An Easy Approach to the Synthesis of Optically Active *vic*-Diols: A New Single-Enzyme System

Olga Bortolini, Giancarlo Fantin, Marco Fogagnolo, Pier Paolo Giovannini, Alessandra Guerrini, and Alessandro Medici*

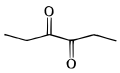
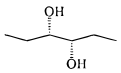
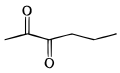
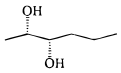
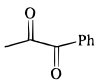
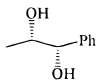
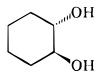
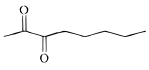
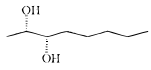
Dipartimento di Chimica, Universita' di Ferrara, Via Borsari 46, 44100 Ferrara, Italy

Received September 25, 1996

The synthesis of enantiomerically pure vicinal diols is an area of growing interest, since these compounds are highly valuable chiral synthons. New efficient processes, allowing for their synthesis, have been recently developed by different authors using transition metal catalysis;[1] however, the major drawback of these procedures is represented by the use of heavy metal catalysts, which may be source of pollution. In this context, enzymes present attractive alternatives for asymmetric synthesis,[2] and numerous illustrations of their applicability to a broad spectrum of synthetic problems, including the obtainment of enantiopure diols,[3] are well documented. Nicotinamide cofactor-dependent oxidoreductase, for example, is a class of enzymes involved in the oxidation of alcohols and reduction of ketones.[4] For the oxidation of alcohols that require nicotinamide adenine nucleotide (NAD) or its phosphate analogue (NADP), or the reduction of carbonyl compounds mediated by the reduced form NADH (NADPH), regeneration of the cofactor is necessary. Enzymatic regeneration of NADH (or NADPH) for synthesis usually employs the glucose/glucose dehydrogenase[5a] or the glucose 6-phosphate/glucose 6-phosphate dehydrogenase[5b] systems. An improvement in the regeneration cycle is represented by the use of single-enzyme systems, where one enzyme can catalyze a desired reaction while simultaneously regenerating the cofactor. Single-enzyme systems have been established for the alcohol dehydrogenases from horse liver,[6] *Thermoanaerobium brockii*,[7] *Lactobacillus kefir*,[8] *Pseudomonas* species,[9] and *Geotrichum candidum*.[10]

Pursuing our interest in the development of new enzymes for organic synthesis, we recently isolated and characterized a diacetyl reductase from *Bacillus stearothermophilus* (BSDR).[11] This enzyme, also in its crude form, is an efficient catalyst for the stereospecific redox reactions of bicyclic octen- and heptenols and ones.[12] In this paper we present the synthesis of several chiral 1,2-diols, two of them new as (*S*,*S*) isomers, that were obtained from α-diketones by reduction with NADH catalyzed by BSDR. In addition, the synthetic potential of the system is illustrated by the synthesis of the pheromone of the grape borer, *Xylotrechus pyrrhoderus*.[13] The cofactor NADH is conveniently and simultaneously regenerated by this single-enzyme system in the presence of a cosubstrates of high synthetic utility.

Table 1. BSDR-Catalyzed Reductions of α-Diketones by the Single-Enzyme System

starting diketone	product	yield %	ee %[a] (abs. conf.)
 1a	 2a	40	>98 (S,S)
 1b	 2b	80	95 (S,S)
 1c	 2c	92	>98 (S,S)
 1d	 2d	80	>98 (S,S)
 1e	 2e	80	95 (S,S)
 1f	 2f	82[b]	>98 (S,S)

[a] Determined by GC analysis on a chiral column, when >98% the (*R*,*R*) isomer is not detected; see the Experimental Section. [b] Reaction time 72 h, 8 mL of enzyme solution.

