofactor.[13] PED alcohol dehydrogenase transfers the deuteride (and analogously the hydride) to and from the *re* face of the cofactor as determined by a single NMR peak at 2.67 ppm. The stereochemistry of the nicotinamide cofactor with horse liver alcohol dehydrogenase,[14] yeast alcohol dehydrogenase,[14] *T. brockii* alcohol dehydrogenase,[5b] *Lactobacillus kefir* alcohol dehydrogenase,[7b] and *Pseudomonas* sp. strain SBD6 alcohol dehydrogenase (PADH)[5] as determined in a comparable manner with the appropriate deuterated alcohol were the same, but opposite that of glucose dehydrogenase (*Bacillus*).[14] The overall stereochemical outcome of reactions catalyzed by PED alcohol dehydrogenase as well as PADH is thus unique as illustrated in Figure 2.

PED alcohol dehydrogenase reactions were run with a number of representative substrates (Table III). Aliphatic and aromatic (*R*) alcohols are formed in good enantiomeric excess. The carbonyl groups of 1-phenyl-1,2-propanedione (**3**) were reduced in a 6:1 ratio in favor of the carbonyl group proximal to the phenyl ring. Good enantioselectivity is seen when the carbonyl is flanked by a methyl group (**2**) or bulkier groups (**4** and **9**). PED alcohol dehydrogenase catalyzed reduction of the *trans*-2-phenylcyclopropanecarboxaldehyde (**5**) discriminates fairly well between the two enantiomers. In all cases, the reductions proceed in high enantiomeric excess except for 3-octanone, where the enzyme does not distinguish between the ethyl and pentyl side chains efficiently.

Table III. Products Prepared from PED Alcohol Dehydrogenase Catalyzed Reactions

substrate	product[a]	% enantiomeric excess	% yield[e]
	 **1**	92[c]	37
	 **2**	94[b]	34
	 **3**	86[b]	83
	 **4**	98[b,d]	79
	 **5**  **6**	65[c] (*S,S*)	36
	 **7**  **8**	92[b]	41
	 **9**	98[b,d]	76
	 **10**	97[b,d]	51
	 **11**	93[b]	48
	 **12**	45[b]	5-10
	 **13**	27[b,d]	43

[a] Absolute configuration was determined by comparison of optical rotation with literature values, see Experimental Section. [b] Enantiomeric excess was determined by conversion of the alcohol to an MTPA ester and analyzed by NMR.[12] [c] Enantiomeric excess was determined by HPLC analysis of a Chiralcel OB column with hexane/2-propanol, see Experimental Section for details. [d] Enantiomeric excess determined by comparison of optical rotation versus literature values. [e] Not optimized. Higher yields can be obtained with more enzyme or longer reaction times.

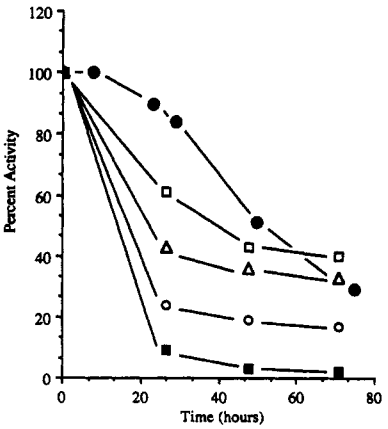
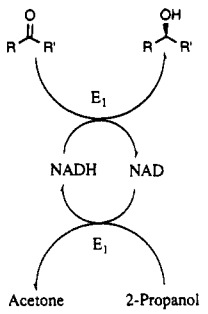


Figure 3. Stability of PED alcohol dehydrogenase at 25 °C (50 mM phosphate buffer, pH 7.1) in the presence of 2-propanol: (●) 0% propanol, (□) 5% propanol, (△) 10% propanol, (○) 15% propanol, (■) 20% propanol.

(11) Walton, D. R. M.; Waugh, F. *J. Organomet. Chem.* **1972**, *37*, 45.
(12) Alcohol dehydrogenases which produce (*R*) alcohols have been discovered but not developed synthetically: (a) Alleman, R. K.; Hung, R.; Benner, S. A. *J. Am. Chem. Soc.* **1988**, *110*, 5555. (b) Hou, C. T.; Patel, R.; Barnabe, H.; Marczak, N. *Eur. J. Biochem.* **1981**, *119*, 359.
(13) Arnold, L. J.; You, K.; Allison, W. S.; Kaplan, N. O. *Bichemistry* **1976**, *15*, 4844.
(14) You, K. *Crit. Rev. Biochem.* **1984**, *17*, 313.

