A Novel Generation of Optically Active Ethyl 4-Chloro-3-hydroxybutyrate as a C4 Chiral Building Unit Using Microbial Dechlorination

Toshio SUZUKI,* Hideaki IDOGAKI and Naoya KASAI*

Research Laboratories of DAISO Co., Ltd. 9 Otakasu-Cho, Amagasaki, Hyogo 660, Japan

Abstract: A novel procedure for the generation of optically active ethyl 4-chloro-3-hydroxybutyrate using bacterial cells was developed. Ethyl (S)-4-chloro-3-hydroxybutyrate was prepared by *Pseudomonas* sp. OS-K-29, which stereoselectively assimilates 2,3-dichloro-1-propanol. The reaction was based on its kinetic dehalogenation for both enantiomers using the resting cells. The obtained 4-chloro-3-hydroxybutyrate had high enantiomeric excess of >98 % with a yield of 33 % at the microbial resolution step. Moreover, several C4 compounds having the 4-chloro-3-hydroxyl function were also resolved and gave good enantiomeric purities (>95 %ee). Ethyl (R)-4-chloro-3-hydroxybutyrate was also obtained with high enantiomeric purity (>98 %ee) using the cells of *Pseudomonas* sp DS-K-NR818. Copyright © 1996 Elsevier Science Ltd

Chiral chloro alcohols having a C4 structural frame such as ethyl 4-chloro-3-hydroxybutyrate (CHBE) and 4-chloro-3-hydroxybutyronitrile (CHBN) serve as intermediates for the synthesis of biologically and pharmacologically important carnitine,[1-3] 4-amino-3-hydroxybutyric acid (GABOB)[4-6] and 4-hydroxy-2-pyrrolidone.[7,8] In particular, R-carnitine (vitamin BT), which is a physiologically significant agent, is involved in the metabolism of long-chain fatty acids through the mitochondrial membrane in the human body.[9] Therefore, several synthetic methods were reported for optically active CHBE and CHBN. The asymmetric reduction of carbonyl compounds with microorganisms and enzymes was known for the synthesis of optically active compounds.[10,11] Shimizu *et al.* found that *Sporobolomyces salmonicolor* and *Trichosporon cutaneum* can transform 4-chloro-3-oxobutanoate to (R)-CHBE and (S)-CHBE through asymmetric reduction, respectively.[12] Ethyl acetoacetate was reduced to the corresponding optically active esters using bakers' yeast.[1,13] With respect to chemical methods, Noyori *et al.* reported the asymmetric reduction of ethyl 4-chloro-3-oxobutanoate to optically active CHBE using the BINAP-based Ru(II) catalysts.[2] These approaches depended on the enzymatic, microbial or chemical reduction of the β-keto esters. Also, the enzymatic synthesis of (R)-CHBN using the halohydrin hydrogen halide-lyase is known.[14]

Recently, we have developed the novel microbial resolution of racemic CHBE to give optically active CHBE by stereoselective dechlorination. Among the many microorganisms screened from soil samples, we found that *Pseudomonas* sp. OS-K-29[15] can stereoselectively degrade (R)-CHBE from the racemate to give the (S)-enantiomer with high enantiomeric purity (>98 %ee) with a yield of 33%. The (R)-enantiomer was then converted into the corresponding diol (41 %ee). We have already reported that this strain, *Pseudomonas* sp. OS-K-29, stereoselectively assimilated (R)-2,3-dichloro-1-propanol with the release of chloride ion so that it was converted to glycerol *via* the corresponding diol, that is, 3-chloro-1,2-propanediol.[16] Moreover, for several C4 compounds having the 4-chloro-3-hydroxyl function, the dechlorinating enzyme of this strain showed stereoselectivity (Table). The resulting (S)-CHBE, *i*-propyl (S)-4-chloro-3-hydroxybutyrate, (S)-

CHBN and (R)-butylene chlorohydrin exhibited excellent enantiomeric purities. However, the C3 compounds such as propylene chlorohydrin and 3-chloro-1,2-propanediol were not good substrates.

