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Bioreduction of β-keto esters with Active Dried Baker's Yeast in Organic Solvent System; Such as n-Hexane, Pentane or Petroleum ether.

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Abstract : Bioreduction with active dried baker's yeast proceeded smoothly in n-hexane, pentane or petroleum ether as an organic solvent system. Ethyl(**1**) and octyl 3-oxohexanoate (**2**) were reduced to (*R*)-ethyl(**3**) and (*S*)-octyl 3-hyroxy-hexanoate(**4**) with high enantiomeric excess, respectively(Received June 27 1994, accepted October 6, 1994).

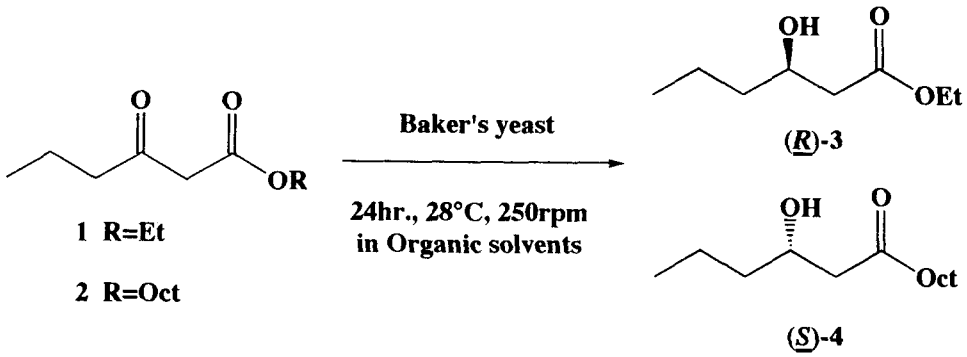
Introduction

Use of enzyme-catalyzed processes to accomplish asymmetric syntheses has provided an effective method of preparing optically pure synthon for synthesis of biologically active substance in recent years.[1] Especially asymmetric microbial reduction of the carbonyl group by common baker's yeast has gained widespread popularity as a useful tool for obtaining optically active alcohols. However, it is usually performed in an aqueous solution of glucose or sucrose as an energy source.[2] For synthetic purpose, yeast as an available reagent/catalyst is required. It needs to keep its activity in organic solvents with a substrate than in an aqueous solution.

Based upon this idea, ethyl(**1**) and octyl 3-oxohexanoate(**2**) were successfully reduced to the corresponding useful chiral synthons, (*R*)-ethyl(**3**) and (*S*)-octyl 3-hydroxyhexanoate(**4**), by using active dried baker's yeast[3] in n-hexane, pentane or petroleum ether as an organic solvent system (Scheme 1).

Materials and Methods

^{1}H and ^{13}C-NMR spectra were recorded on JEOL



Scheme 1. Bioreduction of β-keto ester using organic solvent system.

Key words : Bioreduction, immobilized yeast,
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JNM-GX-270 spectrometers with $CDCl_3$ as solvent, unless otherwise stated, using tetramethylsilane as the internal standard. NMR chemical shifts are given in δ values as ppm downfield of tetramethylsilane. IR spectra were measured on a JASCOIR-810 and Perkin Elmer 881 infra-red spectrometer. Optical rotations were measured using a Perkin Elmer 241MC polarimeter. Mass spectra were recorded on a JEOL HX-105 mass spectrometer.

All organic solvents were distilled prior to use and purification of all compounds was carried out by bulb-to-bulb distillation. Reactions involving chemicals or intermediates sensitive to air and/or moisture were performed under a nitrogen atomsphere.

Preparation of β-keto esters.

2,2-Dimethyl-1,3-dioxane-4,6-dine (meldrum's acid, **5**): Concentrated sulfuric acid (0.6 m*l*) was added to a suspension of malonic acid (20.8 g, 0.20 mol) in acetic acid (30 m*l*, 0.30 mol). The mixture was cooled to 0℃ and aceton (20 m*l*) was added dropwise over a period of 1 hr. The resulting solution was stirred for a further hour then left to stand in a refrigerator. Recrystallization from acetone/water afforder Meldrum's acid **5** (13.1 g, 46%) as needles m.p. 94~96℃. IR ν_{max} (KBr) cm^{-1}: 1780. ^{1}H-NMR (270 MHz) δ: 1.78 (6H, s), 3.61 (2H, s). ^{13}C-NMR (270 MHz) δ: 27.40 (CH_3), 36.05 (CH_2), 106.18 (C-O), 162.94 (C=O).

