177 (17); HRMS calculated for $C_{15}H_{24}O$ 220.1827, found 220.1824.

X-ray Analysis of 1. A crystal of compound 1 measuring approximately 0.40 × 0.45 × 0.65 mm^3 was selected and aligned on a Nicolet R3m/V diffractometer system. Preliminary X-ray photographs displayed orthorhombic symmetry, and accurate lattice constants of a = 6.320 (1), b = 8.252 (2), and c 26.413 (7) Å were determined from a least-squares fit of 25 diffractometer-measured $2q$ values. The empirical formula was $C_{15}H_{24}O$. The crystal density, 1.062 g/cm^3, indicated that four molecules of 1 made up the unit cell. Systematic extinctions were consistent with space group $P2_12_12_1$ (with four molecules per unit cell). All unique diffraction maxima with $2q < 112°$ were collected using variable speed 1° $2q$-q scans and graphite monochromated Cu Kα radiation (1.541 84 Å). Of the 1046 reflections collected, 997 (95%) were judged observed ($|F_o| > 3s(F_o)$) after correction for Lorentz, polarization, and background effects. All non-hydrogen atoms were located by DF-synthesis. Full-matrix least-squares refinements with anisotropic nonhydrogen atoms converged to a crystallographic residual of 0.042 (R_w = 0.053) for the observed data.

(4a*S*,5*R*,8*S*,8a*R*)-8-Isopropyl-5-methyl-3,4,4a,5,6,7,8,8a-octahydro-2-naphthalenecarboxylic Acid (6). To a solution of aldehyde **17** (1.12 mg, 0.005 mmol) in acetonitrile (0.1 mL) was added an aqueous solution of sodium phosphate monobasic (0.6 mM, 10 μL) which had been adjusted to pH 1 with concentrated hydrochloric acid, hydrogen peroxide (30%, 10 μL), and aqueous sodium chlorite (0.1 mL, 1.0 mM). After being stirred for 2 h at room temperature, the reaction mixture was diluted with water and extracted with hexane (×6). The organic solution was dried ($MgSO_4$) and concentrated, yielding **6** (1.03 mg, 0.0044 mmol, 85%) as a white solid: IR (CCl_4) 3075, 2970, 2930, 2880, 1730, 1685, 1640, 1485, 1280 cm^{-1}; ^{1}H NMR δ (200 MHz) 7.02 (br s, 1 H), 2.65 (br s, 1 H), 2.30 (dm, 1 H, J = 17.2 Hz), 2.15 (m, 1 H), 2.04 (m, 1 H), 1.74–1.42 (m, 4 H), 1.37–1.2 (m, 2 H), 1.1–0.9 (m, 2 H), 0.94 (d, 3 H, J = 6.0 Hz), 0.90 (d, 3 H, J = 6.6 Hz), 0.86 (d, 3 H, J = 6.0 Hz), 0.80 (m, 1 H); EIMS m/z (rel intensity) 236 (M$^+$, 10), 193 (56), 150 (40), 147 (83), 137 (36), 107 (50), 105 (53), 95 (76), 93 (42), 91 (74), 81 (93), 79 (86), 77 (60), 41 (100); CIMS m/z (rel intensity) 237 (M$^+$ + 1, 100), 219 (39); HRMS calculated for $C_{15}H_{24}O_2$ 236.1776, found 236.1786.

(4a*S*,5*R*,8*S*,8a*S*)-8-Isopropyl-5-methyl-3,4,4a,5,6,7,8,8a-octahydro-2-naphthalenecarboxylic Acid (5). To a solution of aldehyde 1 (2.35 mg, 0.011 mmol) in acetonitrile (0.2 mL) was added an aqueous solution of sodium phosphate monobasic (0.6 mM, 20 μL) which had been adjusted to pH 1 with concentrated hydrochloric acid, hydrogen peroxide (30%, 20 μL), and aqueous sodium chlorite (0.2 mL, 1.0 mM). After being stirred for 3 h at room temperature the reaction mixture was diluted with water and extracted with hexane (×6). The organic solution was dried ($MgSO_4$) and concentrated, yielding a crude product which contained 10% starting material. Chromatography on silica gel (hexane–ethyl acetate, 92:8) gave **5** (1.77 mg, 7.5 mmol, 70%) as a white solid: IR (CCl_4) 3100, 2975, 2940, 2870, 1730, 1680, 1645, 1435, 1280 cm^{-1}; ^{1}H NMR: δ (200 MHz) 7.22 (br s, 1 H), 2.41 (dm, 1 H, J = 17.7 Hz), 2.22–2.06 (m, 3 H), 1.77–1.66 (m, 3 H), 1.20–0.92 (m, 5 H), 0.91 (d, 6 H, J = 6.5 Hz), 0.85 (m, 1 H), 0.77 (d, 3 H, J = 6.8 Hz); EIMS m/z (rel intensity) 236 (M$^+$, 11), 193 (45), 147 (66), 137 (32), 107 (47), 105 (46), 91 (68), 81 (100), 69 (48), 67 (55), 55 (78), 41 (98); CIMS m/z (rel intensity) 237 (M$^+$ + 1, 100), 219 (31); HRMS calculated for $C_{15}H_{24}O_2$ 236.1776, found 236.1775.