Scheme II.[a]



[a] E_1, PED alcohol dehydrogenase.

For synthesis, the enzyme was prepared as lyophilized crude cell extract and used in soluble form. This enzyme has excellent stability under reaction conditions, obviating the need for immobilization. The enzyme lost no activity after 7 days for the reduction of 3-octanone. The minimum stability of the enzyme was seen with the reduction of trifluoroacetophenone, where PED alcohol dehydrogenase had a half-life of approximately 35 h. Inclusion of 2-propanol in the reaction serves two purposes. Low concentrations of the organic solvent provide long-term stability of PED alcohol dehydrogenase (Figure 3). In addition, PED alcohol dehydrogenase is particularly amenable to synthetic reactions by virtue of cofactor regeneration with inclusion of 2-propanol as cosolvent. In most cases the excess 2-propanol or product acetone does not interfere with product isolation. An important consideration with cofactor-requiring reactions is the cost of cofactor and the concommitant regeneration. This alcohol dehydrogenase constitutes a good example of a one-enzyme-catalyzed reaction, where a single enzyme is responsible for a desired reaction and cofactor regeneration (Scheme II).[15] Similarly, PED alcohol dehydrogenase could also serve to regenerate NADH in two-enzyme systems.

Experimental Section

Materials and Methods. All chemicals are from Aldrich, Fisher, or Sigma unless noted otherwise. NMR spectra were recorded on a 300-MHz spectrometer. (−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride is from Fluka. For the determination of enantiomeric excess, the alcohols were converted to (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters (MTPA esters) and analyzed by NMR spectroscopy,[16] by HPLC analysis on a Daicel Chiralcel OB column, or by comparison of the optical rotations versus known compounds. The optical rotations were determined with 10-cm path-length cells.

Enzyme Assays. *Pseudomonas* sp. strain PED assays were done by combining appropriate aliquots of the following solutions with purified PED alcohol dehydrogenase and monitoring at 340 nm (ϵ_{NADH} 6.22 L mol^{-1} cm^{-1}): 50 mM TRIS buffer pH 8.5, 5 mM NAD, and 10 mM of an appropriate alcohol, or, in the other direction, 50 mM TRIS buffer pH 7.1, 0.45 mM NADH, and 10 mM of an appropriate ketone. Initial rate data were recorded and fitted to the Michaelis Menten equation to determine the kinetic constants. Product inhibition data were used to determine the kinetic mechanism. For substrate specificity studies, 5% (v/v) of DMF was included to aid in the solubility of substrates. In each substrate specificity assay, the concentration of ketone was 10 mM with 0.45 mM of NADH.

Stereochemistry of PED Alcohol Dehydrogenase. The following were combined and stirred at room temperature: 1 mL of 2-propanol-d_8, 150 mg of NAD, and 45 mL of 100 mM ammonium bicarbonate buffer, pH 8. Purified PED alcohol dehydrogenase (20 units) was enclosed in a dialysis bag and placed in the solution. After 2 days, the reaction was 80% complete. The dialysis bag was removed and rinsed with distilled water. The combined aqueous layers were lyophilized. The white powder was applied to a DEAE cellulose column (2 × 16 cm) previously equilibrated with 25 mM ammonium bicarbonate, pH 8. NAD was eluted with 50 mM ammonium bicarbonate, and the reduced cofactor was eluted with 250 mM ammonium bicarbonate buffer, both pH 8. The NADH fractions were combined and lyophilized. ^{1}H NMR (D_2O) 2.67 ppm (s, 1 H).

Enzyme Stability under Reaction Conditions. PED alcohol dehydrogenase solutions as prepared for synthesis in 50 mM phosphate buffer, pH 7.1 (total volume 1 mL), containing various amounts of 2-propanol were stirred at room temperature. Portions of this solution (50 μL) were removed periodically and assayed as described above with 2 mM NAD, 4 mM 2-propanol, in 50 mM TRIS buffer, pH 7.1 with 0.5 M ammonium sulfate.