Results and Discussion

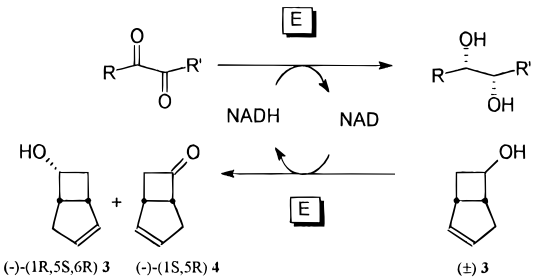
Bacillus stearothermophilus diacetyl reductase (BSDR) is specific for 2,3-butanedione (diacetyl) and 2-hydroxy-3-butanone (acetoin) substrates[11] and, as most alcohol dehydrogenases, catalyzes the transfer of a hydride from the reduced cofactor to the *re*-face of the carbonyl function to give (*S*) alcohols, according to Prelog's rule.[14] Taking into account this specificity, the reduction of different α-diketones bearing aliphatic, cyclic, or aromatic substituents has been studied. Table 1 lists six α-diketones that have been reduced to (*S*,*S*)-1,2-diols by this catalytic system. The overall reaction view is shown in Scheme

* To whom correspondence should be addressed: Tel. 39-532-291172, Fax 39-532-240709, E-mail mda@ifeuniv.unife.it.

(1) (a) Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483. (b) Katsuki, T. *Coord. Chem. Rev.* **1995**, *140*, 189.
(2) *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; VCH: Weinheim, 1995; Vols I and II.
(3) (a) Faber, K.; Mischitz, M.; Kroutil, W. *Acta Chem. Scand.* **1996**, *50*, 249. (b) Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *Tetrahedron* **1996**, *52*, 4593. (c) Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *Tetrahedron Lett.* **1996**, *37*, 3319. (d) Bellucci, G.; Chiappe, C.; Cordoni, A. *Tetrahedron: Asymmetry* **1996**, *7*, 197.
(4) Fang, J.-M.; Lin, C.-H.; Bradshaw, C. W.; Wong, C.-H. *J. Chem. Soc., Perkin Trans. 1* **1995**, 967.
(5) (a) Wang, 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.
(6) Haslegrave, J. A.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 4666.
(7) (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
(8) Bradshaw, C. W.; Hummel, W.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 1532.
(9) (a) Bradshaw, C. W.; Fu, H.; Shen, G.-J.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 1526. (b) Shen, G.-J.; Wang, Y.-F.; Bradshaw, C. W.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1990**, 677.
(10) Nakamura, K.; Kitano, K.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1996**, *37*, 1629.
(11) Giovannini, P. P.; Medici, A.; Bergamini, C. M.; Rippa, M. *Bioorg. Med. Chem.* **1996**, *4*, 1197.
(12) Giovannini, P. P.; Hanau, S.; Rippa, M.; Bortolini, O.; Fogagnolo, M.; Medici, A. *Tetrahedron* **1996**, *52*, 1669.
(13) (a) Mori, K. *Tetrahedron* **1989**, *45*, 3233. (b) Mori, K.; Otsuka, T. *Tetrahedron* **1985**, *41*, 553. (c) Saki, T.; Nakagawa, Y.; Takahashi, J.; Iwabuchi, K.; Ishii, K. *Chem. Lett.* **1984**, 263.
(14) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119.

S0022-3263(96)01838-5 CCC: $14.00

Scheme 1



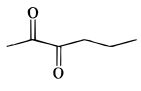
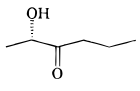
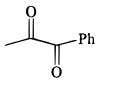
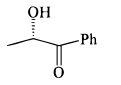
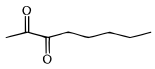
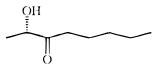
E = *Bacillus stearothermophilus* diacetyl reductase (BSDR)