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Registry No. 1, 126580-54-9; **2**, 126580-64-1; **3**, 100019-20-3; **4**, 99957-14-9; **5**, 126642-62-4; **6**, 126580-56-1; **8**, 14073-97-3; **9**, 74185-00-5; **10a**, 126580-57-2; **10b**, 126642-63-5; 11, 126580-58-3; 12, 126580-59-4; 12 (alcohol), 126580-55-0; 13 (isomer 1), 126580-60-7; 13 (isomer 2), 126642-66-8; **14**, 126580-61-8; **15**, 126580-62-9; **16a**, 126580-63-0; **16b**, 126642-64-6; **17**, 126642-65-7; **21**, 126580-65-2; CH_2I_2, 75-11-6.

Supplementary Material Available: Tables of atomic coordinates, interatomic angles and distances for 1, ^{13}C NMR spectra for compounds **9**, **10a,b**, 11–14, and ^{1}H NMR spectra for compounds 1, **5**, **6**, **15**, **16a,b**, and **17** (18 pages). Ordering information is given on any current masthead page.

Asymmetric Reduction of Aliphatic Short- to Long-Chain β-Keto Acids by Use of Fermenting Bakers' Yeast

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Eleven β-keto acids, ranging from 3-oxobutanoic to 3-oxooctanoic acids, were reduced with fermenting bakers' yeast to the corresponding optically active β-hydroxy acids, which were isolated as the methyl esters. In all cases, the (*R*)-hydroxy acids were obtained in ≥98% ee, except for 3-oxobutanoic acid, which afforded the (*S*)-hydroxy acid in 86% ee. Inhibition of fermentation was observed for 3-oxoundecanoic to 3-oxotetradecanoic acids, leading to no reduction. Lowering of the substrate concentration was found to be appreciably effective in avoiding inhibition.

In a recent communication,[1] we reported that 3-oxooctadecanoic acid (**1k**) was reduced with fermenting bakers' yeast to optically pure (*R*)-3-hydroxyoctadecanoic acid (**2k**), which was transformed to (+)-corynomycolic acid (**4**), known as a cord factor.[2] Other naturally occurring long-chain 3-hydroxyalkanoic acids include (−)-3-hydroxydecanoic acid (found in a secretion by the leaf-cutting ant),[3] (*R*)-3-hydroxytetradecanoic acid (**2i**) (a constituent of lipid A in endotoxin),[4] (*S*)-3-hydroxyhexadecanoic acid (in the fish toxin, pahutoxin (**3**)),[5] and

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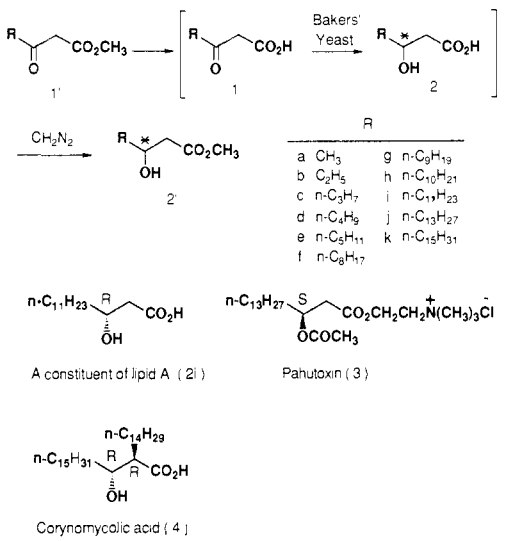
0022-3263/90/1955-3917$02.50/0

Scheme I



(*R*)-3-hydroxyhexadecanoic acid (**2j**) (a constituent of extracellular glycolipids from the red yeast *Rhodotorula*).[6] The acid **2k** is also found as a fatty acid component of eupassofilin from a higher plant.[7] Therefore, preparation of optically pure, long-chain 3-hydroxyalkanoic acids may be useful for the synthesis of these biologically active compounds. In addition, optically active, short-chain 3-hydroxyalkanoic acids or their esters have been widely used as chiral synthons or chiral building blocks.[8] The bakers's yeast reduction of various acetoacetates and their γ-substituted derivatives has been studied extensively,[9,10] but little is known about the reduction of β-keto acids.

