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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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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(1) (a) Wong, C.-H.; Drueckhammer, D. G.; Sweers, H. M. *J. Am. Chem. Soc.* **1985**, *107*, 4028. (B) Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890. (c) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 167. (d) For a general review, see: Chenault, H. K.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1987**, *14*, 147.
(2) (a) Keinan, E.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 3474. (b) Hummel, W. *Appl. Microbiol. Biotechnol.* **1990**, *34*, 15. (c) Hummel, W. *Biotechnol. Lett.* **1990**, *12*, 403.
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(5) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119.
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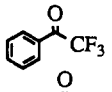
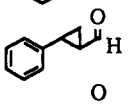
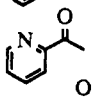
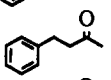
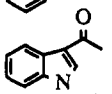
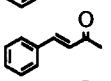
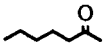
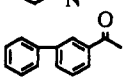
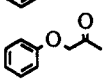
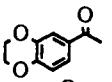
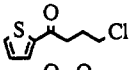
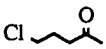
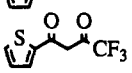
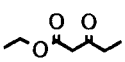
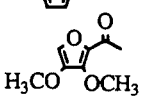
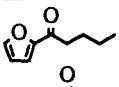
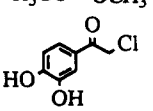
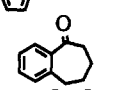
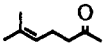
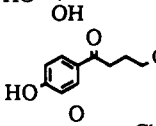
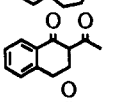
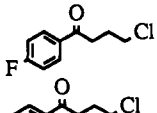
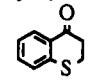
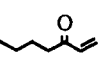
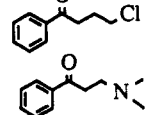
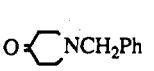
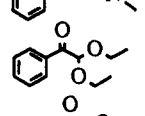
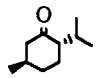
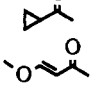
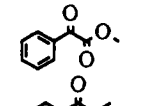
Table I. Relative Rate of *L. kefir* Alcohol Dehydrogenase Catalyzed Reductions of Ketones

compound	rel rate[a]	compound	rel rate[a]	compound	rel rate[a]
	6.5		vs		3.6
	7.6		3.2		0
	9.3		23		73
	2.4		0.2		57
	4.2		100		53
	0		1.4		67
	1.8		0		16.7
	0		0		74
	0		0.43		75
	0		0		82
	vs		0		2.6
	1.1		8.2		5.9
	vs		0.5		0
	vs		3.8		3.5
	0.9		50		0.4
	19.6		25		0.9
					3.7
					1.9
					52

[a] Relative rates were determined as described in the Experimental Section.

Figure 1. Enzyme-catalyzed reactions and cofactor regeneration. E_1 = *L. kefir* alcohol dehydrogenase or E_1 = *T. brockii* alcohol dehydrogenase.

ketones, substitution of the aromatic ring as tested is deleterious to enzyme activity, with two notable exceptions, namely, 3-phenylacetophenone and 3-acetylindole. Other alcohol dehydrogenases such as *T. brockii*[1c] and *Pseudomonas* sp. strain SBD6 (PADH)[3] work best if one side chain is a methyl group. However, *L. kefir* alcohol dehydrogenase accepts a large variety of side chains of differing sizes, including cyclic compounds. Particularly interesting is the ability to reduce the easily prepared[7] trimethylsilyl-protected terminal alkyne ketones. To our knowledge, there are no reports on enzymatic reduction of these types of compounds. Indeed, enzymatic reductions of organometallic compounds are rare.[8] The chiral α,β-alkynyl alcohols have proven very versatile, for example, in the synthesis of hydroxyethylene dipeptide isosteres.[9]

Under substrate specificity assay conditions, an increase in rate was observed for several substrates by inclusion of 10 mM 2-propanol. A typical increase of 15% was seen