The acylated Meldrum's acid (**6**): Pyridine (14.0 g, 0.176 mol) was added dropwise over a period of 20 min. to a solution of Meldrum's acid **5** (10.0 g, 0.069 mol) in dry CH_2Cl_2(30 m*l*) at ca. −5℃ under a nitrogen atomsphere. To a solution of n-butyryl chloride (7.4 g, 0.07 mol) in CH_2Cl_2 (26 m*l*) was added dropwise over a period of 1 hr. The reaction mixture was stirred at 0℃ for 1 hr and then for 1 hr at room temperature, diluted with CH_2Cl_2 (20 m*l*) and poured into cold 1N-HCl solution (50 m*l*). The organic phase was separated and the further extracted with CH_2Cl_2. The combined organic extracts were washed with 1N-HCl, water, brine, and dried over anhydrous $MgSO_4$. After evaporating the solvent, the acylated Meldrum's acid **6** (13.8 g, 96%) was obtained as a brown oil, which was not purified further. ^{1}H-NMR (270 MHz) δ: 1.02 (3H, t, J=6.98 Hz), 1.76 (s, 6H), 1.78 (2H, sextet, J=6.98 Hz), 3.12 (2H, t, J=6.96 Hz).

Ethyl3-oxohexanoat(**1**): Treatment of the acylated Meldrum's acid (**6** 21.4 g, 0.1 mol) with dry ethanol (250 m*l*) under reflux followed by removal of the solvent under reduced pressure afforded a dark red oil. Distillation under aspirator yielded β-keto ester **1** (14.2 g, 89% from **6**), b.p.$_{18}$~95℃. HRMS; Found: M^+ 158.0932, $C_8H_{14}O_3$ requires 158.0943. IR ν_{max} (film)cm^{-1}: 1748, 1718. ^{1}H-NMR (270 MHz) δ: 0.94 (3H, t, J=6.9 Hz), 1.28 (3H, t, J=7.2 Hz), 1.63 (2H, sextet, J=7.3 Hz), 2.53 (2H, t, J=7.3 Hz), 3.44 (2H, s), 4.19 (2H, 1, J=7.1 Hz), ^{13}C-NMR (270 MHz) δ: 13.10 (CH_3), 13.72 (OCH_2CH_3), 16.71 (CH_2CH_3), 44.53 ($CH_2XH_2CH_3$), 48.96 (CH_2CO), 60.98 (OCH_2CH_3), 164.08 (c=o), 202.46 (C=O).

Octyl3-oxohexanoate(**2**): Treatment of the acylated Meldrum's acid (**6**, 10.71 g, 0.05 mol) in dry benzene (130 m*l*) with octanol (6.53 g, 0.052 mol) under reflux followed by removal of the solvent *in vacuo* afforded a red oil. Bulb to bulb distillation under reduced pressure yielded β-keto ester **2** (10.21 g, 84% from **6**). HRMS; Found: M^+ 242.1873, $C_{14}H_{26}O_3$ requires 242.1882. IR ν_{max}(film)cm^{-1}L 1732, 1714. ^{1}H-NMR (270 MHz) δ: 0.86 (3H, t, J=7.3 Hz), 0.93 (3H, t, J=7.1 Hz), 1.06~1.43 (10H, m), 1.53~1.71 (4H, m), 2.52 (2H, t, J=7.2 Hz), 3.43 (2H, s), 4.12 (2H, t, J=7.1 Hz).

Bioreduction of β-keto esters in n-hexane, pentane or petroleum ether; General procedure.

Active dried baker's yeast (4.3 g) was suspended in 60 m*l* of organic solvent in the presence of 2 %v/v of water. The β-keto esters (**1** and **2**, 5 mmol) were added to the suspension and the mixture shaken (ca. 250 rpm) for 24 hr at 28℃. The reaction mixture filtered and the solvent removed *in vacuo* yielding a green oil. Bulb to bulb distillation afforded the corresponding products as a colourless oil.