The use of acetoacetic acid as substrate for bakers' yeast reduction was first reported by Friedmann,[11] who investigated the stereochemical course of its bioreduction. Later Lemieux and Giguere[12] extended the work to 3-oxohexanoic and 3-oxooctanoic acids and found that these higher homologues were reduced to the corresponding

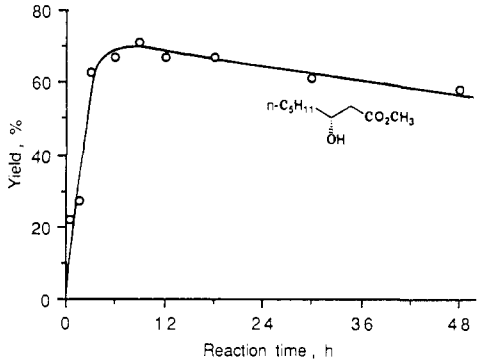


Figure 1. Time course of the reduction of 3-oxooctanoic acid (**1e**) with fermenting bakers' yeast at 28 °C and pH 5. The product was esterified with diazomethane and purified by LC.

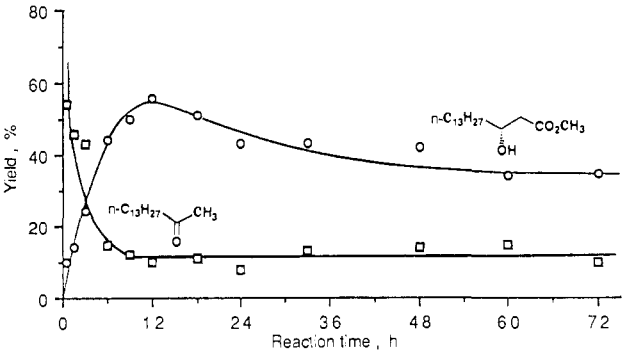


Figure 2. Time course of the reduction of 3-oxohexadecanoic acid (**1j**) with fermenting bakers' yeast at 28 °C and pH 5. The product was esterified with diazomethane and purified by LC. The decarboxylation product, 2-pentadecanone, was analyzed by GC.

(*R*)-(−)-3-hydroxy acids in contrast to the (*S*)-(+)-3-hydroxy acid from acetoacetic acid. Recently, the bakers' yeast reduction of 3-oxo-6-alkenoic,[13a,b] 6-(benzyloxy)-3-oxohexanoic,[13a] and (*S*)-4-methyl-3-oxohexanoic[13c] acids has been studied by Hirama and co-workers. The optical purity of the reduction products was excellent (≥98% ee), although the chemical yields were low to moderate (≤59%).

Recent advances in the asymmetric chemical reduction of β-keto esters have been quite remarkable.[14,15] Noyori and co-workers have demonstrated that chiral RuX_2[binap] complexes catalyze hydrogenation of β-keto esters to β-hydroxy esters with ≥96% yield and 97–100% ee.[15e,f] However, it is our belief that the potential of bakers' yeast as a chiral reducing reagent has not been fully explored yet.

In the present work, results of a comprehensive study of the bakers' yeast reduction of 3-oxoalkanoic acids ranging from 4 to 18 carbons are presented in terms of yield, optical purity, and configuration under various conditions. The results are also discussed in light of similar

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Table I. Asymmetric Reduction of 3-Oxoalkanoic Acids 1 to 3-Hydroxyalkanoic Acids 2 with Fermenting Bakers' Yeast

$RCOCH_2CO_2H$ (1)	$RCH(OH)CH_2CO_2CH_3$ (2′)				
	% yield[a]			$[\alpha]_D$, deg	
R	(9–12 h)[b]	(48 h)[c]	% ee[d]	(c, $CHCl_3$)[e]	config[f]
a CH_3	2	3	86		
	13[g]		86	+36.2 (1.17)[l]	*S*
b C_2H_5	18	21	98	−35.2 (0.50)[m]	*R*
	13[g]				
c n-C_3H_7	37	34	>98	−28.5 (1.15)[n]	*R*
d n-C_4H_9	69	52	>98	−27.1 (1.52)	*R*
e n-C_5H_{11}	71	58	>98	−22.7 (1.04)[o]	*R*
f n-C_8H_{17}	51[h]	36	>98	−22.4 (1.34)	*R*
g n-C_9H_{19}		2 (89)			
	19[i]	15 (28)[i]	>98	−21.1 (1.51)	*R*
		17 (64)[j]			
h n-$C_{10}H_{21}$		0 (90)			
		22 (20)[i]	>98	−19.6 (1.14)	*R*
		16 (77)[j]			
i n-$C_{11}H_{23}$	22[h]	0 (69)			
	19[i]	41 (14)[i]	>98	−18.5 (1.05)	*R*
		25 (26)[k]			
j n-$C_{13}H_{27}$	56 (10)	42 (14)	>98	−16.6 (1.82)[p]	*R*
k n-$C_{15}H_{31}$	40 (28)	44	>98	−15.8 (1.10)[q]	*R*