On the other hand, the preparation of (R)-CHBE using the resting cells of Pseudomonas sp DS-K-NR818,[15] which was isolated as a mutant of Pseudomonas sp. OS-K-29, also gave excellent enantiomeric purity (>98 %ee). The yield was estimated to be average 40%. As well as the original strain stereoselectively degrading (R)-CHBE, the stereoselectivity of Pseudomonas sp. DS-K-NR818 for the C4 structural compounds was very excellent (Table). Interestingly, the (S)-enantiomers of alkyl 4-chloro-3-hydroxybutyrate were all converted into (S)-3-hydroxy-γ-butyrolactone[17] releasing the corresponding alcohol and chloride ion. The enantiomeric purity of the (S)-3-hydroxy-γ-butyrolactone obtained from racemic CHBE was estimated to be very low (62.5 %ee). This reaction was considered to be quite novel and provided interesting dechlorination of the chlorinated alcohols. So, the details about this mechanism are now in progress.

Table Stereoselectivity for several chlorinated alcohols by two kinds of resting cells, Pseudomonas sp. OS-K-29 and DS-K-NR818

Substrate	Residual[a] ratio (%)	Optical purity (% ee)	Product[b]
Pseudomonas sp. OS-K-29			
1-Chloro-2-hydroxypropane	49	-	1,2-Propanediol
3-Chloro-1,2-propanediol	47	-	Glycerol
2,3-Dichloro-1-propanol	37	99.9 (S)	3-Chloro-1,2-propanediol, glycerol
1-Chloro-2-hydroxybutane	46	95.6 (R)	1,2-Butanediol
4-Chloro-3-hydroxybutyronitrile	40	94.5 (S)	3,4-Dihydroxybutyronitrile
Methyl 4-chloro-3-hydroxybutyrate	56	56.0 (S)	Methyl 3,4-dihydroxybutyrate
Ethyl 4-chloro-3-hydroxybutyrate	35	98.5 (S)	Ethyl 3,4-dihydroxybutyrate
i-Proryl 4-chloro-3-hydroxybutyrate	35	98.6 (S)	i-Propyl 3,4-dihydroxybutyrate
Pseudomonas sp. DS-K-NR818			
2-Chloro-1-hydroxypropane	49	98.3 (R)	Not detected
3-Chloro-1,2-propanediol	42	98.7 (S)	Not detected
2,3-Dichloro-1-propanol	41	99.9 (R)	Not detected
2-Chloro-1-hydroxybutane	49	98.4 (R)	Not detected
Methyl 4-chloro-3-hydroxybutyrate	40	98.5 (R)	3-Hydroxy-γ-butyrolactone
Ethyl 4-chloro-3-hydroxybutyrate	42	98.4 (R)	3-Hydroxy-γ-butyrolactone
i-Proryl 4-chloro-3-hydroxybutyrate	42	98.7 (R)	3-Hydroxy-γ-butyrolactone

The resolution reaction was carried out in a 300 ml Erlenmeyer flask with 50 ml of 2 mM phosphate buffer (pH 7.2) containing 1 %(v/v) above racemic substrate, 1 %(w/v) $CaCO_3$ and 1g bacterial cells (wet weight). The flask was incubated at 30 °C for 24-48hr with a 130-rpm shaking rate. The resting cells were prepared in the same manner as described in the text.

a) At initial time, residual ratio is indicated to be 100 %.

b) Identification of respective reaction products was carried out by gas chromatography.

Production of optically active CHBE by the cells

The production of (S)-CHBE was carried out as follows: The resting cells were prepared in a 50-l jar fermenter (Model KMJ-501-MGU, Mitsuwa Rikagaku Co., Ltd., Japan) with 35 l of a nutrient medium containing 1 %(w/v) each of polypeptone, yeast extract and glycerol (pH 7.2). The cultivation was done under