(*R*)-Ethyl-3-hydroxyhexanoate(**3**): $[\alpha]_D^{20}$ −22.7℃ (c 1.08 in $CHCl_3$), lit[4] $[\alpha]_D$ −22.1° (c 1.04 in $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3450, 1728, ^{1}H-NMR (270

MHz) δ: 0.96 (3H, t, J=7.1 Hz), 1.26 (3H, t, J=7.1 Hz), 1.34~1.56 (4H, m), 2.36~2.58 (2H, m), 2.96 (1H, br.s), 4.01~4.05 (1H, m), 4.17 (2H, q, J=7.1 Hz). ^{13}C-NMR (270 MHz) δ: 13.34 (CH_3), 14.16 (OCH_2CH_3), 18.69 (CH_2), 38.92 (CH_2), 41.50 (CH_2 CO), 60.45 (OCH_2CH_3), 67.81 (HCOH), 173.20 (C=O). MS *m/z*: 160 (M^+)

(*S*)-Octyl-3-hydroxyhexanoate(**4**): $[\alpha]_D^{20}$+13.1° (c 1.15 in $CHCl_3$), lit[5] $[\alpha]_D$+10.94 (c, 2.13 in $CHCl_3$), IR ν_{max}(film)cm^{-1}; 3448, 1732. ^{1}H-NMR (270 MHz) δ: 0.93 (3H, t, J=7.1 Hz), 0.98 (3Gm tm H=7.1 Hz), 1.19~2.66 (18H, m), 2.82 (1H, br.s), 3.98~4.09 (1H, m), 4.12 (2H, t, J=7.1 Hz). ^{13}C-NMR (270 MHz) δ: 13.68 (CH_3), 13.92 (CH_3), (each CH_2) 18.52, 22.49, 25.78, 28.42, 29.13 (2xCH_2), 31.68, 38.54, 41.49, 64.73, 67.72 (HCOH), 172.91 (C=O), MS *m/z*: 244 (M^+).

Results and Discussion

A convenient route for the synthesis of a range of β-keto ester **1** and **2** was from acylation of Meldrum's acid **5**, the product from the acid catalyzed reaction of malonic acid and acetone. ^{1}H-NMR of this acylated Meldrum's acid (**6**) indicated that it exists predominantly in the enol tautomer. In the spectrum of this enol tautomer (**6**), enone form showed as a singlet at δ 3.66 ppm (ca, 7%). Reaction of this acylater Meldrum's acid (**6**) with ethanol or octanol under reflux yielded, on removal of the solvent and distillation, ethyl (**1**) or octyl 3-oxohexanoate (**2**) in over 84% from Meldrum's acid. A general mechanism for these reaction can be written through the expected intermediary metabolite **7** (Scheme. 2).

In n-hexane, pentane or petroleum ether as organic solvent, microbial reduction of β-keto ester **1** and **2** with active dried Baker's yeast (*Saccharomyces cerevisiae*) gave (*R*)-ethyl (**3**) and (*S*)-octyl 3-hydroxy-hexanoate (**4**), respectively, in high enantiomeric excess after 24 hr at 28℃. The active dried Baker's yeast was commercially and cheaply available pure strain.[3] The absolute stereochemistry was

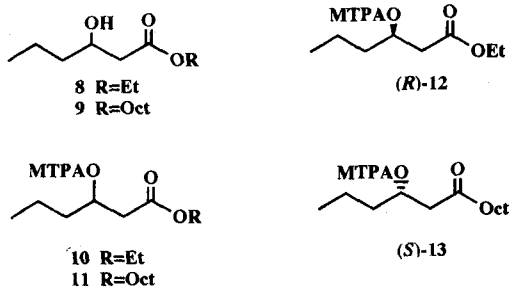
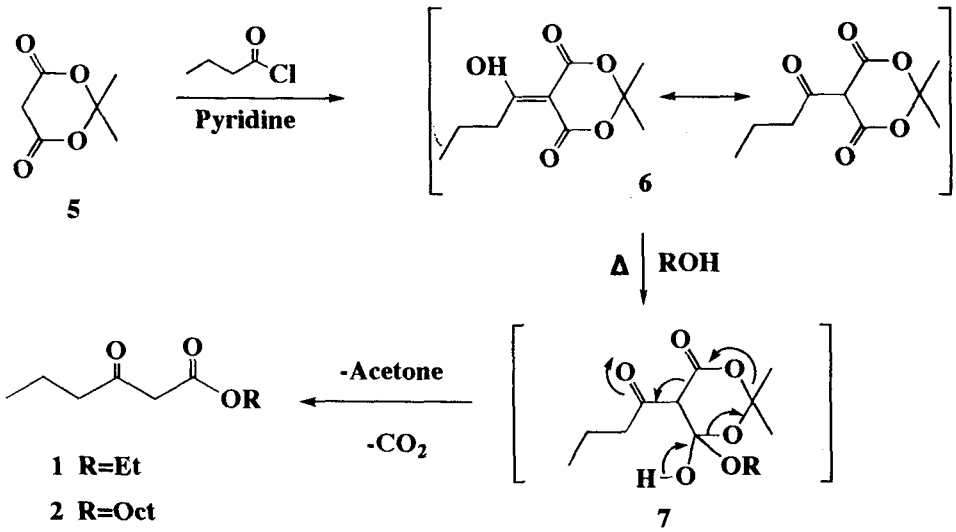