[a] Isolated yields by LC. Recoveries of the decarboxylated methyl ketones are given in parentheses. [b] Reaction time of 9 h for **1a–f** and 12 h for **1g,i–k**. [c] Reaction time of 48 h. [d] Determined by ^1H NMR using (+)-Eu(hfc)$_3$. [e] At 23–27 °C. [f] Determined by comparing the specific rotations of **2** or **2′** with those reported. [g] Extracted by using the Soxhlet extractor after centrifugation. [h] Amount of bakers' yeast used initially was again added when the fermentation ceased owing to the addition of the substrate. [i] Aqueous substrate solution was added dropwise over 8 h. [j] Substrate was added in four portions at 0, 3, 9, and 18 h. [k] Substrate was added dropwise over 2 h. [l] Rotation of (*S*)-**2′a**: $[\alpha]^{23-25}_D$ +33.3° (*c* 1.2, $CHCl_3$), prepared by bakers' yeast reduction of **1a**, ref 12. [m] Rotation of (*R*)-**2′b**: $[\alpha]^{23}_D$ −35.7° (*c* 1, $CHCl_3$), prepared by depolymerization of a mixed biopolymer, ref 22; $[\alpha]^{23}_D$ −37.8° (*c* 1.30, $CHCl_3$), prepared by a microbial process and purified by crystallization to 100% ee (HPLC), ref 21b; $[\alpha]^{23}_D$ −37.0° (*c* 2.00, $CHCl_3$), prepared from a chiral β-keto sulfoxide, ref 23. [n] Rotation of (*R*)-**2′c**: $[\alpha]^{23-25}_D$ −27.1° (*c* 1, $CHCl_3$), prepared by bakers' yeast reduction of **1c**, ref 12. [o] Rotation of (*R*)-**2′e**: $[\alpha]^{23-25}_D$ −18.4° (*c* 1, $CHCl_3$), prepared by bakers' yeast reduction of **1e**, ref 12. [p] Rotation of (*R*)-**2′j**: $[\alpha]^{25}_D$ −14.3° (*c* 2.5, $CHCl_3$), prepared from (+)-methyl hydrogen β-acetoxyglutarate, ref 6. [q] Rotation of (*R*)-**2′k**: $[\alpha]^{25}_D$ −15.0 (*c* 1.9, $CHCl_3$), isolated from the red yeast *Rhodotorula*, ref 6; $[\alpha]^{23}_D$ −15.1° (*c* 0.56, $CHCl_3$), prepared from a chiral β-keto sulfoxide, ref 23.

reduction of γ- and δ-keto acids reported previously.[16] Novel aspects of the bakers' yeast reduction of β-keto acids are also disclosed, in an attempt to demonstrate that the bioreduction remains a convenient and useful method in organic syntheses.

Results and Discussion

Conditions for Fermentation and Reduction. Using the substrate **1k**, we examined the conditions for yield optimization. The best conditions required the use of 6 g of pressed yeast per mmol of substrate, 3 g of glucose, a pH value of 4.8–6.0, a temperature of 27–28 °C, and a reaction time of 12 h. Glucose was added successively as it was consumed. A decrease in yield was observed in the absence of glucose, even when several times the amount of yeast was used. The optimal pH was maintained by addition of KH_2PO_4 during the whole period of fermentation. Deviations from the pH range resulted in low yields.[1] Higher temperature (36–38 °C) or extremely long reaction time (6–7 days) with additional amounts of yeast afforded lower yields (10–36%).

The time course of the reduction was followed using **1e** and **1j** as shown in Figures 1 and 2. The maximum yield was found at 9 h for **1e** and at 12 h for **1j**. Therefore, a reaction time of 9 h was used for **1a–e** and that of 12 h was used for **1f–k**. For comparison, the reaction time of 48 h was also adopted. Dry bakers' yeast also gave yields comparable to those obtained with pressed yeast.

Table II. Decarboxylation of Acetoacetic Acid (1a) in Deuterium Oxide[a]

	% decarboxylation			
time, h	pD 2–3	4–5	5–6	7–9[b]
2	15	3	1	1
5	41	22	2	1
10	59	22	7	3
24	79	26	7	4

[a] At 30 °C. The % decarboxylation was measured by ^1H NMR. [b] By the titration of CO_2 evolved, the decarboxylation was determined to be 8.5% in water at pH 8 after 48 h. See: Passingham, B. J.; Barton, R. N. *Anal. Biochem.* **1975**, *65*, 418.

