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Stereocontrolled reduction of α-keto esters with thermophilic actinomycete, *Streptomyces thermocyaneoviolaceus* IFO 14271[1]

Kohji Ishihara [a,*], Hitomi Yamaguchi [b], Hiroki Hamada [c], Nobuyoshi Nakajima [d], Kaoru Nakamura [b]

[a] *Department of Chemistry, Kyoto University of Education, Fushimi-ku, Kyoto 612-8522, Japan*
[b] *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*
[c] *Department of Applied Science, Okayama University of Science, Ridai-cho, Okayama 700-0005, Japan*
[d] *Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1112, Japan*

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Abstract

The reduction of aliphatic and aromatic α-keto esters was carried out using a thermophilic actinomycete, *Streptomyces thermocyaneoviolaceus* IFO 14271, as a biocatalyst. Ethyl 3-methyl-2-oxobutanoate, methyl benzoylformate, and ethyl benzoylformate were reduced to the corresponding (*R*)-alcohols with > 98% enantiomeric excess (ee) at 37°C, while the reduction in the presence of glutamic acid gave the (*S*)-hydroxy esters in excellent ee (> 99%). Ethyl 2-oxopropanoate and ethyl 2-oxobutanoate were also reduced to the corresponding (*S*)-alcohols with > 99% ee in the presence of an amino acid such as asparagine or aspartic acid.

Keywords: Keto ester; Bacteria; Thermophile; Actinomycete; Stereocontrolled reduction

1. Introduction

Biotransformation has been widely studied for the preparation of chiral compounds [1–5]. In particular, yeast and fungi have been often used for the stereoselective reduction of prochiral ketones to obtain optically active alcohols, such as α- and β-hydroxy esters [6–15]. To date, only a few studies of the reduction of various carbonyl compounds with other microorganisms such as bacteria have been reported [16–23]. Among them, the reduction of keto esters by an anaerobic thermophile, *Thermoanaerobactor brockii* DSM 1457, has been reported [16,17]. The commercial TBADH (the alcohol dehydrogenase from *T. brockii*) has high thermostability and high stereoselectivity toward various ketones [24,25]. Therefore, such an enzyme is quite available for organic syntheses. However, little information is known about the reduction of carbonyl compounds using other thermophiles as biocatalysts. We previously reported that aerobic thermophilic bacteria such as *Bacillus stearothermophilus* (DSM 297) and

* Corresponding author. Tel.: +81-75-644-8265; fax: +81-75-645-1734.
E-mail address: kishi@wsml.kyokyo-u.ac.jp (K. Ishihara).

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Pseudonocardia thermophila (IFO 12133) have high reducing abilities toward several keto esters and produced the corresponding chiral alcohols [22,23].

In this paper, we report the stereocontrolled reduction of aliphatic and aromatic α-keto esters with a thermophilic actinomycete, *Streptomyces thermocyaneoviolaceus* IFO 14271.

2. Experimental

2.1. Instruments

Gas chromatography was performed on GL Science GC-353 (DB-Wax, J&W Scientific, USA, 0.25 mm × 30 m; CP-Chirasil-DEX CB, Chrompack, Netherlands, 0.25 mm × 25 m) and Shimadzu GC-9A (Chiraldex G-TA, Astec, USA, 0.25 mm × 40 m) gas chromatographs.

2.2. Materials

S. thermocyaneoviolaceus IFO 14271 used in this study was purchased from the Institute for Fermentation, Osaka (IFO). Ethyl pyruvate (**1a**) was purchased from Wako, Japan. Ethyl 3-methyl-2-oxobutanoate (**1f**) was purchased from Aldrich, USA. Ethyl benzoylformate (**1h**) and methyl benzoylformate (**1g**) were obtained from Tokyo Kasei Kogyo, Japan. Ethyl 2-oxobutanoate (**1b**), ethyl 2-oxopentanoate (**1c**), ethyl 2-oxohexanoate (**1d**), ethyl 2-oxoheptanoate (**1e**), and α-hydroxy esters (**2a–h**) were prepared according to the literature procedure [6]. Bactopeptone and yeast extract were purchased from Difco Laboratories, USA. Meat extract (Ehlrich) was obtained from Kyokuto Pharmaceuticals, Japan. Extrelut® was purchased from Merck, Germany. All other organic reagents and solvents were purchased from Wako, and Nacalai Tesque, Japan.

2.3. Growth medium and cultivation

The following chemicals are per 1 l of distilled water: Bactopepton, 15 g; glycerol, 2 g; KH_2PO_4, 2 g; K_2HPO_4, 2 g; yeast extract, 2 g; meat extract, 2 g; and $MgSO_4 \cdot 7H_2O$, 0.1 g. The pH of the culture medium was adjusted to 7.2 with 0.1 M KOH solution. The actinomycete was aerobically incubated in the medium (800 ml) for 14 h at 45°C with vigorous shaking in a 2-l baffled flask.