Fig. 1. The products of MTPA esters and racemic hydroxy esters.



Scheme 2. Preparation of ethyl(**1**) and Octyl(**2**) 3-oxohexanoate.

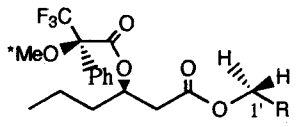
Table 1. Bioreduction of β-keto esters **1** and **2** with dry active Baker's yeast in organic solvent

Solvent	(*R*)-**3**			(*S*)-**4**		
	%Yield	%ee	$[\alpha]_{20}^{D}$	%Yield	%ee	$[\alpha]_{20}^{D}$
n-Hexane	68	95	−22.0	12	80	+12.3
n-Petane	43	≥94	−21.9	−	−	−
Petroleum ether	62	98	−22.7	13	85	+13.1

Table 2. ^{1}H-NMR data of MTPA esters

Compoung No.	Chemical shift, ppm			
	Methyl ester*		1'-Proton	
10	3.52	3.56	4.01	4.11
11	3.52	3.54	3.99	4.07
(*R*)-12	3.56		4.11	
(*S*)-13	3.52		3.99	

The MTPA esters was purified by preparative t.l.c. plate (Merk kieselgel $60F_{254}$, 20×20×0.1 cm)



assigned by comparison of the optical rotation with the literature.[4,5] The enantiomeric purity was determined from ^{1}H-NMR of the corresponding MTPA esters.[6] Reduction of β-keto esters **1** and **2** with $NaBH_4$-THF provided the corresponding racemic hydroxy esters **8** and **9**, respectively, as standards. Comparison of methyl ester signals and 1'-proton signals from their ^{1}H-NMR in the chiral products **12-13** with that of the MTPA ester from racemic materials]-[[clearly showed the level of enantiomeric excess (Fig. 1). Bioreduction of octyl 3-oxohexanoate (**2**) attempt to use substrate control to influence the stereochemical outcome of the reaction. However, the isolated yield was only 13%. A number of possible modifications were not attempted to increase the yield. The results are summarized in Tables 1 and 2.

A recent study of bioreduction in this type system has been carried out by four reports.[7-10] The use of immobilized yeast was reported to give improvements. not only in opitical purity but also in ease of product isolation, dependent on the nature of the polimeric matrix used. Immobilizing the yeast, as before, with magnesium alignate and performing the reaction with a high concentration of metal ions provide the opposite enantiomer to the reaction run under normal conditions.[9] In the presence of magnesium or calcium ion, potassium, with chloride as the counter ion the optical purity of the products increased as a function of the molarity. But, immobilization of Baker's yeast is time consuming and expensive.

From bioreduction utilizing active dried Baker's yeast in organic solvent system, compared with water system, has been found to have advantage in terms of the separation of products from the catalyst. And, this system with commercially available yeast would be a simpler and more cost effective approach. This type system should prove useful in asymmetric syntheses of natural products, especially from the polyketide biosynthetic pathway. The investigation on the mechanism in the yeast cell is now in progress.

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유기용매에서 활성 빵효모를 이용한 β-*keto ester*의 생물학적 환원

고병섭(성균관대학교 기초과학연구소)

초록 : 활성 빵효모를 이용한 생물학적 환원은 n-hexane, pentane과 석유에테르와 같은 유기용매에서 순조롭게 진행되어, ethyl(1)과 octyl 3-oxohexanoate(2)는 높은 %ee로 환원되었다. 활성 빵효모에 의한 생물학적 환원은 물보다 유기용매에서 생성물의 정제가 간편하고 경제적 효과가 크다는 이점이 있었다. 이러한 생물학적 환원 방법은 광학활성 천연물의 합성에 이용할 수 있으리라 생각되어 진다.