Chemical Yields. The results of the bakers' yeast reduction of β-keto acids **1a–k** are presented in Table I. The products were isolated as methyl esters. The yield was low with lower aliphatic β-keto acids **1a** and **1b** and increased rapidly to a maximum with 3-oxooctanoic acid (**1e**). A similar behavior was observed previously with γ- and δ-keto acids and attributed to sluggishness of the reduction for the short-chain keto acids.[16] In the present case, however, the low yield could largely be attributed to losses during the extraction process. This was ascertained by pilot experiments in which a sample of racemic 3-hydroxybutanoic acid was recovered from an aqueous solution by a continuous extraction method[17] in the presence (31% recovery) and in the absence of bakers' yeast (46% recovery). The 15% difference in recovery was the loss due to uptake and/or adhesion to the yeast cells. Additional losses in yield were incurred probably due to decarboxylation[18] of the β-keto acid **1a**. We examined the decarboxylation of **1a** in deuterium oxide at pD 2–7 by using ^1H NMR spectroscopy. The results shown in Table II indicate that **1a** was decarboxylated rather slowly to the extent of 7% at pD 5–6 in 10 h. This is in good agreement with an estimation of 18% (difference between the 31% recovery and the 13% reduction yield), although the conditions of the decarboxylation experiment are considerably different from those of the bakers' yeast reduction.

Unexpectedly, β-keto acids **1f–i** with an alkyl group of n-C_8H_{17} to n-$C_{11}H_{23}$ inhibited the fermentation so severely that glucose was not consumed after addition of those acids.[19] The isolated yields in these cases were 36% for n-C_8H_{17}, 0–2% for n-C_9H_{19} and n-$C_{10}H_{21}$, and 0–12% for n-$C_{11}H_{23}$. This was circumvented by adding dropwise an aqueous solution of the substrate over 8 h or in four portions at reaction times of 0, 3, 9, and 18 h. The yields are shown in Table I. The fermentation was still suppressed in these cases, but it was not inhibited.[20] The yield was further improved by adding an additional amount (6 g) of yeast when the fermentation was suppressed after addition of about one-tenth of the substrate

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(20) The amounts of glucose consumed per g of pressed yeast during 48 h after addition of the substrates were R = C_1, 2.5 g; C_2, 2.5 g; C_3, 3.0 g; C_4, 2.0 g; C_5, 1.3 g; C_8, <0.25 g; C_9, <0.5 g, <0.5 g (dropwise addition); C_{10}, 1.0 g (dropwise addition); C_{11}, 1.0 g (dropwise addition); C_{13}, 2.5 g; C_{15}, 2.8 g.

solution. The improved yields were 51% for **1f** and 22% for **1i**.

The longer alkyl groups of n-$C_{13}H_{27}$ and n-$C_{15}H_{31}$ for **1j** and **1k** showed no inhibitory effect on fermentation as indicated by the glucose consumption (2.5–2.8 g). Their lower yields (40–56%) could be attributed to uptake of the substrate and/or product to the yeast cells.

Time Course of the Reduction. Figures 1 and 2 reveal several interesting facts. The reduction is rather rapid and reaches a maximum yield after 9–12 h for both the medium- and the long-chain substrates **1e** and **1j**. Apparently β-keto acid **1j** is reduced more rapidly than a δ-keto acid (5-oxohexadecanoic acid)[16] of comparable size, which can be attributed to the proximity of the carboxyl group to the ketone carbonyl group. The decrease in the yield for both **1e** and **1j** in the course of longer reaction times, which were not observed for the δ-keto acid, may be due to loss of β-hydroxy acids by yeast cell metabolism. The recovery of methyl ketone (2-pentadecanone) indicated decomposition of the substrate by decarboxylation during the reduction and the workup. As can be seen in Figure 2, the recovery of methyl ketone decreases rapidly as the reduction proceeds rapidly, and a minimum recovery of about 10% is reached after about 12 h, when a maximum yield of 56% for the reduction was attained. Apparently, 10% of the substrate was decarboxylated before the reduction was completed in 12 h, which is comparable with 7–18% loss deduced for acetoacetic acid during the reduction.

Optical Purities. The optical purities were ≥98% ee in all cases, except for **2'a** (Table I). This high purity is common for β-,[13] γ-,[16] and and δ-keto[16] acids as substrates and is higher than that obtained with the corresponding esters. For instance, the ethyl ester of **1b** was reported to give the corresponding (*R*)-β-hydroxy ester only in 40% ee.[9d] Optically pure (*R*)-**2b** or its derivatives have been obtained via elaborate methods.[9g,21-23]

On the other hand, methyl ester **1'b** was reported to give **2'b** in varying optical purities of 33–54% ee with pressed bakers' yeast and of 7–12% ee with dry bakers' yeast.[9o] In contrast, using **1b** as substrate, we obtained **2'b** invariably in 98% ee with both pressed and dry bakers' yeast. This difference between the keto acid and the keto ester can be explained by assuming that the keto acid is reduced by a single enzyme or enzymes of the same steric outcome. It is known that the keto esters are reduced by both D and L enzymes,[9k] and the contribution of each enzyme is considered to vary from pressed to dry yeast.[9o]

The optical purity of **2'a** was found to be 86% ee. This result is not clear, but may be explained by investigating related enzyme(s).