1. As the diketone is stereospecifically reduced to (*S*,*S*)-1,2-diol by the diacetyl reductase, NAD is formed, which in turn is reduced to NADH by the same enzyme in the presence of the cosubstrate *endo*-bicyclo[3.2.0]hept-2-en-6-ol, (±) **3**. BSDR accepts a large variety of side chains of different size, including cyclic compounds, and all diketones are reduced to 1,2-diols in high enantiomeric excess and good chemical yield. A relatively easy workup affords the diols **2a**−**f**, in the configuration reported in Table 1. In addition, the kinetic resolution of the cosubstrate (±)-**3** is obtained during NADH regeneration. The resulting ketone *endo*-(−)-(1*S*,5*R*)-bicyclo[3.2.0]hept-2-en-6-one (**4**), an important starting material in the synthesis of prostaglandins,[15] and the remaining alcohol (−)-(1*R*,5*S*,6*R*)-**3** may be almost quantitatively recovered from the reaction mixture in an enantiomeric excess higher than 98%, by virtue of the strict stoichiometry of the reaction.[16]

From a mechanistic point of view the reaction takes place through two consecutive enantioselective reductions, with formation of the monoreduced product, α-hydroxy ketone, that is further reduced to 1,2-diol. By a careful control of the experimental conditions, in particular cosubstrate concentration and reaction time, the diketones may be chemo- and enantioselectively reduced to (*S*)-α-hydroxy ketones; however there is a clear distinction between symmetric (R = R′) and asymmetric (R ≠ R′) starting substrates. Symmetric diketones, due to the statistical factor, are reduced faster but in poor yield, and the product of monoreduction is always contaminated by large amounts of diol. On the other hand, α-hydroxy ketones are obtained, from asymmetric diketones, in good enantiomeric and chemical yields. Particularly interesting is the ability to reduce, in first place, the carbonyl group proximal to the smaller substituent (methyl) in **1c**, **1d**, and **1f**. The middle substrate was reported to be reduced to (R)-hydroxy ketone at the carbonyl group close to the phenyl substituent in the presence of *Pseudomonas* sp. alcohol dehydrogenase.[4,9a]

Contrary to the facile workup found during 1,2-diol isolation, the synthetic-scale preparation of α-hydroxy ketones by this single-enzyme system showed several unexpected difficulties, related to product separation. To overcome the problem, the single-enzyme system has been replaced with a double-enzyme system consisting of the substrate, NADH-dependent BSDR and the classical glucose 6-phosphate/glucose 6-phosphate dehydrogenase.[5b] This second set of results is collected in Table 2. The chemoselective reduction of the carbonyl group closer to the smaller substituent and the formation of α-hydroxy ketones with (*S*) stereochemistry are peculiar to the double-enzyme catalytic cycle, as well.

Table 2. BSDR-Catalyzed Reductions of α-Diketones by the Double-Enzyme System

starting diketone	product	yield %	ee %[a] (abs. conf.)
 **1a**	 **5a**	96	>98 (S)
 **1d**	 **5d**	92	>98 (S)
 **1f**	 **5f**	65[c]	>98 (S)

[a] Determined by GC analysis on a chiral column, when >98% the (*R*) isomer is not detected; see the Experimental Section. [b] The reduction affords also the diol **2f** in 30% yield.

The synthetic potential of the single- and double-enzyme systems has been illustrated during the synthesis of the male sex pheromone of the grape borer *Xylotrechus pyrrhoderus*[13] identified as a two-component mixture of **2f** and **5f**.[13c] The synthetic route to **2f** and **5f** employed the commercially available, low-cost 1-octyn-3-ol that is transformed to 3-hydroxy-2-octanone and oxidized to the α-diketone **1f**. The latter product is chemo- and enantioselectively reduced to **2f**, Table 1, or **5f**, Table 2, by the appropriate single- or double-enzyme system, in high chemical and enantiomeric yield.

In conclusion, two concise and technically simple procedures for the synthesis of chiral 1,2-diols and α-hydroxy ketones have been uncovered. Work is in progress to further exploit their synthetic utility.