(7) Walton, D. R. M.; Waugh, F. *J. Organomet. Chem.* **1972**, *37*, 45.
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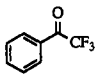
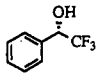
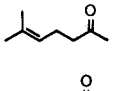
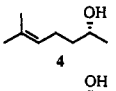
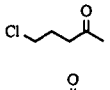
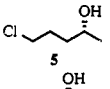
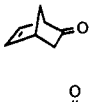
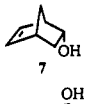
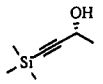
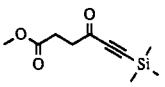
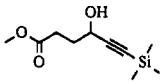
(9) Herold, P.; Duthaler, R.; Rihs, G.; Angst, C. *J. Org. Chem.* **1989**, *54*, 1178.

Table II. *L. kefir* Catalyzed Reductions

starting material	product[a]	% yield[b]	% enantiomeric excess
	 1	71	>99[c]
	 2	60	>97[d]
	 3	65	95[d]
	 4	58	>99[d,e]
	 5	52	>97[d]
	 6	46	>97[d]
	 7	39	>97[f]
	 8	25	94[d]
	 9	15	97[d]

[a] Absolute stereochemistry was determined by comparison of the optical rotation versus literature values. [b] Not optimized. Higher yields can be obtained with more enzyme or longer reaction time. [c] Enantiomeric excess was determined by HPLC analysis on Chiralcel OB column. [d] Enantiomeric excess determined by conversion to a MTPA ester. [e] Enantiomeric excess determined by comparison of optical rotation. [f] Enantiomeric excess determined by NMR analysis of the endo/exo ratio.

for acetophenone reduction. Therefore, 2-propanol accomplishes several tasks for synthesis. First, it increases the rate of reduction. Second, by acting as a cosolvent, 2-propanol can aid in the solubility of some substrates. Third, by regenerating NADPH, 2-propanol can also force the reaction to completion.

The synthetic-scale reductions of several compounds was undertaken in order to illustrate the usefulness of *L. kefir* alcohol dehydrogenase. The results are summarized in Table II. All products are formed in excellent enantiomeric excess. The enzyme was used either as the crude cell extract or partiallly purified form. The reactions were run up to 10-mmol scale. A larger scale synthesis is feasible as *L. kefir* alcohol dehydrogenase has already been shown to be amenable to large-scale synthesis in a membrane reactor.[2c]

The stereochemistry with respect to the cofactor was determined by incubating *L. kefir* alcohol dehydrogenase, NADP, and 2-propanol-d_8. The stoichiometric enzymatic transfer of the deuteride to the cofactor is monitored by NMR. The diastereotopic protons of NADPH at C4 differ by 0.1 ppm, 2.67 ppm for the *pro-S* hydrogen, and 2.77 ppm for the *pro-R* hydrogen.[10] Thus, by observing the presence of a peak at 2.67 ppm after transfer of the deuteride to the cofactor, we conclude that the enzyme

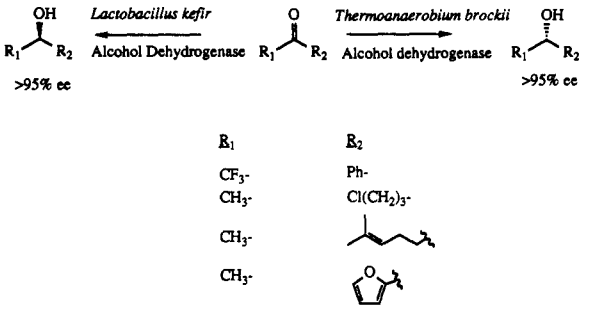


Figure 2. Alcohol dehydrogenases that are enantiocomplementary.

transfers the *pro-R* hydride of NADPH to the *si* face of carbonyls to give (*R*) alcohols. The overall stereochemistry is different from that of many known alcohol dehydrogenases.[3c] This enzyme is therefore enantiocomplementary to the alcohol dehydrogenase from *T. brockii* and horse liver (Figure 2).