Absolute Configuration. The absolute configurations of **2**, except for **2a**, were determined to be *R* by comparing the specific rotations of free acids **2i**[15a,24] and **2j**[15a] and methyl esters **2'b**,[23] **2'c**,[12] **2'e**,[12] **2'j**,[6] and **2'k**[6,23] with those reported. The configurations of **2'd**, **2'f**, **2'g**, and **2'h** were reasonably determined to be *R* by comparing their specific rotations with those of the (*R*)-hydroxy esters **2'** mentioned above. The configuration of **2'a** produced by the bakers' yeast reduction was reported in 1951 to be *S* by Lemieux and Giguere.[12] We obtained a comparable value of the specific rotation for **2'a**, as compared to that reported by them. They were not able to determine the optical purities at that time but were interested in the observation that 3-oxohexanoic and 3-oxooctanoic acids **1c** and **1e** are reduced to (*R*)-(−)-3-hydroxy acids **2c** and **2e** opposite to the reduction of **1a**. In the present work, it became clear that an almost complete change in configuration occurs between **1a** and **1b** from 86% ee *S* to 98% ee *R*. To explain the change in terms of the Prelog rule, one must assume that the ethyl group is larger than the carboxyl group in **1b**. This is obviously difficult. As discussed in the cases of γ- and δ-keto acids,[16] the carboxyl group must be taken as a functional group that determines the stereochemical course of the reduction. Therefore, we consider that **1a** is mainly reduced by *S* enzyme(s) to give the *S* configuration while **1b** and others are exclusively reduced by *R* enzyme(s) to give the *R* configuration.

Table III. Methyl 3-Oxoalkanoates (1') and Optically Pure Methyl 3-Hydroxyalkanoates (2')

	$RCOCH_2CO_2CH_3$ (1')[a]		$RCH(OH)CH_2CO_2CH_3$ (2')[b]
R	yield,[c] %	bp (Torr) or mp, °C	bp (Torr) or mp, °C
b C_2H_5	62	90–100 (7)	80–120 (12)
c n-C_3H_7	69	90–100 (5)	60–70 (2)
d n-C_4H_9	63	120–130 (5)	65–75 (2)
e n-C_5H_{11}	55	80–90 (3)	110–120 (5)
f n-C_8H_{17}	57	110–120 (2)	155–165 (3)
g n-C_9H_{19}	56	145–155 (2)	26.5–28.5
h n-$C_{10}H_{21}$	56	21.6–23.8	34.5–35.7
i n-$C_{11}H_{23}$	79	28.0–30.0[d]	39.4–40.6
j n-$C_{13}H_{27}$	85	40.2–41.4[e]	47.6–48.6[f]
k n-$C_{15}H_{31}$	79	46.2–48.0	55.6–56.0[g]

[a] Compounds 1'**b**,**c**,**e** were reported in ref 27 and 1'**g**,**i**,**j** in ref 15a. Satisfactory analytical data (±0.3% for C and H) were obtained for 1'**c**,**d**,**e**,**f**,**h**,**i**,**k**. [b] Satisfactory analytical data (±0.3% for C and H) were obtained for **2'd–k**. [c] Based on the starting ester 1'**a** after distillation or LC. [d] Reference 15a, mp 30 °C. [e] Reference 15a, mp 41 °C. [f] Reference 6, mp 48–49 °C. [g] Reference 6, mp 55.5–56.5 °C.

Preparation of (+)-Corynomycolic Acid (4).[23,25] In a recent communication,[1] we reported that the optically pure acid 4 was prepared by α-alkylation of the dianion[26] of **2'k** with tetradecyl iodide, followed by hydrolysis. This method is simple and straightfoward, although the anti/syn ratio of the alkylation was 94/6.

Conclusions. 3-Oxobutanoic acid (**1a**) was reduced to (*S*)-3-hydroxybutanoic acid (**2a**) (isolated as methyl ester **2'a**), but the yield was low (13%) and the optical purity was not very high (86% ee). In contrast, 3-oxopentanoic acid (**1b**) was reduced to (*R*)-3-hydroxypentanoic acid (**2b**) (isolated as **2'b**) in 20% yield with 98% ee. This very high optical purity is attractive if the low yield can be tolerated, since it seems to be difficult to obtain optically pure (*R*)-**2b** or its derivatives. Similarly, the other higher homologues (*R*)-**2c–k** can be obtained by the present method in 19–71% yields with >98% ee. Efforts must be made to improve the chemical yield.

