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Microbial reduction of α-chloroketone to α-chlorohydrin

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Microbial reduction of α-chloroketone to α-chlorohydrin was studied as one of the approaches for construction of the chiral center of the corresponding epoxide. About 100 microorganisms covering many species of *Candida*, *Pichia*, *Hansenula*, *Geotrichum*, *Rhodococcus* and *Aureobasidium* were screened to reduce the α-chloroketone stereospecifically. Many strains provided the *R*-α-chlorohydrin with 100% enantiomeric excess (ee), e.g., *Candida sonorensis* SC 16117, *Geotrichum candidum* SC 5469, *Rhodotorula glutinis* SC 16293, *Sphingomonas paucimobilis* SC 16113, *Pichia silvicola* SC 16159 and *Rhodococcus equi* SC 15835. Few microorganisms showed preferential formation of *S*-α-chlorohydrin after reduction. Among them, *Pichia pinus* SC 13864 and two *Pichia methanolica* strains SC 16116 and SC 13860 were the best, providing the *S*-α-chlorohydrin with ee of 88%, 79% and 78%, respectively. The enantiospecificity of the reduction by these *Pichia* species can be modified by changing the pH or prior heat treatment of the cells and *S*-α-chlorohydrin with ≥95% ee was obtained by appropriate modification of reaction conditions. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 259–262.

Keywords: microbial reduction; α-chloroketone; α-chlorohydrin; stereospecific reduction

Introduction

Chiral epoxides are important intermediates for the synthesis of numerous biologically active compounds. Microbial reduction of α-haloketones can provide chiral α-halohydrins [1,2,5,8], which could then be converted chemically to the chiral epoxides. Previously, we have reported the enzymatic preparation of chiral intermediates for the synthesis of antiviral [12], anticancer [10], antihypertensive [14] antiatherosclerotic [11] and β-blocker [13] drugs using oxidoreductases. The present report describes the reduction of 1-{2′,3′-dihydrobenzo[*b*]furan-4′-yl}-1-oxo-2-chloroethane (identified as "α-chloroketone" henceforth) (**1**) to chiral 1-{2′,3′-dihydrobenzo[*b*]furan-4′-yl}-2-chloroethan-1-ol (identified as "α-chlorohydrin" henceforth) (**2**) by various microorganisms.

Materials and methods

Chemicals

Authentic samples were prepared by the Process Research and Development department of Bristol-Myers Squibb. The structural identity and purity of each compound was established by spectroscopic and other physical and chemical methods. Other chemicals were purchased from VWR and/or Aldrich.

α-Chloroketone (1)

^{1}H NMR (300 MHz): δ 3.55 (2H, t, *J*=9 Hz), 4.65 (2H, t, *J*=9 Hz), 4.75 (2H, s), 7.0 (1H, d, *J*=7 Hz), 7.23 (1H, m), 7.3 (1H, d, *J*=7 Hz).

α-Chlorohydrin (2)

^{1}H NMR (500 MHz): δ 3.1 (2H), 3.51 (2H), 4.45 (2H), 4.72 (1H), 6.6 (1H), 6.78 (1H), 7.02 (1H). The assignments were established by a ^{1}H–^{1}H COSY experiment.

The enantiomeric purities of *S*- (**2a**) and *R*-α-chlorohydrins (**2b**) were established by converting them to the respective epoxides [7] as follows. A solution (1 ml) of potassium *tert*-butoxide in tetrahydrofuran was added to a cooled (0°C) solution of the respective α-chlorohydrin (**2**) (10 mg) in methyl *tert*-butyl ether (1 ml). The mixture was stirred under anhydrous condition at 0°C for 1 h. After removal of solvent, the enantiomeric identity of the epoxide was determined by HPLC analysis [7].

Microorganisms

Microorganisms were obtained from our culture collection. The SC number denotes the number in the BMS culture collection. Some microorganisms were obtained from ATCC or other sources.

Analytical methods

The amounts of α-chloroketone (**1**) and α-chlorohydrin (**2**) were determined by HPLC on a reversed phase C-8 column (15 cm×0.46 cm) kept at 50°C using acetonitrile–water (1:1) as mobile phase at a flow rate of 0.5 ml/min and detection at 220 nm. The α-chlorohydrin and α-chloroketone eluted at 5 min and 9 min, respectively.