Experimental Section

Materials and Methods. *Bacillus stearothermophilus* is available from American Type Culture Collection (ATCC 2027) and was grown as previously described.[12] For synthetic reactions, the wet cells (20 g) obtained from four portions of 250 mL cultures are separated upon centrifugation and washed with 200 mL of 0.15 M NaCl. The cells are suspended in 100 mL of TEA-HCl buffer, treated with 40 mg of lysozyme for 60 min at 22 °C, and recentrifuged. The supernatant (enzyme solution) was used without further treatment. Typically 1 mL of enzyme solution contains approximately 0.2 unit of enzyme (1 unit = 1 μmol of *endo*-bicyclo[3.2.0]hept-2-en-6-ol oxidized per minute).[12] The TEA-HCl buffer is composed of 50 mM triethanolamine, containing 0.1 mM EDTA, 1 mM β-mercaptoethanol, and HCl to adjust the pH at 7.5. NADH and glucose 6-phosphate were purchased from Sigma. *Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase is from Boehringer Mannheim. *endo*-Bicyclo-[3.2.0]hept-2-en-6-ol, (±)-**3**, was obtained from the commercially available bicyclo[3.2.0]hept-2-en-6-one (Merck) by reduction with $NaBH_4$ and separation of the *endo*:*exo* isomers (9:1 ratio) by chromatography on SiO_2.[17] Alternatively, the bicyclo[3.2.0]hept-2-en-6-one may be synthesized by cycloaddition of cyclopentadiene with dichloroketene, followed by reduction, in 80% yield.[18]

(15) (a) Newton, R. F.; Roberts, S. M. *Tetrahedron* **1980**, *36*, 2163. (b) Hart, T. W.; Comte, M.-T. *Tetrahedron Lett.* **1985**, *26*, 2713. (c) Riefling, B. F. *Tetrahedron Lett.* **1985**, *26*, 2063.

(16) The use of the less valuable but more common 2-hexanol, 2,3-butanediol, or propan-2-ol as cosubstrate for NADH regeneration gave unsatisfactory results.

(17) Newton, R. F., Paton, J.; Reynolds, D. P.; Young, S.; Roberts, S. M. *J. Chem. Soc., Chem. Commun.* **1979**, 908.

(18) (a) Depres, J.-P.; Greene, A. E.; Crabbe, P. *Tetrahedron* **1981**, *37*, 621. (b) Ghosez, L.; Montaigne, R.; Roussel, A.; Vanlierde, H.; Mollet, P. *Ibid.* **1971**, *27*, 615. (c) Corey, E. J.; Arnold, Z.; Hutton, J. *Tetrahedron Lett.* **1970**, 307.

Except for 2,3-octanedione, all other diketones and **2a**,**e** are commercially available products purchased from Aldrich and used upon distillation. Enantiomeric separations and excesses were determined by GC on a Megadex 5 column containing dimethyl-*n*-pentyl-*β*-cyclodextrin in OV 1701 from Mega s.n.c. The absolute configurations are determined by comparison of the sign of the specific rotations with the reported values, when available. In the other cases, the absolute configurations are assigned on the basis of the GC retention time in a homologous series, upon analysis of the racemates. The entry ee > 98% of Tables 1 and 2 means that the (*R*,*R*)-diols or the (*R*)-α-hydroxy ketones are not detected in our experimental conditions. The ^{1}H and ^{13}C NMR spectra were obtained on a 300 MHz instrument.

General Procedure for 1,2-Diols (Single-Enzyme System). A portion of the enzyme solution (4 mL) was added to a flask containing 1 mmol of ketone substrate, 20 mg of NADH, and 4 mmol of cosubstrate in 120 mL of TEA-HCl buffer. Note that, due to kinetic resolution, half of the cosubstrate is elaborated, and consequently 4 equiv is required to complete the reduction. After the proper time (48 h) the reaction mixture was saturated with NaCl and extracted with ethyl acetate (3 × 20 mL). The combined and dried organic layers were evaporated and the products separated by chromatography on SiO_2, using ethyl ether−cyclohexane (1:1) as eluent.