Experimental Section

All boiling and melting points are uncorrected. ^1H NMR spectra were obtained at 60, 100, 200, and 500 MHz. ^{13}C NMR spectra were obtained at 126 MHz.

Materials. Methyl acetoacetate (1'**a**) and ethyl acetoacetate were purchased from Tokyo Kasei. Other methyl 3-oxoalkanoates (1'**b–k**) were prepared by the reaction of dianion of 1'**a** with alkyl

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Table IV. ^{1}H NMR Chemical Shifts (δ) of the Ester Methyl Groups in Racemic $RCH(OH)CH_2CO_2CH_3$ (2′) in the Presence of (+)-Eu(hfc)$_3$[a]

R	(+)-Eu-(hfc)$_3$, mol %	δ	R	(+)-Eu-(hfc)$_3$, mol %	δ
CH_3	20	4.02, 4.15	n-C_8H_{17}	30	4.72, 4.96
	30	4.18, 4.33	n-$C_{11}H_{23}$	30	4.18, 4.34
n-C_3H_7	30	4.18, 4.31[b]	n-$C_{13}H_{27}$	30	4.22, 4.38
n-C_4H_9	30	4.15, 4.35	n-$C_{15}H_{31}$	10	4.02, 4.13
n-C_5H_{11}	30	4.22, 4.38		20	4.29, 4.41

[a] In CCl_4-$CDCl_3$ (3:1) at room temperature (200 MHz for R = CH_3 and 100 MHz for the others). [b] At 55 °C.

bromides or iodides according to the method reported.[27] Their yields and physical data are listed in Table III. Pressed and dry bakers' yeasts were purchased from Oriental Yeast. A chiral shift reagent, tris[[3-(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]europium(III) [(+)-Eu(hfc)$_3$] was purchased from Aldrich.

Bakers' Yeast Reduction of Acetoacetic and 3-Oxopentanoic Acids 1a,b. Use of the Soxhlet Extractor. A typical procedure is as follows. In 4 mL of ethanol was dissolved 520 mg (4.0 mmol) of ethyl acetoacetate with 8 mL of 1 M KOH. The solution was stirred overnight at room temperature. After evaporation of the ethanol under reduced pressure, the residue was added with 72 mL of water to the vigorously fermenting culture suspension, which involved 24 g of pressed bakers' yeast, 90 mg of KH_2PO_4, and 12 g of glucose in 72 mL of water at 28 °C. The suspension was adjusted to pH 5 with KH_2PO_4 and stirred for 9 h, during which time 48 g of glucose was added in four portions as the glucose was consumed. The consumption was checked by use of glucose test paper (Diasticks II, Miles-Sankyo). Then the suspension was adjusted to pH 7 with $NaHCO_3$ and centrifuged (10000 rpm, 20 min) to give 300 mL of the supernatant. This was concentrated to 20 mL under reduced pressure and acidified to pH 2 with concentrated H_3PO_4. This solution was taken up in 80 mL of anhydrous Na_2SO_4. Then the powder was extracted with ether by use of the Soxhlet extractor for 12 h. The ether extract was treated with diazomethane and the crude product obtained was purified by LC (Merck silica gel 60, hexane–AcOEt 20:1→5:1) to afford 63 mg (13%) of (*S*)-methyl 3-hydroxybutanoate (**2′a**): $[\alpha]^{28}_D$ +36.2° (*c* 1.17, $CHCl_3$). The ee was determined to be 86% by measuring the 200-MHz ^{1}H NMR spectra in the presence of 20 mol % (+)-Eu(hfc)$_3$ in CCl_4-$CDCl_3$ (1:1).

Bakers' Yeast Reduction of 3-Oxoalkanoic Acids 1a–k. Conventional Extraction. A typical procedure is as follows. After being stirred for 9–48 h or more, the culture suspension was cooled in an ice bath and stirred with 24 g of Celite per 12 g of yeast for 6 h. The mixture was filtered and the filtrate was acidified to pH 2 with 10% HCl. This was extracted with ethyl acetate and the Celite was washed with acetone. The combined organic extracts were washed with saturated aqueous NaCl and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residue was treated with diazomethane and passed through a silica gel column (Katayama gel 60, hexane–AcOEt 15–20:1→10–15:1) to give optically active or pure methyl 3-hydroxyalkanoate **2′a–k**. Methyl ketones as decarboxylation products were detected by GLC with the aid of authentic samples. Analytically pure samples of 2′ were obtained by HPLC (Yanapac silica gel SA-1, 7.5 × 200 mm, hexane–AcOEt 4–5:1). **Dropwise addition of substrates 1g,h,i was carried out as follows**: The hydrolyzates of **1g–i** were concentrated to remove ethanol, diluted with 36 mL of water, and added dropwise to the fermenting yeast suspension from a dropping funnel over 8 h.