The unusual stereospecificity, broad substrate specificity, and cofactor regenerative ability make *L. kefir* alcohol dehydrogenase a useful synthetic catalyst. Work is in progress to further exploit its synthetic utility.

Experimental Section

Materials and Methods. *L. kefir* is available from American Type Culture Collection (ATCC 34511) and was grown as recommended by ATCC or as described previously.[2b] Lactobacillus MRS broth is available from Fisher. (−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA) is available from Fluka. All other compounds were from Aldrich or Sigma unless stated otherwise. NMR spectra were recorded on a 300-MHz spectrometer. High resolution mass spectra were done by the in house facility at Scripps Research Institute. Enantiomeric excess was determined by HPLC analysis on a Daicel Chiralcel OB column or by NMR analysis of the (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters.[11]

Enzyme Assays. The change in absorbance of NADPH was monitored at 340 nm (ϵ_{NADPH} 622 M^{-1} mm^{-1}) after addition of purified enzyme to a cuvette containing 0.4 mM NADPH and 10 mM of a ketone in 50 mM TRIS buffer, pH 7.1, with 5% DMF. Relative rates were determined by arbitrarily setting the rate of reduction for 1-phenoxy-2-propanone to be 100.

***L. kefir* Alcohol Dehydrogenase Catalyzed Reactions. General Procedure.** For synthetic reactions, the wet cells (previously stored at −80 °C) were suspended in 0.1 M phosphate buffer, pH 8.5, containing 5 mM dithiothreitol (1 g wet cells/3 mL buffer), added to an equal volume of 0.1-mm glass beads, and disintegrated 3 × 3 min at 0 °C in a bead beater. The total volume was typically 50 mL. Cell debris was removed by centrifugation for 25 min at 15 000 rpm. A portion of the supernatant (8 mL) was added to a flask containing 2.5 mmol of a ketone substrate, 15 mg of NADPH, and 1 mL of 2-propanol in 8 mL of 50 mM phosphate 2 mM magnesium chloride buffer, pH 7.1. When problems with subtrate solubility were encountered, the reaction was layered with 10 mL of hexane. After the reaction was complete as determined by lack of further product formation (12–36 h), the aqueous layer was extracted with ethyl ether, 3 × 15 mL. The combined and dried (Na_2SO_4) organic layers were evaporated to a residue and purified by silica gel chromatography (hexane/ethyl ether).

(*S*)-1-Phenyl-2,2,2-trifluoroethanol (1): 71% yield; >99% ee as determined by HPLC analysis on a Chiralcel OB column, hexane/2-propanol 98:2; with a flow rate of 1 mL/min the retention times were 10.65 min for (−)(*R*) and 11.69 min for (+)(*S*); ^1H NMR ($CDCl_3$) δ 3.15 (bs, 1 H), 4.95 (t, 1 H), 7.40 (m, 5 H). Spectroscopic properties were compared versus literature values for absolute configuration determination ($[\alpha]_D$ +8.6° (*c* = 4.25,

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benzene), 8% ee of the (*S*) enantiomer).[12]

(*R*)-1-(2-Pyridyl)ethanol (2): 60% yield; >97% ee as determined by conversion to a MTPA ester and comparison of the methyl peaks, δ 1.62 and 1.69 for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{25}_D$ +48° (*c* = 0.75, $CDCl_3$); ^1H NMR ($CDCl_3$) δ 1.52 (d, 1 H), 4.33 (s, 1 H), 4.91 (m, 1 H), 7.22 (m, 1 H), 7.30 (d, 1 H), 7.71 (m, 1 H), 8.57 (d, 1 H). Absolute configuration was determined by comparison of the optical rotation with literature assignments ($[\alpha]_D$ +14.7° (*c* = 4.35, ethanol), 22% ee for the (*R*) isomer).[13]