General Procedure for α-Hydroxy Ketones (Double-Enzyme System). A portion of the enzyme solution (2 mL) was added to a flask containing 1 mmol of ketone substrate, 20 mg of NADH, 1 mmol of glucose 6-phosphate, and 10 μL of glucose 6-phosphate dehydrogenase in 100 mL of TEA-HCl buffer. After the proper time (24 h) the reaction mixture was saturated with NaCl and extracted with ethyl acetate (3 × 20 mL). The combined and dried organic layers were evaporated and the products separated by chromatography on SiO_2, using ethyl ether−cyclohexane (1:1) as eluent.

Synthesis of 2,3-Octanedione (1f). 1-Octyn-3-ol (32 mmol) was added dropwise to a well-stirred boiling solution composed of HgO (32 mmol), H_2SO_4 (1.7 mL), and H_2O (50 mL), with continuous steam-distillation.[19] The distillate was saturated with NaCl, and the upper layer was separated and combined with the ethereal extracts of the aqueous layer. The combined and dried organic layers were evaporated, affording the 3-hydroxy-2-octanone as yellow oil, in 90% yield: ^{1}H NMR ($CDCl_3$) 4.20 (t, J = 6 Hz, 1H), 2.20 (s, 3H), 1.80 (s, 1H, exchanges with D_2O), 1.60−1.40 (m, 2H), 1.40−1.20 (m, 6H), 0.90 (t, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$) 210.0, 76.8, 33.4, 31.6, 25.1, 24.3, 22.4, 13.9. The crude 3-hydroxy-2-octanone was oxidized to the α-diketone **1f** by the Jones reagent[20] and purified by column chromatography on SiO_2, using ethyl acetate as eluent, in 85% yield: ^{1}H NMR ($CDCl_3$) 2.70 (t, J = 7 Hz, 2H), 2.15 (s, 3H), 1.65−1.55 (m, 2H), 1.40−1.20 (m, 6H), 0.90 (t, J = 6 Hz, 3H); ^{13}C NMR ($CDCl_3$) 199.5, 197.6, 35.6, 31.2, 23.7, 22.7, 2.3, 13.8. Anal. Calcd C, 67.57; H, 9.93, O, 22.49. Found: C, 68.30; H, 9.85.

(*S*,*S*)-2,3-Butanediol (2a). The synthesis of this product followed the general procedure described above. After the proper time the reaction mixture was evaporated under vacuum, the residue was dissolved in ethyl acetate methanol (10%) and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The low chemical yield (30%) is due to the high solubility of **2a** in water. Racemization or formation of complex stereoisomeric mixtures is not observed:[21] $[\alpha]^{20}_D$ = +13.0 (neat); ^{1}H and ^{13}C NMR are consistent with commercial sample.

(*S*,*S*)-3,4-Hexanediol (2b): $[\alpha]^{20}_D$ = (−) 12.4 (*c* = 2.0, chloroform); ^{1}H NMR ($CDCl_3$) 3.35 (m, 2H), 2.40 (br s, 2H, exchange with D_2O), 1.65−1.40 (m, 4H), 1.00 (t, J = 7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) 75.5, 26.4, 9.9; IR (neat) 3400.

(*S*,*S*)-2,3-Hexanediol (2c): $[\alpha]^{20}_D$ = (−)16.1 (*c* = 1.2, chloroform); ^{1}H NMR ($CDCl_3$) 3.60 (m, 1H), 3.35 (m, 1H), 2.40 (br s, 1H, exchanges with D_2O), 2.30 (br s, 1H, exchanges with D_2O), 1.40−1.60 (m, 4H), 1.20 (d, J = 7.0 Hz, 3H), 0.90 (t, J = 7.5, Hz, 3H); ^{13}C NMR ($CDCl_3$) 75.0, 69.9, 34.5, 18.2, 17.8, 13.1; IR ($CHCl_3$) 3400.