aerobic conditions; agitation, 320 rpm; aeration, 15 l/min; temperature, 30 °C; pH, 7.2). Three hundred milliliters of the seed culture cultivated in a 500-ml Erlenmeyer flask containing 100 ml of the nutrient medium was inoculated into the above 35 l of nutrient medium. After a 20hr cultivation, 951 g of the wet cells was harvested by continuous centrifuge (26,000 g, 5-10 °C, flow rate, 6 l/hr). The resting cells were suspended in a 100 l of resolution solution containing 0.02 %(w/v) $Na_2HPO_4 \cdot 12H_2O$, 0.02 %(w/v) K_2HPO_4, 0.04 %(w/v) $NaH_2PO_4 \cdot 2H_2O$, 0.05 %(w/v) $MgSO_4 \cdot 7H_2O$ and 1% (v/v) racemic CHBE.[18] The resolution reaction was done under the following conditions: agitation, 150 rpm; temperature, 30 °C; pH was controlled at 6.7 with 7.9 N-NaOH (25 %(w/w)). The residual amount of CHBE and its enantiomeric purity and configuration were measured by gas chromatography.[19] The products of the resolution reaction were identified by gas chromatography.[20] If more detailed data were needed for identification, GC/MS[20] and NMR analyses were done. Forty hours later, the resolution reaction was stopped, and at that time the residual amount of CHBE and its enantiomeric purity were estimated to be 33 % and 99.1 %ee, respectively. The cells were removed using the UF membrane system (Model UW-1A, Nitto Denko Co., Tokyo, Japan) and the resulting supernatant was subjected to an active carbon column (10 cm diameter and 127 cm length) to recover the obtained CHBE. The elution was done with acetone (20 l) and more than 95% of the absorbed CHBE (313 g) was recovered. After removing the solvent by evaporation, the CHBE was purified by distillation *in vacuo* (95 °C/3 mmHg). Finally, 181 g of *(S)*-CHBE was obtained in a yield of 15 % without loss of ee. Specific rotation of the obtained CHBE was estimated to be $[\alpha]_D^{20}$ = -13.1 (*c* 1.2, CH_3OH), confirming that the configuration was *S* form (*S* form, $[\alpha]_D^{20}$ = -12.9 (*c* 3.3, CH_3OH))[12]. In addition, production of *(R)*-CHBE was also done using *Pseudomonas* sp. DS-K-NR818 in the same manner as described above. In the case of the 100 l scale reaction, 360 g of *(R)*-CHBE with an enantiomeric purity of 99.2 %ee was finally prepared. Its specific rotation was estimated to be $[\alpha]_D^{20}$ = 13.7 (*c* 1.2, CH_3OH) (*R* form, $[\alpha]_D^{20}$ = 12.0 (*c* 3.3, CH_3OH))[12].

Our method for the production of optically active CHBE was considered to be novel and provided an effective resolution. Using this method, the resulting *(S)*- and *(R)*-CHBE showed higher enantiomeric purities (>98 %ee), respectively, compared to those methods utilizing the biological and chemical asymmetric reduction as mentioned above. In addition, racemic CHBE as a substrate was supplied from propylene *via* epichlorohydrin using petroleum chemicals. So, optically active CHBE as a useful C4 chiral synthon was economically produced by this method. More detailed research is now in progress for industrial production.

References and Notes

1. Zhou, B.; Gopalan, A. S.; VanMiddlesworth, F.; Shieh, W-R.; Sih, C. J., *J. Am. Chem. Soc.* **1983**, *105*, 5925-5926.

2. Kitamura, M.; Ohkuma, T.; Takaya, H.; Noyori, N., *Tetrahedron Lett.* **1988**, *29*, 1555-1556.

3. Kasai, N.; Sakaguchi, K., *Tetrahedron Lett.* **1992**, *33*, 1211-1212.

4. Jung, M. E.; Shaw, T. J., *J. Am. Chem. Soc.* **1980**, *102*, 6304-6311.

5. Bock, K.; Lundt, I.; Pedersen, C., *Acta Chem. Scand.* **1983**, *37*, 341-344.

6. Rossiter, B. E.; Sharpless, K. B., *J. Org. Chem.* **1984**, *49*, 3707-3711.

7. Pifferi, G.; Pinza, M., *Farmaco, Ed. Sci.* **1977**, *32*, 602-613.

8. Pellegata, R.; Pinza, M.; Pifferi, G., *Synthesis* **1978**, 614-616.

9. Fraenkel, G.; Friedman, S. In Vitamins and Hormones, Haaris, R. S.; Marrian, G. F.; Thimann, K. V. (EDs.), Academic Press, New York, 1957, Vol. 15, pp73-118.

10. Whitesides, G. M.; Wong, C-H., *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 617-638.

11. Yamada, H.; Shimizu, S., *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 622-642.

12. Shimizu, S.; Kataoka, M.; Morishima, A.; Katoh, M.; Morikawa, T.; Miyoshi, T.; Yamada, H., *Biotechnology Lett.* **1990**, *12*, 593-598.