The enantiomeric composition of α-chlorohydrin (**2**) was determined by HPLC on a Chiralcel AD column (25×0.46 cm) using hexane–ethanol (97:3) as eluent at a flow rate of 1 ml/min and monitoring by UV at 210 nm. The *S*- (**2a**) and *R*-α-chlorohydrin (**2b**) enantiomers eluted as two separate peaks at 18 and 22 min, respectively, with baseline separation. α-Chloroketone (**1**) eluted at 13 min in this system.

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Table 1 Microorganisms reducing α-chloroketone (**1**) to *S*-α-chlorohydrin (**2a**)

Microorganism	SC number	Chlorohydrin in final mixture (%)	Total recovery (%)	Ketone recovered (%)	Chlorohydrin yield (%)	*S*-chlorohydrin ee (%)
P. pinus	13864	86	46	6	40	88
P. methanolica	16116	77	53	12	41	79
P. methanolica	13860	97	64	2	62	78
Pichia membranafaciens	13859	73	44	12	32	43
Mortierella ramanianna	13843	97	56	2	55	72
M. ramanianna	13842	97	75	2	73	70
Candida parapsilosis	16347	44	49	27	22	73
Candida utilis	13984	3	56	54	2	49
Hansenula polymorpha	13895	96	88	4	85	60
H. polymorpha	13896	88	5	1	4	69
Arthrobacter simplex	6379	49	70	36	34	53

Growth of microorganisms

The medium for growing microorganisms contained malt extract 10 g, yeast extract 10 g, peptone 1 g, and dextrose 20 g dissolved in distilled water to a total volume of 1 l, adjusted to pH 7.0 and autoclaved at 121°C for 20 min.

Sterilized medium (100 ml in a 500-ml flask) was inoculated with microorganisms from vials and grown with shaking at 200 rpm at 28°C for 72 h. Cells were harvested by centrifugation (16,000×*g* for 20 min). All cells were stored in a −70°C freezer before use.

General method of microbial reduction of α-chloroketone to α-chlorohydrin

Cells were suspended in 10 ml of 100 mM phosphate buffer pH 7 containing 250 mg of glucose in a 50-ml Erlenmeyer flask. A solution of α-chloroketone (**1**) (10 mg) in 50 μl dimethyl formamide was added and placed on a shaker at 200 rpm at 28°C. After 24 h, the reaction mixture was extracted with ethyl acetate (20 ml). One 2-ml portion of the ethyl acetate extract was evaporated, the residue was dissolved in 1 ml of acetonitrile and analyzed by reversed phase HPLC to determine the amount of α-chloroketone (**1**) and α-chlorohydrin (**2**). Another 2-ml portion of the ethyl acetate extract was evaporated, the residue was dissolved in a mixture of hexane–isopropanol (90:10, 1 ml) and analyzed by HPLC on the Chiralcel AD column to determine the enantiomeric purity of the α-chlorohydrin (**2**).

The results of screening experiments were confirmed by repeating the experiments with successful microorganisms. All experiments beyond initial screening were confirmed by running duplicate experiments.

Table 2 Microorganisms reducing the α-chloroketone (**1**) to *R*-α-chlorohydrin (**2b**)

Microorganism	SC number	Chlorohydrin in final mixture (%)	Total recovery (%)	Ketone recovered (%)	Chlorohydrin yield (%)	*R*-chlorohydrin ee (%)
Candida maltosa	16112	43	47	27	20	82
C. sonorensis	16117	82	84	15	69	100
C. parapsilosis	16347	44	49	27	22	73
G. candidum	5469	79	48	10	37	100
Pichia anomala	16142	88	100	12	88	70
P. anomala	16143	72	49	14	35	69
P. anomala	16139	91	72	6	65	86
P. anomala	16140	90	81	8	73	92
P. anomala	16145	95	77	4	73	87
P. methanolica	13825	96	64	2	62	87
P. silvicola	16159	40	80	48	32	100
Hansenula anomala	13830	79	96	20	76	78
H. anomala	16158	97	89	3	86	59
H. anomola	13832	53	8	3	4	79
Hansenula fabianii	13894	97	57	2	56	86
Hansenula saturnus	13829	98	96	2	94	91
H. polymorpha	13805	5	60	57	3	100
R. equi	15835	84	75	12	63	100
R. erythropolis	13845	99	87	1	86	76
R. erythropolis	16236	85	66	10	56	76
R. glutinis	16293	93	63	4	58	100
S. paucimobilis	16113	49	9	5	4	100
Streptomyces sp.	13955	94	80	4	75	84
Trigonopsis variablis	16071	96	63	3	60	93
Arthrobacter globiformis	13958	9	100	91	9	100
Mycobacterium neoaurum	16052	99	82	1	81	95
Lodderomyces elongisporo	16110	91	64	6	58	88