PED Alcohol Dehydrogenase Catalyzed Reduction of Ketones. The enzyme was prepared by suspending the wet cells in 50 mM phosphate buffer, pH 7.5 (1 g wet cells/5 mL buffer), breaking in an Amicon SLM French press (23 000 psi), and centrifuging at 15 000 rpm for 75 min. The supernatant was lyophilized and used as the source of enzyme. In a typical procedure, the carbonyl compound (5 mmol) was stirred at room temperature with 50 mg of NAD, 4 mL of 2-propanol, 75 mL of 50 mM phosphate buffer pH 7.1, 1.0 g of enzyme preparation, and 25 mL of hexane. The pH of the reaction was maintained constant by addition of 1 N NaOH. After the reaction stopped as determined by lack of further product formation, the product was isolated by removal of the hexane layer and extraction of the aqueous layer with ethyl ether (3 × 75 mL). The combined organic layers were dried over sodium sulfate and evaporated and the residue was purified on silica gel (hexane/ethyl ether).

(*S*)-1-Phenyl-2,2,2-trifluoroethanol (1): 37% yield; 92% ee as determined by HPLC on a Chiralcel OB column 98:2 hexane/2-propanol; with a flow rate of 1 mL/min the retention times were 10.42 min for (−)(*R*) and 11.26 min for (+)(S); ^{1}H NMR ($CDCl_3$) δ 3.15 (s, 1 H), 4.95 (q, 1 H), 7.40 (m, 5 H). Spectroscopic properties were the same as determined previously.[17] $[\alpha]^{23}_D$ +99° (*c* = 2.1, $CHCl_3$) (literature value $[\alpha]_D$ +8.6° (*c* = 4.25, benzene), 8% ee).[17]

(*R*)-1-Phenylethanol (2): 34% yield; >97% ee as determined by conversion a MTPA ester and comparison of the methyl group integrations, δ 1.58 and 1.65 for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{20}_D$ +50.3° (*c* = 6.7, $CDCl_3$); ^{1}H NMR $CDCl_3$ δ 1.47 (d, 3 H), 2.25 (s, 1 H), 4.79 (quartet, 1 H), 7.28 (m, 5 H). ^{1}H NMR was identical to the commercially available racemic compound. Absolute configuration was determined by literature assignments of optical rotation ($[\alpha]^{25}_D$ +42.9° (*c* = 3.36, $CHCl_3$), 79% ee).[18]

(*S*)-1-Hydroxy-1-phenyl-2-propanone (3): 83% yield; 86% ee as determined by conversion to a MTPA ester and comparison of the methyl peaks, δ 2.12 and 2.19 for the (*S*) and (*R*) isomers, respectively; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H), 5.11 (s, 1 H), 7.37 (m, 5 H). ^{1}H NMR was the same as reported previously and showed a 6:1 ratio for the two regioisomers (1-hydroxy-1-phenyl-2-propanone/2-hydroxy-1-phenyl-1-propanone).[19a] Absolute configuration was determined by comparison of literature assignments of optical rotation for the (*R*) enantiomer ($[\alpha]^{20}_D$ −380° (*c* = 1, $CHCl_3$), >95% ee).[19b]

(*S*)-Methyl mandelate (4): 79% yield; >97% ee as determined by comparison of the optical rotation, $[\alpha]^{25}_D$ +173° (*c* = 2.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3 H), 5.20 (d, 1 H), 7.44 (m, 5 H). ^{1}H NMR was the same as the commercially available sample. Absolute stereochemistry was assigned on the basis of the literature values of optical rotation ($[\alpha]_D$ +99.1° (*c* = 2.25, $CHCl_3$), 59% ee).[18]

(*S,S*)-(2-Phenylcyclopropyl)methanol (5): 36% yield; 65% enantiomeric excess as determined by HPLC on Chiralcel OB

(15) Two other enzymes have been used synthetically in such a manner, see refs 3 and 5.

(16) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

(17) Peters, H. M.; Fiegl, D. M.; Mosher, H. S. *J. Org. Chem.* **1968**, *33*, 4245.

(18) Ziffer, H.; Kawai, K.; Kasui, M.; Imuta, M.; Froussios, C. *J. Org. Chem.* **1983**, *48*, 3017.