(*R*)-1-(2-Furanyl)ethanol (3): 65% yield; 95% ee as determined by conversion to a MTPA ester and comparison of the methyl peaks, δ 1.62 and 1.69 for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{22}_D$ +22.0° (*c* = 2.67, $CHCl_3$); ^1H NMR ($CDCl_3$) 1.55 (d, 3 H), 1.68 (s, 1 H), 4.88 (m, 1 H), 6.22 (d, 1 H), 6.31 (m, 1 H), 7.38 (d, 1 H). ^1H NMR is consistent with the reported values for the (*S*) enantiomer.[14] Absolute configuration was determined by comparison of the optical rotation with literature assignments ($[\alpha]_D$ +5.0° (*c* = 3.14, $CHCl_3$), 22% ee of the (*R*) isomer).[13,14]

(*R*)-6-Methyl-5-hepten-2-ol (4): 58% yield; 100% ee as determined by comparison of the optical rotation of the (*S*) enantiomer (literature $[\alpha]_D$ +10.76° ($CHCl_3$), 99% ee),[1c] and conversion to the MTPA ester followed by comparison of the methyl peaks at δ 1.20 and 1.27 for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{23}_D$ −14.8° (*c* = 5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.20 (d, 3 H), 1.52 (m, 2 H), 1.65 (s, 3 H), 1.71 (s, 3 H), 2.08 (m, 2 H), 3.82 (m, 1 H), 5.13 (t, 1 H). ^1H NMR is consistent with the commercially available compound.

(*R*)-5-Chloro-2-pentanol (5): 52% yield; >97% ee as determined by conversion to a MTPA ester followed by comparison of the methyl peaks at δ 1.28 and 1.36 for the (*S*) and (*R*) enantiomers, respectively; ^1H NMR ($CDCl_3$) δ 1.23 (d, 3 H), 1.61 (m, 2 H), 1.87 (m, 2 H), 3.60 (t, 2 H), 3.85 (m, 1 H). Absolute configuration was determined by comparison of the optical rotation with literature assignments ($[\alpha]_D$ +15.58° ($CHCl_3$), 98% ee of the (*S*) isomer).[2a]

(*R*)-1-Cyclopropyl-1-ethanol (6): 46% yield; >97% ee as determined by conversion to a MTPA ester followed by comparison of the methyl peaks at δ 1.26 and 1.33 for the (*S*) and (*R*) isomers, respectively; ^1H NMR ($CDCl_3$) δ 0.18 (m, 2 H), 0.29 (m, 2 H), 0.89 (m, 1 H), 1.28 (d, 3 H), 3.07 (m, 1 H). ^1H NMR is consistent with the commercially available sample. Absolute configuration was assigned on the basis of optical rotation of the (*R*) enantiomer ($[\alpha]_D$ −7.55° ($CHCl_3$), 44% ee).[1c]

(*R*)-5-Norbornen-2-ol (7): 39% yield; >97% ee as determined by NMR ratio of endo/exo isomers, δ 4.48 and 3.83 for the endo and exo isomers, respectively; ^1H NMR ($CDCl_3$) δ 0.78 (m, 1 H), 1.28 (m, 2 H), 1.49 (m, 1 H), 2.12 (m, 1 H), 2.83 (s, 1 H), 3.01 (s, 1 H), 4.48 (m, 1 H), 6.11 (m, 1 H), 6.46 (m, 1 H). ^1H NMR is consistent with the commercially available sample.

(*R*)-1-(Trimethylsilyl)-1-butyn-3-ol (8): 25% yield; 94% ee as determined by conversion to MTPA ester followed by comparison of the methyl peaks at δ 1.30 and 1.37 for the (*S*) and (*R*) isomers, respectively; $[\alpha]^{23}_D$ +36° (*c* = 0.47, $CDCl_3$); ^1H NMR ($CDCl_3$) δ 0.17 (s, 9 H), 1.44 (d, 3 H), 1.61 (s, 1 H), 4.52 (quartet, 1 H). Spectroscopic properties were consistent with those reported previously for the (*S*) enantiomer ($[\alpha]^{25}_D$ −25.9° (*c* = 3.12, $CHCl_3$), 95% ee).[15]