(*S*,*S*)-1,2-Dihydroxy-1-phenylpropane (2d): $[\alpha]^{20}_D$ = +55.9 (*c* = 1.9, chloroform);[22] ^{1}H NMR ($CDCl_3$) 7.30−7.38 (m, 5H), 4.36 (d, J = 7.3 Hz, 1H), 3.84 (dq, J = 6.4 Hz, 1H), 2.78 (m, 1H, exchanges with D_2O), 2.62 (m, 1H, exchanges with D_2O), 1.05 (d, J = 6.4 Hz, 3H); IR (neat) 3580.

(*S*,*S*)-1,2-Cyclohexanediol (2e): $[\alpha]^{20}_D$ = +39.0 (*c* = 1.6, H_2O), ^{1}H and ^{13}C NMR consistent with commercial sample.

(*S*,*S*)- 2,3-Octanediol (2f). $[\alpha]^{20}_D$ = −17.9 (*c* = 1.14, cholroform);[13b] ^{1}H ($CDCl_3$) 3.60 (m, 1H), 3.30 (m, 1H), 2.70 (br s, 2H, exchange with D_2O), 1.10−1.70 (m, 8H), 1.35 (d, J = 6.0 Hz, 3H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 76.3, 70.9, 33.3, 32.0, 25.3, 22.6, 19.5, 14.1.

(*S*)-2-Hydroxy-3-hexanone (5a): $[\alpha]^{20}_D$ = +57 (*c* = 2.5, chloroform); ^{1}H NMR ($CDCl_3$) 4.10 (q, J = 7.5 Hz, 1H), 3.10 (br s, 1H), 2.35−2.55 (m, 2H), 1.60−1.79 (m, 2H), 1.40 (d, J = 7.0 Hz, 3H), 0.90 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) 212.0, 72.5, 39.4, 19.8, 17.0, 13.7; IR ($CHCl_3$) 3450, 1700.

(*S*)- 2-Hydroxy-3-phenyl-3-propanone (5d): $[\alpha]^{20}_D$ = −64.4 (*c* = 7.8, chloroform);[22] ^{1}H NMR ($CDCl_3$) 7.26−7.90 (m, 5H), 5.10 (q, J = 7.0 Hz, 1H), 3.60 (s, 1H, exchanges with D_2O), 1.40 (d, J = 7.0, 3H); ^{13}C NMR ($CDCl_3$) 202.3, 133.9, 128.6−128.8, 69.2, 22.2; IR (neat) 3450, 1670.

(*S*)-2-Hydroxy-3-octanone (5f): $[\alpha]^{20}_D$ = +65.8 (*c* = 1.8, chloroform);[13b] ^{1}H NMR ($CDCl_3$) 4.20−4.30 (m, 1H), 3.70 (s, 1H, exchanges with D_2O), 2.30−2.60 (m, 2H), 1.20−1.85 (m, 6H), 1.35 (d, J = 7.0 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 212.8, 72.6, 37.5, 31.4, 23.3, 22.4, 19.8, 13.9.

Acknowledgment. We are grateful to Professor Mario Rippa, this University, for the helpful and stimulating discussion.

JO9618381

(19) Ansell, M. F.; Hickinbottom, W. J.; Hyatt, A. A. *J. Chem. Soc.* **1955**, 1592.

(20) Jones, E. R. H.; Meakins, G. D.; Pragnell, W. E.; Muller W. E.; Wilkins *J. Chem. Soc., Perkin Trans. 1* **1974**, 2376.

(21) Ikeda, H.; Sato, E.; Sugai, T.; Ohta, H. *Tetrahedron* **1996**, *52*, 8113 and references therein.

(22) Takeshita, M.; Sato, T. *Chem. Pharm. Bull.* **1989**, *37*, 1085.