(19) (a) Davis, F. A.; Sheppard, A. C.; Lal, G. S. *Tetrahedron Lett.* **1989**, *30*, 779. (b) Fuganti, C.; Graselli, P.; Poli, G.; Servi, S.; Zorzella, A. *J. Chem. Soc., Chem. Commun.* **1988**, 1619.

column 95:5 hexane/2-propanol; With a flow rate of 1 mL/min the retention times were 9.68 min for (-)(*R,R*) and 10.88 min for (+)(*S,S*); ^{1}H NMR ($CDCl_3$) δ 0.7–2.0 (m, 4 H), 2.8 (s, 1 H), 3.5 (d, 2 H), 7.1 (m, 5 H). ^{1}H NMR was identical to the previously reported data.[20] Absolute stereochemistries of the all-trans products were determined by comparison of optical rotation versus known compounds ($[\alpha]_D$ -84° (c = 28 mg/mL, CCl_4) for the (*R,R*) isomer).[20]

(*R*)-Phenylcyclopropylmethanol (8): 41% yield; 92% ee as determined by conversion to a MTPA ester and comparison of the methoxy peaks, δ 3.55 and 3.61, or the methine proton, δ 5.46 and 5.38, for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{25}_D$ +27.99° (c = 1.072, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.25–7.44 (m, 5 H), 3.98–4.00 (d, 1 H), 1.19–1.29 (m, 1 H), 0.37–0.65 (m, 4 H). ^{1}H NMR is consistent with commercially available compound. Absolutes stereochemistry is assigned on the basis of correlation of the stereospecificity of the enzyme with other substrates.

(*S*)-Methyl 4-chloro-3-hydroxybutanoate (9): 76% yield; >98% ee and absolute stereochemistry were determined by comparison of the optical rotation ($[\alpha]^{23}_D$ -11.7° (c = 5.75, $CHCl_3$), 55% ee for the *S* isomer);[21] $[\alpha]^{25}_D$ -21.64° (c = 0.67, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.65 (dd, 2 H), 3.62 (dd, 2 H), 3.74 (s, 3 H), 4.28 (m, 1 H).

(*R*)-6-Methyl-5-hepten-2-ol (10): 51% yield; >97% ee as determined by conversion to a MTPA ester and comparison of the methyl peaks, δ 1.20 and 1.27 for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{23}_D$ -14° (c = 1.66, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 3 H), 1.52 (m, 2 H), 1.65 (s, 3 H), 1.71 (s, 3 H), 2.08 (m, 2 H), 3.83 (m, 1 H), 5.15 (t, 1 H). ^{1}H NMR is consistent with the commercially available compound. Absolute configuration was assigned by comparison of the optical rotation for the (*S*) enantiomer ($[\alpha]_D$ +10.76° ($CHCl_3$), 99% ee).[3a]

(*R*)-5-Chloro-2-pentanol (11): 48% yield; 93% ee as determined by optical rotation and conversion to a MTPA ester and comparison of the methyl peaks, δ 1.28 and 1.36 for the (*S*) and (*R*) isomers, respectively, $[\alpha]^{23}_D$ -14.9° (c = 0.19, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.23 (d, 3 H), 1.62 (m, 2 H), 1.88 (m, 2 H), 3.59 (t, 2 H), 3.86 (m, 1 H). Absolute configuration was assigned on the basis of literature values of optical rotation of the (*S*) enantiomer ($[\alpha]_D$ +15.58° ($CHCl_3$), 98% ee).[3b]

(*R*)-α-Butyl-2-furanmethanol (12): 5–10% yield; 45% ee as determined by conversion to a MTPA ester and comparison of the methoxy peaks, δ 3.46 and 3.53 for the (*S*) and (*R*) isomers, respectively; ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 3 H), 1.34 (m, 4 H), 1.86 (m, 2 H), 4.68 (t, 1 H), 6.24 (d, 1 H), 6.31 (m, 1 H), 7.36 (d, 1 H). ^{1}H NMR is consistent with previously reported data.[22a] Absolute streochemistry was assigned in accord with published values of optical rotation ($[\alpha]_D$ +9.2° (c = 1.07, $CHCl_3$), 94% ee).[22b]