Results and discussion

Screening of microorganisms for the reduction of α-chloroketone

Microorganisms were screened for their abilities to stereospecifically reduce the α-chloroketone (**1**). One hundred microorganisms covering many species of *Candida*, *Pichia*, *Hansenula*, *Geotrichum*, *Rhodococcus* and *Aureobasidium* were screened.

Only a few microorganisms showed a higher amount of *S*-α-chlorohydrin (**2a**) after reduction (Table 1). Among them, *Pichia pinus* SC 13864 and two *Pichia methanolica* strains SC 16116 and SC 13860 were the best, providing the *S*-α-chlorohydrin (**2b**) with enantiomeric excess (ee) of 88%, 79% and 78%, respectively. The chlorohydrin yield was about 40% for *P. pinus* SC 13864 and *P. methanolica* SC 16116, and 62% for *P. methanolica* SC 13860.

Many microbes reduced the α-chloroketone (**1**) to the *R*-α-chlorohydrin (**2b**) with high ee (Table 2). With some, e.g., *Candida sonorensis* SC 16117, *Geotrichum candidum* SC 5469, *Rhodotorula glutinis* SC 16293, *Sphingomonas paucimobilis* SC 16113, *Pichia silvicola* SC 16159, and *Rhodococcus equi* SC 15835, only the *R*-chlorohydrin peak was seen in the chiral HPLC suggesting an ee of 100%. With many other cultures, the *R*-α-chlorohydrin (**2b**) was produced with more than 90% ee. The extent of conversions were also high (50–95%) in many cases.

The reductases present in many microorganisms, therefore, prefer reducing the α-chloroketone (**1**) according to Prelog's rule providing the *R*-α-chlorohydrin (**2b**). The reduction of α-chloroketone (**1**) to provide *S*-α-chlorohydrin (**2a**) will follow anti-Prelog's rule. Although a number of microorganisms were reported to have anti-Prelog reductases [3,4,6], they do not show high specificities in the reduction of α-chloroketone (Figure 1) (**1**) to provide the *S*-α-chlorohydrin (**2a**).

Modification of conditions for reduction by P. pinus SC 13864

Attempts were made to improve the ee of the *S*-α-chlorohydrin (**2b**) obtained by reduction of the α-chloroketone (**1**) by *P. pinus* SC 13864 by modification of conditions. The modifications attempted involved varying the starting pH from 5 to 9, heating the cells for a short period of time before reduction, using other additives besides glucose for cofactor regeneration, and use of aqueous–organic biphasic systems for the reduction. Ethanol, isopropanol or sodium formate were ineffective as additives for cofactor regeneration. Reduction was completely inhibited in aqueous–organic solvent biphasic system with the solvents heptane, toluene and methyl *tert*-butyl ether. Varying the starting pH had a significant effect on the stereospecificity of the reduction (Figure 2). The ee of the *S*-α-chlorohydrin (**2b**) increased with increasing pH and reached an optimum of 95% at pH 8.0. The ee of 90% at pH 9.0 was also very close to that at pH 8.0.