2.4. Reduction of α-keto esters with actinomycete

The saline-washed cell (0.5 g), harvested by filtration in vacuo, was resuspended in a large test tube (φ30 × 200 mm) containing 20 ml of saline, then the substrate (0.15 mmol) and glycerol (or additive) were added and incubated. A portion of the reaction mixture was filtered using an Extrelut® short column and extracted with ether, and then concentrated under reduced pressure.

2.5. Analysis

The conversions of the products (**2a–h**) were determined using a GLC equipped with a capillary DB-WAX column (0.25 mm × 30 m, 110–220°C). Chemical yields were determined also GLC equipped with a capillary DB-WAX column with the internal standard (**2a**: tetradecane, **2b**: pantadecane, **2c**: hexadecane, **2d**: diethyl succinate, **2e**: pentadecane, **2f**: pentadecan, **2g**: tetradecane, **2h**: diethyl succinate). The enantiomeric excesses (ee) of the products **2** were determined using a GLC equipped with an opti-

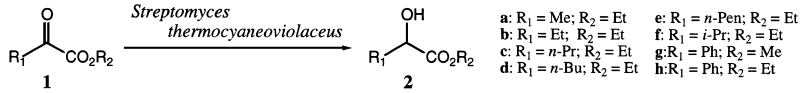

Fig. 1. Reduction of α-keto esters with the thermophilic actinomycete.

Table 1
The reduction of α-keto esters **(1)** to the corresponding alcohols **(2)** with *Streptomyces thermocyaneoviolaceus* IFO 14271[a]

Product	37°C				45°C				55°C			
	Conv.(%)[b]	Yield (%)[b]	ee (%)[c]	(*R*/*S*)[c]	Conv.(%)[b]	Yield (%)[b]	ee (%)[c]	(*R*/*S*)[c]	Conv.(%)[b]	Yield (%)[b]	ee (%)[c]	(*R*/*S*)[c]
2a	> 99	42	68	*S*	> 99	41	74	*S*	> 99	34	96	*S*
2b	> 99	45	35	*S*	> 99	43	62	*S*	> 99	35	88	*S*
2c	> 99	51	37	*S*	> 99	49	22	*S*	99	33	20	*S*
2d	> 99	53	28	*S*	> 99	49	18	*S*	98	31	15	*S*
2e	> 99	43	41	*S*	> 99	45	23	*S*	95	35	17	*S*
2f	> 99	57	> 99	*R*	> 99	53	63	*R*	98	35	45	*R*
2g	> 99	68	98	*R*	> 99	50	62	*R*	98	46	53	*R*
2h	> 99	65	> 99	*R*	> 99	52	52	*R*	97	41	44	*R*

[a] Substrate (7.5 mM), glycerol (250 mM), and saline (20 ml) were added to the wet cell (0.5 g) and the reaction mixture was incubated for 20 h.
[b] Conversion and chemical yields were measured by GLC analysis.
[c] Enantiomeric excesses and configuration were measured by GLC analysis with optically active capillary columns.

cally active capillary CP-Chirasil-DEX CB (0.25 mm × 25 m, 70–130°C) (**2a–e**, **2g**, **2h**) column and Chiraldex G-TA (0.25 mm × 40 m, 90°C) (**2f**) column. The absolute configuration of the isomer was determined by comparing its retention time with those of authentic samples [6].

3. Results and discussion

3.1. Effect of temperature on stereoselectivity

The reduction of the α-keto esters (**1a–h**) with *S. thermocyaneoviolaceus* IFO 14271 at various temperatures was investigated (Fig. 1).

As shown in Table 1, (**1f–h**) were reduced to the corresponding α-hydroxy esters with high enantioselectively (> 99% ee, 98% ee, and > 99% ee, respectively) at 37°C.

The stereochemistry of the produced alcohols tended to change toward the (*S*)-configuration at high temperature. Interestingly, ethyl pyruvate (**1a**) and ethyl 2-oxobutanoate (**1b**) were reduced to the corresponding (*S*)-hydroxy esters (**2a** and **2b**) with high enantioselectively (96% ee and 88% ee, respectively) at 55°C, compared with the reduction at 37°C (68% ee and 35% ee, respectively). Such change in the stereoselectivity of the produced alcohols at various temperatures has been few reported [9,26]. These results (in Table 1) suggest that the (*R*)-hydroxy ester-producing enzyme(s) ((*R*)-producing enzyme(s)) and (*S*)-producing enzyme(s) are present in the *S. thermocyaneoviolaceus* cell and the thermostability of the (*S*)-producing enzyme is superior to that of the (*R*)-producing enzyme(s). The reduction of **1c–e** at high temperature (55°C) gave the corresponding alcohols (**2c–e**) in low yield (17–20%). These results suggest the participation of several (*R*)-producing enzymes having the different substrate specificity and thermostability in the reduction.