(*R*)-3-Octanol (13): 43% yield; 27% ee as determined by comparison of optical rotation, $[\alpha]^{20}_D$ -3.5° (c = 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 6 H), 1.22 and 1.38 (m, 10 H), 3.48 (m, 1 H). ^{1}H NMR is consistent with commercially available compound. Absolute configuration was determined by comparison of literature values of optical rotation ($[\alpha]^{25}_D$ +7.3° (c = 6, ether), 57% ee for the (*S*) enantiomer).[23]

Synthesis of 2-Phenylcyclopropanecarboxaldehyde. Potassium hydroxide (152 g, 272 mmol) was dissolved in 400 mL of water at 0 °C. The reaction was layered with 400 mL of ethyl ether and 20 g (136 mmol) of 1-methyl-3-nitro-1-nitrosoguanidine was added and stirred until evolution of gas ceased (25 min). The ether layer was removed and the aqueous layer washed with 1 × 150 mL of ether. The fresh yellow CH_2N_2 solution was added in small portions to 20 g of 1-carboxy-2-phenylcyclopropane in 200 mL of dry ether until the yellow color stayed for a few minutes. The solvent was evaporated and the methyl ester purified by silica gel chromatography, 1:2 hexane/ethyl ether to yield 18.5 g (105 mmol), 85%. The methyl ester (4.0 g, 23 mmol) in 100 mL of anhydrous ether was cooled in a liquid nitrogen/methanol/ethyl ether bath in a three-neck flask; then 57 mL (57 mmol) of 1 M diisobutylaluminum hydride in hexane was added dropwise over 4 h, not allowing the temperature inside the flask to reach above -100 °C. Water (7 mL) was added to quench the reaction immediately followed by Rochelles salt and the mixture was allowed to warm to room temperature. The reaction was extracted with 3 × 125 mL of ethyl ether. The dried ether extracts were evaporated and chromatographed on silica gel (1:1 hexane/ethyl ether) to yield 2.69 g (80%): ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 1 H), 1.66 (m, 1 H), 2.12 (m, 1 H), 2.58 (m, 1 H), 7.20 (m, 5 H), 9.24 (m, 1 H). ^{1}H NMR are consistent with literature values.[20b]

Synthesis of Terminal Alkynyl Ketones. General Procedure. The following were combined in a dry flask under nitrogen at 0 °C: 23 mmol of bis(trimethylsilyl)acetylene, 23 mmol of an acid chloride, and 80 mL of dichloromethane. Aluminum trichloride (3.07 g) was added over 35 min under nitrogen at 0 °C. The reaction was stirred overnight and allowed to warm to room temperature. The excess aluminum trichloride was destroyed by addition of 100 mL of 1 N HCl followed by extraction with 3 × 100 mL of ethyl ether. The dried organic layers (Na_2SO_4) were evaporated and the residue was purified by vacuum distillation or silica gel chromatography (1:9 ethyl ether/hexane). The trimethylsilyl group was removed by adding 15 mL of 0.1 M borax to a solution of the ketone (1 g) in 80 mL of methanol. The solution was allowed to sit at room temperature for 15 min and the reaction was quenched with 75 mL of ice-cold 1 N HCl. The dried ethyl ether extractions, 3 × 75 mL (Na_2SO_4), were evaporated to dryness. Purification was accomplished by either vacuum distillation or silica gel chromatography (1:4 ethyl ether/hexane).

1-Butyn-3-one: 66% yield; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3 H), 3.27 (s, 1 H). ^{1}H NMR consistent with commercial sample.

1-Hexyn-3-one: 69% yield; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3 H), 1.70 (m, 2 H), 2.54 (t, 2 H), 3.23 (s, 1 H). ^{1}H NMR are consistent with literature values.[24]

Methyl 4-oxo-5-hexynoate: 38% yield; ^{1}H NMR ($CDCl_3$) δ 2.64 (t, 2 H), 2.91 (t, 2 H), 3.28 (s, 1 H), 3.68 (s, 3 H). ^{1}H NMR data are the same as reported previously.[25]