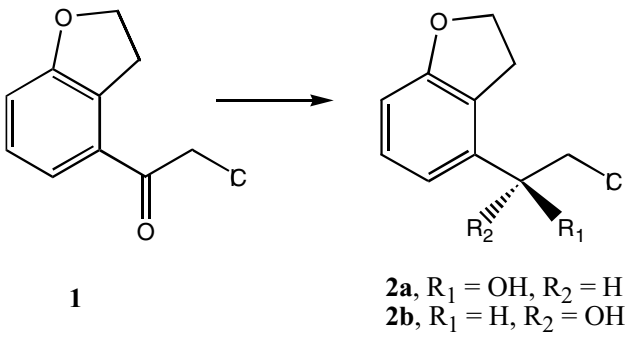

Figure 1

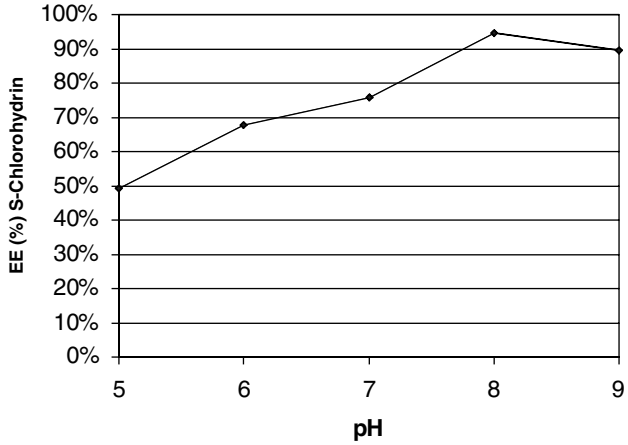

Figure 2

Heating the cells at 50°C for 15 min resulted in the improvement of ee to about 96% (Table 3). *P. pinus* SC 13864 probably has a small amount of heat-labile reductase providing the *R*-α-chlorohydrin (**2a**). The cells after heat treatment contained mostly one reductase giving the *S*-α-chlorohydrin (**2b**) with higher ee. Stereochemical control of reduction by prior heat treatment of microbial cells has been reported with other microorganisms [9]. Prolonged heating, however, deactivated the S-reductase and resulted in no reduction of the α-chloroketone (**1**).

Modification of conditions for reduction by P. methanolica SC 13860

Similar attempts to improve the enantiomeric excess of the product *S*-α-chlorohydrin (**2b**) with *P. methanolica* SC 13860 led to interesting results. Again, varying the starting pH had a significant effect on the enantiospecificity (Table 4). The yield and ee of the *S*-α-chlorohydrin **2b** was best between pH 6 to 8 with an optimum at pH 7. The ee was significantly reduced at pH 9.0. Unlike *P. pinus* SC 13864, the reduction with *P. methanolica* SC 13860 was not favored even under slightly basic conditions. Running the reaction for a longer time (i.e., a second day) resulted

Table 3 Effect of prior heating of cells on the reduction

Results after 1 day	Relative chlorohydrin production	ee *S*-chlorohydrin %
P. pinus cells	100	76
P. pinus cells — Heat treated	112	96
P. methanolica cells	100	100
P. methanolica cells Heat treated	76	8

Table 4 Effect of pH on reduction by *P. methanolica* SC 13860

Medium	Time day	Chlorohydrin in final mixture (%)	Chloroketone in final mixture (%)	Total recovery ketone+alcohol (%)	Chlorohydrin yield (%)	ee *S*-chlorohydrin (%)
pH 6	1	74	26	82	60	95
	2	93	7	71	66	23
pH 7	1	67	33	82	55	100
	2	96	4	62	60	47
pH 8	1	63	37	68	42	100
	2	91	9	37	34	20
pH 9	1	77	23	71	55	10
	2	93	7	44	41	29

in very poor ee and with very little additional conversion (over the first day). This might be due to reversible conversion between alcohol and ketone resulting in lower ee but no significant effect on yield.

Heating the *P. methanolica* SC 13860 cells at 50°C for 15 or 30 min lowered the ee of *S*-α-chlorohydrin (**2b**) (Table 3). This indicates that in *P. methanolica* SC 13860, the reductase responsible for the conversion of the α-chloroketone (**1**) to *S*-α-chlorohydrin (**2b**) is less heat stable. Therefore, heat treatment resulted in formation of more *R*-α-chlorohydrin (**2a**) isomer. This was the opposite of what was seen with *P. pinus* SC 13864 where the reductase for *S*-chlorohydrin formation was more heat stable.

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