3.2. Effect of substrate concentration on stereoselectivity

The effects of the substrate concentration on the reduction of three α-keto esters (**1a**, **1f**, **1h**) with the actinomycete are summarized in Table 2.

For the reduction of **1a**, when the concentration was low, the substrate was reduced to the corresponding alcohol with high 86% ee (*S*), however, the enantioselectivity decreased to a low ee (47% ee) with increasing concentration. On the other hand, **1f** and **1h** were reduced to the corresponding alcohols that had the (*S*)-configuration with increasing concentration. These results suggest that the (*R*)-producing enzyme(s) has a larger K_m value than that of the (*S*)-producing enzyme(s) during the reduction of **1a**, while on the contrary, the (*S*)-producing enzyme(s) has a large K_m value toward **1f** and **1h**.

3.3. Effect of additives on stereoselectivity

Table 3 shows the effect of various additives (mainly amino acids) on the reduction of **1** with

Table 2
Effect of substrate concentration on the stereoselectivity of the reduction[ab]

Product	Concentration of substrate (**1**)									
	2.5 mM		5.0 mM		7.5 mM		10 mM		15 mM	
	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]
2a	86	*S*	79	*S*	74	*S*	54	*S*	47	*S*
2f	73	*R*	68	*R*	63	*R*	61	*R*	54	*R*
2h	69	*R*	65	*R*	62	*R*	45	*R*	41	*R*

[a] Substrate, glycerol (250 mM), and saline (20 ml) were added to the wet cell (0.5 g) and the reaction mixture was incubated for 20 h at 45°C.

[b] Conversions were > 99% in all cases (by GLC analysis).

[c] Enantiomeric excesses and configuration were measured by GLC analysis with optically active capillary columns.

Table 3
Effect of additives on the stereoselectivity of produced α-hydroxy esters[ab]

Product	Additives (500 mM)													
	Alanine		Glycine		Asparagine		Glutamic acid		Aspartic acid		Lactic acid		Malic acid	
	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]	ee (%)[c]	(*R*/*S*)[c]
2a	84	*S*	83	*S*	90	*S*	89	*S*	90	*S*	72	*S*	85	*S*
2b	94	*S*	88	*S*	> 99	*S*	82	*S*	> 99	*S*	84	*S*	98	*S*
2c	> 99	*S*	75	*S*	> 99	*S*	> 99	*S*	> 99	*S*	46	*S*	> 99	*S*
2d	7	*S*	30	*S*	28	*S*	27	*S*	86	*R*	71	*S*	43	*R*
2e	10	*S*	55	*S*	12	*S*	16	*R*	5	*S*	10	*S*	30	*R*
2f	35	*R*	40	*R*	15	*R*	> 99	*S*	36	*R*	41	*R*	20	*R*
2g	82	*R*	79	*R*	86	*S*	> 99	*S*	20	*R*	10	*R*	17	*R*
2h	60	*R*	58	*R*	> 99	*S*	> 99	*S*	35	*R*	30	*R*	26	*R*

[a] Substrate (7.5 mM), additive (only asparagine: 100 mM), and saline (20 ml) were added to the wet cell (0.5 g) and the reaction mixture was incubated for 30 h at 37°C.
[b] The conversions were > 99% in all cases (by GLC analysis).
[c] Enantiomeric excesses and configuration were measured by GLC anlaysis with optically active capillary columns.

S. thermocyaneoviolaceus. The stereoselectivity of **2b** and **2c** were increased by the addition of alanine, asparagine, glutamic acid, aspartic acid, and malic acid.

In particular, the reduction in the presence of asparagine and aspartic acid gave the corresponding (*S*)-alcohol in excellent enantioselectively (> 99% ee). It is noted that **1f**, **1g**, and **1h** were reduced to the (*R*)-alcohols with the actinomycete cell in the absence of additives, while the reduction in the presence of glutamic acid gave the antipodal (*S*)-alcohols in > 99% ee. Furthermore, the reduction of **1d** in the presence of aspartic acid gave the (*R*)-α-hydroxy ester (**2d**) in high ee. The mechanism of the stereoselectivity change in the produced alcohol is not clear. It seems that the stereochemistry of the produced alcohols is reflected by the difference in the resistibility toward additives and the coenzyme specificity of between (*R*)- and (*S*)-producing enzyme(s).

To gain insight into the mechanistic interpretation of the actinomycete reduction, further detailed studies including purification of the enzymes, which contribute to the reduction system, are currently under investigation and will be reported in our following paper.

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