Registry No. 1, 340-06-7; 2, 1517-69-7; 3, 53439-91-1; 4, 21210-43-5; 5, 110659-58-0; 6, 10488-04-7; 7, 110548-56-6; 8, 110548-55-5; 9, 86728-93-0; 10, 58917-27-4; 11, 76188-95-9; 12, 120409-96-3; 13, 70492-66-9; NAD, 53-84-9; 2,2,2-trifluoroacetophenone, 434-45-7; acetophenone, 98-86-2; 1-phenyl-1,3-propanedione, 579-07-7; methyl 2-oxo-2-phenylethanoate, 15206-55-0; *trans*-2-phenylcyclopropanecarboxaldehyde, 34271-31-3; 2-propanol, 67-63-0; methyl 4-chloro-3-oxobutanoate, 32807-28-6; 6-methyl-5-hepten-2-one, 110-93-0; 5-chloro-2-pentanone, 5891-21-4; 2-(1-pentanoyl)furan, 3194-17-0; 3-octanone, 106-68-3; 2-acetylpyridine, 1122-62-9; 1-propanoylbenzene, 93-55-0; (4-chloro-1-butanoyl)benzene, 939-52-6; [3-(dimethylamino)-1-propanoyl]benzene, 3506-36-3; (2,2-diethoxyacetyl)benzene, 6175-45-7; 4-(2-chloroacetyl)-2-hydroxyphenol, 99-40-1; 4-(4-chloro-1-butanoyl)phenol, 7150-55-2; 1-(4-chloro-1-butanoyl)-4-fluorobenzene, 3874-54-2; 2-(4-chloro-1-butanoyl)thiophene, 43076-59-1; 2-(3-oxo-4,4,3-trifluoro-1-buanoyl)thiophene, 326-91-0; 2-acetylfuran, 1192-62-7; 2-acetyl-3,4-dimethoxyfuran, 113452-64-5; benzocycloheptan-1-one, 826-73-3; [(2-oxopropyl)oxy]benzene, 621-87-4; (3-oxobutyl)benzene, 2550-26-7; (3-oxo-1-butynyl)-benzene, 1817-57-8; 6-acetylbenzo-1,4-dioxane, 2879-20-1; 3-acetylindole, 703-80-0; (3-oxo-1-butenyl)benzene, 122-57-6; 2-acetyl-1,2,3,4-tetrahydro-2-naphthalenone, 17216-08-9; thiochroman-4-one, 3528-17-4; 2-butanone, 78-93-3; 2-pentanone, 107-87-9; 2-hexanone, 591-78-6; 2-heptanone, 110-43-0; 2-octanone, 111-13-7; 3-oxo-2-butanol, 513-86-0; 3-butyn-2-one, 1423-60-5; 3-chloro-2-butanone, 4091-39-8; acetylcyclopropane, 765-43-5; 3-(methylthio)-2-butanone, 53475-15-3; 2-methyl-3-oxo-1-butanol, 3393-64-4; 1,1-dimethoxyethanone, 6342-56-9; 3-penten-2-one, 3102-33-8; 4-methoxy-3-buten-2-one, 4652-27-1; 5-hexen-2-one,

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109-49-9; 5-methyl-4-hexen-2-one, 28332-44-7; 4-methoxy-3-penten-2-one, 2845-83-2; 2,4-pentanedione, 123-54-6; 3-chloro-2,4-pentanedione, 1694-29-7; methyl 3-oxobutanoate, 105-45-3; 2,5-hexanedione, 110-13-4; 4-oxopentanoic acid, 123-76-2; 2-oxopentanoic acid, 13088-48-7; 2-(methoxycarbonyl)cyclopentanone, 10472-24-9; 2-methyl-1,3-cyclopentanedione, 765-69-5; ethyl 3-oxopentanoate, 4949-44-4; 1-octen-3-one, 4312-99-6; 1,3-dichloro-2-propanone, 534-07-6; 1-chloro-3-butyn-2-one, 138261-29-7; methyl 4-oxo-5-hexynoate, 118622-32-5; 1-hexyn-3-one, 689-00-9; *N*-benzyl-4-piperidinone, 3612-20-2; *trans*-2-isopropyl-5-methylcyclohexanone, 89-80-5; 2-cyclohexenone, 930-68-7; 3-methyl-2-cyclohexenone, 1193-18-6; 3-chloro-2-norbornanone, 61914-03-2; 2-acetyl-5-norbornene, 5063-03-6; 5-norbornen-2-one, 694-98-4; methyl 2-phenylcyclopropanecarboxylate, 20030-70-0; bis(trimethylsilyl)acetylene, 14630-40-1; acetyl chloride, 75-36-5; 3-carbomethoxypropionyl chloride, 1490-25-1; butanoyl chloride, 141-75-3; alcohol dehydrogenase, 9031-72-5; cyclopropyl phenyl ketone, 3481-02-5.