Methyl 4-hydroxy-1-(trimethylsilyl)-5-hexynoate (9): 15% yield; 97% ee as determined by conversion to a MTPA ester followed by comparison of the methoxy peaks at δ 3.40 and 3.46; ^1H NMR ($CDCl_3$) δ 0.19 (s, 9 H), 1.99 (m, 2 H), 2.53 (m, 2 H), 3.28 (s, 1 H), 3.63 (s, 3 H), 4.42 (m, 1 H); HRMS expected, 237.0923; observed, 237.0931. Absolute stereochemistry has not been determined. ^1H NMR is the same as in literature.[16]

Synthesis of TMS-Protected Terminal Alkyne Ketones. The procedures for the synthesis of these compounds were accomplished as described previously.[7]

1-(Trimethylsilyl)-1-hexyn-3-one:[17] 64% yield; ^1H NMR ($CDCl_3$) δ 0.17 (s, 9 H), 0.88 (t, 3 H), 1.62 (m, 2 H), 2.47 (t, 2 H); HRMS expected, 169.1049; observed, 169.1052.

4,4-Dimethyl-1-(trimethylsilyl)-1-pentyn-3-one: 66% yield; ^1H NMR ($CDCl_3$) δ 0.25 (s, 9 H), 1.20 (s, 9 H); HRMS expected, 183.1205; observed, 183.1216. ^1H NMR is the same as reported previously.[18]

5-Methyl-1-(trimethylsilyl)-1-hexyn-3-one:[17,19] 66% yield; ^1H NMR ($CDCl_3$) δ 0.19 (s, 9 H), 0.88 (d, 6 H), 2.18 (m, 1 H), 2.38 (d, 2 H); HRMS expected, 183.1205; observed, 183.1200.

Methyl 4-oxo-6-(trimethylsilyl)-5-hexynoate: 45% yield; ^1H NMR ($CDCl_3$) δ 0.25 (s, 9 H), 2.65 (t, 2 H), 2.89 (t, 2 H), 3.69 (s, 3 H); HRMS expected, 213.0947; observed, 213.0947. ^1H NMR is the same as reported previously.[20]

1-(Trimethylsilyl)-4-hexen-1-yn-3-one:[21] 84% yield; ^1H NMR ($CDCl_3$) δ 0.23 (s, 9 H), 1.98 (d, 3 H), 6.14 (d, 1 H), 7.19 (m, 1 H); HRMS expected, 167.0892; observed, 167.0881.

4-Chloro-1-(trimethylsilyl)-1-butyn-3-one:[22] 48% yield; ^1H NMR ($CDCl_3$) δ 0.24 (s, 9 H), 4.20 (s, 2 H); FTIR 2150 cm^{-1} (m, sharp), 1680 (s), 1400 (m, sharp), 1260 (m, sharp).

Determination of the Stereospecificity of Hydride Transfer. The following were combined and stirred at room temperature: 100 mg of NADP, 500 μL of 2-propanol-d_8, and 1 mL of partially purified *L. kefir* alcohol dehydrogenase[2b] (28 U/mL, 50.9 U/mg in 50% glycerol/phosphate buffer with 0.5 mM $MgCl_2$) in 5 mL of 50 mM ammonium bicarbonate buffer, pH 8, containing 1 mM $MgCl_2$. After 3 days, the reaction was lyophilized and applied to a 25 × 1-cm DEAE cellulose column. The unreacted NADP was eluted with 50 mM ammonium bicarbonate and the reduced cofactor was subsequently washed off the column with 250 mM ammonium bicarbonate buffer, pH 8. The NADPH fractions were combined, lyophilized, and lyophilized two more times from deuterium oxide. ^1H NMR (D_2O) 2.66 ppm (s, 1 H).