13. Santaniello, E.; Casati, R.; Milani, F., *J. Chem. Res. (S)*, **1984**, 132-133.

14. Nakamura, T.,; Nagasawa, T.; Yu, F.; Watanabe, I.; Yamada, H., *Biochem. Biophys. Res. Commun.* **1991**, *180*, 124130.

15. These two kinds of strains, *Pseudomonas* sp. OS-K-29 and *Pseudomonas* sp. DS-K-NR818, were deposited to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan with accession numbers of FERM BP-994 and FERM BP-5491, respectively.

16. Kasai, N.; Tsujimura, K.; Unoura, K.; Suzuki, T., *Agric. Biol. Chem.* **1990**, *54*, 3158-3190.

17. The NMR analytical data were as follows: ^{1}H NMR (CDCl$_3$, 250 MHz), δppm: 2.54 (d, 1H, J=18 Hz); 2.79 (dd, 1H, J=18 Hz, J=6 Hz); 3.29 (s, 1H, OH); 4.34 (d, 1H, J=10 Hz); 4.46 (dd, 1H, J=10 Hz, J=4.5 Hz); 4.6-4.7 (m, 1H), ^{13}C NMR (CDCl$_3$, 250 MHz), δppm: 177, 76.3, 67.2, 37.7.

18. Braun, G., *J. Am. Chem. Soc.* **1930**, *52*, 3167-3176.

19. Assay of degradation reaction was done with a gas chromatograph (Model GC-9A, Shimadzu, Kyoto, Japan) equipped with a PEG 20M-HP (5%, 60/80 mesh, GL Science Co., Ltd., Tokyo, Japan) column (3.2 mm diameter and 1 m length). The conditions of the gas chromatography were as follows: sample size, 1 μl; injection and detector temperature, 240 ºC; column temperature, 180 ºC; carrier gas, nitrogen; flow rate, 50 ml min^{-1}; detector, flame ionization detector (FID). Determination of the ee and configuration for several chlorinated alcohols except for 3-chloro-1,2-propanediol was done by gas chromatography analysis (Model G-3000, Hitachi, Tokyo, Japan). The conditions were as follows: sample size, 1 μl; column, CHIRALDEX^R-G-TA capillary column (0.25 mm diameter, 30 m length, Astec Inc., NJ, USA); column temperature, 70-150 ºC; injection and detector temperature, 150 ºC; detector, FID; carrier gas, nitrogen; split ratio, 1:100. Determination of ee for 3-hydroxy-γ-butyrolactone was done in the same conditions described above after acetylation with treatment of acetyl chloride. In the case of 3-halogeno-1,2-propanediol, the ee was determined with gas chromatograph equipped with CHIRALDEX^R-A-PH capillary column (0.25 mm diameter, 30 m length, Astec Inc., NJ, USA) after conversion to glycidol with aqueous NaOH solution. The conditions were as follows: sample size, 1 μl of isopropanol solution; column temperature, 45 ºC; injection and detector temperature, 150 ºC; detector, FID; carrier gas, nitrogen; split ratio, 1:100.

20. Identification of products by enzymatic reaction was done using a gas chromatograph (Model GC-14A, Shimadzu, Kyoto, Japan) equipped with a capillary column (0.25 mm diameter, 30 m length, TC-1701, GL Science Co., Ltd., Tokyo, Japan). The analytical conditions were as follows: sample size, 1 μl; injection and detector temperature, 250 ºC; column temperature, 70 ºC for 3 min and 70 to 250 ºC at a rate of 10 ºC min^{-1}; carrier gas, nitrogen; flow rate, 1 ml min^{-1}; split ratio, 100:1, detector, FID. GC/MS was done by the chemical ionization (CI) method. The instrument (Model JMS-AX505W, JEOL, Tokyo, Japan) with a capillary column (0.22 mm diameter, 25 m length, CBP20-M25-025, Shimadzu, Kyoto, Japan) was operated using CH$_2$Cl$_2$ as reagent gas. The conditions were as follows: sample size, 1 μl; injection temperature, 230 ºC, column temperature, 200 ºC; carrier gas, helium gas; flow rate, 1 ml min^{-1}; split ratio, 60:1.