Supplementary Material Available: Detailed information on the isolation and characterization of the *Pseudomonas* sp. strain PED as well as the purification of PED alcohol dehydrogenase and ^{1}H NMR spectra of (*S*)-1-phenyl-2,2,2-trifluoroethanol, (*R*)-1-phenylethanol, (*S*)-1-hydroxy-1-phenyl-2-propanone, (*S*)-methyl mandelate, (*S,S*)-(2-phenylcyclopropyl)methanol, (*R*)-phenylcyclopropylmethanol, (*S*)-methyl 4-chloro-3-hydroxybutanoate, (*R*)-6-methyl-5-hepten-2-ol, (*R*)-5-chloro-2-pentanol, (*R*)-3-octanol, and 2-phenylcyclopropanecarboxaldehyde (14 pages). Ordering information is given on any current masthead page.

Lactobacillus kefir Alcohol Dehydrogenase: A Useful Catalyst for Synthesis

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Received September 17, 1991

The alcohol dehydrogenase from *Lactobacillus kefir* simultaneously catalyzes carbonyl reductions and NADPH regeneration in the presence of 2-propanol. Representative synthesis of a number of chiral alcohols was accomplished in good yield and high enantiomeric excess (94–99%). This NADPH-requiring enzyme transfers the *pro-R* hydride from the cofactor to the *si* face of carbonyls to give (*R*) alcohols. The enzyme exhibits a very broad substrate specificity and high enantioselectivity for the synthesis of chiral aromatic, cyclic, polycyclic, and aliphatic alcohols.

Introduction

Enzymatic regeneration of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for synthesis has been limited primarily to the glucose/glucose dehydrogenase,[1a] glucose-6-phosphate/glucose-6-phosphate dehydrogenase,[1b] and 2-propanol/*Thermoanaerobium brockii* alcohol dehydrogenase[1c] systems. Glucose dehydrogenase and glucose-6-phosphate dehydrogenase in some instances complicates product isolation due to contamination by the substrate or byproduct from the regeneration system. An improvement in the regeneration technology is through the use of single-enzyme systems where one enzyme can catalyze a desired reaction while simultaneously regenerating the cofactor. Single-enzyme systems based on *T. brockii* alcohol dehydrogenase and the recently discovered *Lactobacillus kefir* alcohol dehydrogenase have been used advantageously in this manner (Figure 1).[2] These two enzymes may also be effectively utilized for the regeneration of NADPH in multiple-enzyme systems. Single-enzyme systems with regeneration of NADH have also been reported from two different *Pseudomonas* sp.[3]

The synthetically useful alcohol dehydrogenases, including that from yeast,[4] horse liver,[4] and *T. brockii*[3b] transfer the *pro-R* hydride to the *re* face of the carbonyl to give (*S*) alcohols, a process described by Prelog's rule.[5] In contrast, *L. kefir* alcohol dehydrogenase and the two the alcohol dehydrogenases from *Pseudomonas* sp.[3] exhibit anti-Prelog specificity, both transferring the *pro-R* hydride to form (*R*) alcohols. In addition to the interesting stereochemistry, *L. kefir* alcohol dehydrogenase has very broad substrate specificity encompassing cyclic, aromatic, and aliphatic ketones. We present here the overall stereochemistry of *L. kefir* alcohol dehydrogenase catalyzed reactions and the synthetic utility of the enzyme. A number of substrates were reduced on a laboratory scale, all in high enantiomeric excess (94–99%) and in good yield.

Results and Discussion

Unlike the commercially available alcohol dehydrogenases which generally do not accept bulky side chains,[1c,6] *L. kefir* alcohol dehydrogenase exhibits a very broad substrate specificity (Table I). It accepts a wide range of aromatic, cyclic, and aliphatic ketones. Some limitations are observed as shown in Table I. For aromatic

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