Time Course of the Reduction. This was obtained by carrying out the runs with respective reaction times.

Determination of the Optical Purity (% ee) by ^{1}H NMR Spectroscopy. The methyl esters 2′ were dissolved in a mixture of CCl_4–$CDCl_3$ (3:1, dried over molecular sieves 3A) and the ^{1}H NMR spectra were measured in the presence of 10–30 mol % (+)-Eu(hfc)$_3$ at 100 MHz. The esters 2′ showed a sharp singlet around δ 4.0–4.4 for the ester methyl group. The appearance of a signal due to the enantiomer was checked with the aid of a pair of signals due to the racemic methyl ester 2′, which is shown in Table IV.

Decarboxylation of Acetoacetic Acid in D_2O. In 5 mL of methanol, 1.16 g (10 mmol) of methyl acetoacetate was dissolved with 10 mL of 1 M NaOH. The solution was stirred overnight and evaporated to dryness. A 196-mg sample of the residual sodium salt was dissolved in 2 mL of D_2O and neutralized to pD 7.01 with 0.1 mL of 1 M DCl. A 0.4-mL aliquot of this solution was transferred to an ^{1}H NMR sample tube immediately. The decrease of the signal for the acetyl methyl group at δ 2.26 and the increase for acetone-*1,1,1-d*$_3$ at δ 2.19 were monitored at 30 °C over 24 h at 60 MHz using sodium 3-(trimethylsilyl)-propanesulfonate as internal standard. To the remaining stock solution of pD 7.01 was added 0.35 mL of 1 M DCl to prepare a solution of pD 5.08. In a similar way, the decarboxylation at pD 5.08 was monitored. A solution of pD 3.91 was prepared by adding 0.2 mL of 1 M DCl and that of pD 1.96 was prepared with 0.2 mL of 1 M DCl successively. The pD of solution increased as the decarboxylation proceeded. The pD ranges are shown in Table II. The % formation of acetone-*1,1,1-d*$_3$, which is equal to the % decarboxylation, was calculated by using the intensity of the signals cited above. The pD was calculated by adding 0.4 unit to the observed pH meter reading.[28] The pH measurements were carried out by using a Horiba H-7LD pH meter equipped with a Horiba combined glass-calomel electrode.

(*R*)-3-Hydroxytetradecanoic Acid (2i). In 2 mL of ethanol was dissolved 101 mg (0.39 mmol) of **2′i** with 1 mL of 1 M KOH. This was stirred overnight at 30 °C, acidified with hydrochloric acid, and evaporated under reduced pressure. The residue was mixed with water and extracted with methylene chloride to give 66 mg (69%) of **2i**: mp 71.0–71.5 °C (lit. mp 73–74 °C,[24] 72.0 °C[15a]); $[\alpha]^{22}_D$ −16.2° (*c* 1.00, $CHCl_3$) [lit. $[\alpha]^{25}_D$ −16° (*c* 2, $CHCl_3$),[24] $[\alpha]^{20}_D$ −16.2° (*c* 1, $CHCl_3$)[15a]]; IR (KBr) 3550, 3500–2400, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (br t, 3 H), 1.1–1.7 (m, 20 H), 2.38–2.62 (m, 2 H), 4.01 (m, 1 H), 6.0 (br s, 2 H).

(*R*)-3-Hydroxyhexadecanoic Acid (2j). The methyl ester **2′j** (201 mg, 0.76 mmol) was hydrolyzed in ethanol (3 mL)–1 M KOH (3 mL) at 30 °C for 24 h with stirring to yield **2j** (171 mg, 90%): mp 76.8–77.8 °C (lit.[15a] mp 76.8 °C); $[\alpha]^{22}_D$ −19.2° (*c* 1.18, $CHCl_3$) [lit.[15a] $[\alpha]^{20}_D$ −13.8° (*c* 1, $CHCl_3$)]; IR (KBr) 3550, 3500–2400, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (br t, 3 H), 1.1–1.7 (m, 24 H), 2.40–2.57 (m, 2 H), 4.00 (m, 1 H), 6.0 (br s, 2 H).

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Supplementary Material Available: IR and ^{1}H NMR spectra of 1′ and 2′ and experimental procedures for the preparation of (+)-corynomycolic acid [(2*R*,3*R*)-4] with data of 500-MHz ^{1}H and 126-MHz ^{13}C NMR (5 pages). Ordering information is given on any current masthead page.

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