Registry No. 1, 340-06-7; 2, 27911-63-3; 3, 27948-61-4; 4, 58917-27-4; 5, 76188-95-9; 6, 6516-09-2; 7, 106928-44-3; 8, 121522-26-7; 9, 118800-11-6; NADPH, 53-57-6; alcohol dehydrogenase, 9031-72-5; trifluoroacetophenone, 434-45-7; 2-acetylpyridine, 1122-62-9; 2-acetylfuran, 1192-62-7; 6-methyl-5-hepten-2-one, 110-93-0; 5-chloro-2-pentenone, 5891-21-4; 1-cyclopropylethanone, 765-43-5; 5-norbornen-2-one, 694-98-4; 4-(trimethylsilyl)-3-buten-2-one, 34564-67-5; methyl 4-oxo-6-(trimethylsilyl)-5-hexynoate, 88761-59-5; acetophenone, 98-86-2; 3-acetylindole, 703-80-0; 3-phenylacetophenone, 3112-01-4; 6-acetylbenzo-1,4-dioxane, 2879-20-1; 2-acetyl-3,4-dimethoxyfuran, 113452-64-5; 2-chloro-3′,4′-dihydroxyacetophenone, 99-40-1; 4-chloro-4′-hydroxybutyrophenone, 7150-55-2; 4-chloro-4′-fluorobutyrophenone, 3874-54-2; 4-chloroacetophenone, 99-91-2; 3-(dimethylamino)propiophenone, 3506-36-3; 2,2-dimethoxyacetophenone, 6956-56-5; methyl oxophenylacetate, 15206-55-0; 1-phenyl-1,2-propanedione, 579-07-7; cyclopropyl phenyl ketone, 3481-02-5; 2-phenylcyclopropanecarboxaldehyde, 67074-44-6; 4-phenyl-2-butanone, 2550-26-7; (*E*)-4-phenyl-3-buten-2-one, 1896-62-4; phenoxyacetone, 621-87-4; 2-(4-chloro-1-oxobutyl)-thiophene, 43076-59-1; 2-(1,3-dioxo-4,4,4-trifluorobutyl)thiophene, 326-91-0; 2-(1-oxobutyl)furan, 4208-57-5; benzo-2-cycloheptan-1-one, 826-73-3; 2-acetyl-1,2,3,4-tetrahydro-1-naphthalene, 17216-08-9; thiochroman-4-one, 3528-17-4; *N*-benzyl-4-piperidinone, 3612-20-2; *trans*-2-isopropyl-5-methylcyclohexanone, 89-80-5; 4-*tert*-butylcyclohexanone, 98-53-3; 3-chlorononorbornanone, 61914-03-2; 2-formyl-5-norbornene, 5453-80-5; 4-cyclopentene-1,3-dione, 930-60-9; 2,4-pentanedione, 123-54-6; 2-heptanone, 110-43-0; 2-hexanone, 591-78-6; ethyl 3-oxopentanoate, 4949-44-4; 1,1-dimethoxypropanone, 6342-56-9; 5-hexen-2-one, 109-49-9; 1-octen-3-one, 4312-99-6; 4-methoxy-3-buten-2-one, 4652-27-1; 1-(trimethylsilyl)-1-hexyn-3-one, 80352-59-6; 4,4-dimethyl-1-

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(trimethylsilyl)-1-pentyn-3-one, 53723-94-7; 5-methyl-1-(trimethylsilyl)-1-hexyn-3-one, 65149-29-3; 1-(trimethylsilyl)-4-hexen-1-yn-3-one, 53723-96-9; 4-chloro-1-(trimethylsilyl)-1-butyn-3-one, 18245-82-4; 2-propanol, 67-63-0.

Supplementary Material Available: ^{1}H NMR spectra of (*S*)-1-phenyl-2,2,2-trifluoroethanol, (*R*)-1-(2-pyridyl)ethanol, (*R*)-1-(2-furanyl)ethanol, (*R*)-6-methyl-5-hepten-2-ol, (*R*)-5-chloro-2-pentanol, (*R*)-5-norbornen-2-ol, (*R*)-1-(trimethylsilyl)-1-butyn-3-ol, methyl 4-hydroxy-1-(trimethylsilyl)-5-hexynoate, 1-(trimethylsilyl)-1-hexyn-3-one, 4,4-dimethyl-1-(trimethylsilyl)-1-pentyn-3-one, 5-methyl-1-(trimethylsilyl)-1-hexyn-3-one, methyl 4-oxo-6-(trimethylsilyl)-5-pentynoate, 3-oxo-1-(trimethylsilyl)-4-hexen-1-yne, and 4-chloro-1-(trimethylsilyl)-1-butyn-3-one (14 pages). Ordering information is given on any current masthead page.

Para Fluorination by *N*-Fluorobis[(trifluoromethyl)sulfonyl]imide: Synthesis of 10β-Fluoro-3-oxo-1,4-estradiene Steroids

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When 1,3,5(10)-estratrien-3-ols are treated with *N*-fluorobis[(trifluoromethyl)sulfonyl]imide in chloroform solution the ortho and para fluorination products are formed. In contrast, when acetic acid is used as a solvent, fluorination in the para position occurs selectively and 10β-fluoro-3-oxo-1,4-estradiene derivatives are formed in high yields.

The first studies on electrophilic fluorination of estrogen steroids date back to the late fifties and were performed by using perchloryl fluoride.[2,3] Several other reagents providing a "positive fluorine" have been used subsequently to prepare A-ring fluorinated steroids starting from estrogens.[4-10]

Recently, attention has been refocused on this area by the observation that introduction of fluorine in position 2 of 17β-estradiol does not affect the hormonal activity, but reduces its tumorigenicity.[11-13]

As a part of our ongoing study of the synthetic potential of the *N*-fluorobis[(trifluoromethyl)sulfonyl]imide **1**[14] we have investigated the reaction of estrogens **2a–e** with this electrophilic fluorinating agent.

In this paper we describe how under proper reaction conditions a para fluorination occurs selectively and the 10β-fluoro-3-oxo-1,4-estradiene steroids **3a–e** can be obtained in high yields.

Results and Discussion

Synthetic Aspects. When 1,3,5(10)-estratriene-3,17β-diol 17-propionate (**2a**) was treated with the *N*-fluoroimide **1** in chloroform solution a clean reaction occurred at room temperature to give the 2-fluoro-1,3,5(10)-estratriene-3,17β-diol 17-propionate (**3a**), the 4-fluoro isomer **4a**, and the 10β-fluoro-3-oxo-1,4-estradien-17β-ol 17-propionate (**5a**).

Table I

compd	solvent	(3 + 4):5 ratio	3:4 ratio
2a	chloroform	31:69	41:59
	acetonitrile	38:62	46:54
	dioxane	41:59	42:58
	acetic acid	14:86	32:86
2b	chloroform	44:56	43:57
	acetic acid	<2:>98	
2c	chloroform	47:53	45:55
	acetic acid	<2:>98	
2d	chloroform	32:68	40:60
	acetic acid	<2:>98	
2e	acetic acid	<2:>98	

The para fluorination, i.e., the entrance of the halogen on C-10 to give **5a** through an ipso process, is favored with respect to the ortho fluorination, which gives **3a** and **4a**, and a low selectivity exists between the two ortho positions (see Table I). Furthermore, the reaction forming **5a** is completely stereoselective as fluorine enters exclusively from the β-face of the steroid.[15]

Similar regio- and stereoselectivities were observed when dioxane and acetonitrile were employed as solvents, while the use of acetic acid consistently favored the formation of the para fluorination product **5a**.

When estrone **2b**, 17α-estradiol 17-acetate **2c**, and 3,16α,17β-estratriol 16,17-diacetate **2d** were treated with the *N*-fluoroimide **1** they showed a behavior similar to that of estradiol **2a**. In chloroform solution the monofluoro derivatives **3b–d**, **4b–d**, and **5b–d** were formed for all these substrates and their ratios were similar to those obtained starting from **2a**.

Interestingly, the change of regioselectivity induced by acetic acid was much sharper for these estrogens as **5b–d** were the exclusive products when this solvent was employed.

It was also possible to fluorinate 4-nitroestrone **2e**. Its reaction with the *N*-fluoroimide **1** was slower than that of **2a–d** clearly as a consequence of the presence of the deactivating residue in position four. In acetic acid solu-

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