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Preparation of (S)-2,3-dichloro-1-propanol by *Pseudomonas* sp. and its use in the synthesis of (R)-epichlorohydrin

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SUMMARY

Pseudomonas sp. OS-K-29 assimilated (R)-2,3-dichloro-1-propanol preferentially as the sole source of carbon. Isolation of optically pure (S)-2,3-dichloro-1-propanol with 100% enantiomer excess (e.e.) from the racemate was done based on this bacterial assimilation using immobilized-cells of OS-K-29 with calcium-alginate. The overall examination of the reactor involved 19 batches for 50 days without loss of its activity. Highly pure (R)-epichlorohydrin with 99.5% e.e. was prepared from the (S)-2,3-dichloro-1-propanol with treatment of aqueous NaOH. This new method is simple and useful for manufacturing optically active (S)-2,3-dichloro-1-propanol and (R)-epichlorohydrin.

INTRODUCTION

Optically active epichlorohydrin is a useful intermediate for the synthesis of optically active adrenergic beta-blockers, L-carnitine and platelet activating factor (PAF), agrochemicals, ferro-electric liquid crystals, and chiral polymers [1–4]. Optically active epichlorohydrin was made from D-mannitol by synthetic methods [5–6], and was technically difficult [7].

Racemic epichlorohydrin is made via 2,3-dichloro-1-propanol and 1,3-dichloro-2-propanol synthesized from propylene in the petrochemical industry as shown in Fig. 1. 2,3-Dichloro-1-propanol has an asymmetric carbon atom in its center and is considered a desirable precursor for the preparation of optically active epichlorohydrin.

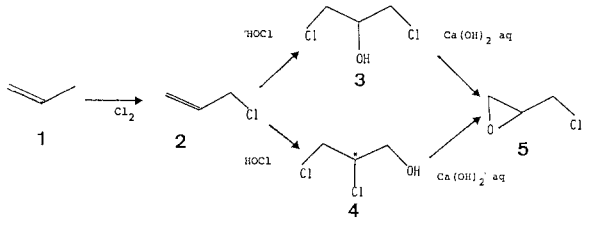

Fig. 1. Scheme of the industrial manufacture of epichlorohydrin. Numbers: 1. propylene; 2. allylchloride; 3. 1,3,-dichloro-2-propanol; 4. 2,3-dichloro-1-propanol; 5. epichlorohydrin.

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For the resolution of 2,3-dichloro-1-propanol, some biochemical methods have been reported. Iriuchijima et al. reported asymmetric hydrolysis of 1-acetoxy-2,3-dichloropropane with pancreatic or *Mucor* sp. lipases [8], and Cambou et al., described the synthesis of optically active esters of 2,3-dichloro-1-propanol by yeast lipase [9]. These reports indicate that the utilization of enzymes in synthetic chemical fields is useful, however, optically pure 2,3-dichloro-1-propanol was not obtained. Thus, alternative methods to prepare it are needed.

We have previously reported the isolation of a bacterium from soil capable of assimilating 2,3-dichloro-1-propanol [10].

In this report, we describe the use of the bacterium for manufacturing optically pure (S)-2,3-dichloro-1-propanol in a bioreactor and its subsequent use in synthesis of optically pure (R)-epichlorohydrin.

MATERIALS AND METHODS

Microorganism

The microorganism used was the strain *Pseudomonas* sp. OS-K-29, isolated as a 2,3-dichloro-1-propanol assimilating bacterium from soil [10].

Chemicals

2,3-Dichloro-1-propanol was from Tokyo Kasei Kogyo Co., Tokyo, Japan. (R)-(2-methoxy-2-(trifluoromethyl)-phenyl acetic acid) (MTPA) and (S)-MTPA were purchased from Merk Co. Tris-[3-(heptafluoropropylhydroxy)-methene-*d*-camphorato]Europium (III)

($Eu(hfc)_3$) was purchased from Aldrich Co. Other materials used were of chemical grade.

Cultivation

The cells were cultured in 30 l nutrient medium, consisting of 1.0% (w/v) peptone, 1.0% (w/v) yeast extract and 1.0% (w/v) glucose (initial pH 7.0) in a 50-l jar-fermentor for 24 h (cultural conditions; temperature 30 °C, agitation 300 rpm, aeration 20 l/min). The cells (600 g wet weight) were harvested by centrifugation.

Immobilized cells

20 l of 2% sodium alginate solution and 600 g of the harvested cells were mixed and the mixture was added to 40 l of 2% calcium chloride solution. The prepared immobilized cells beads (2–4 mm in diameter, 20 l) were washed with water.

Reaction with the bioreactor

Microbial resolution of 2,3-dichloro-1-propanol was carried out with 80 l of synthetic medium, consisting of: 0.05% (w/v) $(NH_4)_2SO_4$, 0.05% (w/v) K_2HPO_4, 0.05% (w/v) $MgSO_4 \cdot 7H_2O$, 0.001% (w/v) $FeSO_4 \cdot 7H_2O$, 0.0001% (w/v) $CuSO_4 \cdot 5H_2O$, 0.0001% (w/v) $MnSO_4 \cdot 5H_2O$, 0.1% (w/v) $CaCO_3$ and 0.2% (v/v) (RS)-2,3-dichloro-1-propanol, in a 120-l bioreactor equipped with a stainless steel palanquin packed with 20 l of the immobilized cells beads. Reactor conditions were as follows: temperature, 30 °C; agitation, 300 rpm; aeration, 20 l/min.

Estimation of 2,3-dichloro-1-propanol

The reaction was monitored by gas chromatography as described previously [10].

Synthesis of (R)-epichlorohydrin

(S)-2,3-Dichloro-1-propanol (100 g) and sodium hydroxide (37.2 g, 1.5 N aq.) were reacted with vigorous mixing at 20 °C for 30 min. The synthesized (R)-epichlorohydrin was extracted with diethylether, dried, and distilled at atmospheric pressure (bp 118 °C, yield 74%).

Analysis for determining chiral purity

The chiral purity of the 2,3-dichloro-1-propanol was determined by analysis of ^{1}H-NMR spectra and HPLC of the Mosher's ester of the product as well as measurement of optical rotation comparing with the known derivatives of the product [8]. The Mosher's esters of the product or (RS)-2,3-dichloro-1-propanol were prepared by the method of Dale et al. [11].

1-Acetoxy-2,3-dichloro-propane and dichloro-*N*-phenylcarbonate were prepared according to Iriuchijima et al. [8]. Chiral purity of the prepared epichlorohydrin was determined by ^{1}H-NMR spectra analysis using a chiral shift reagent and complexation gas chromatography. Analysis was conducted with ^{1}H-NMR by the method of Baldwin et al. [5].

Bis-3-(heptafluorobutyryl)-1R-camphorates of Co(II) and a capillary column (0.25 mm × 50 m) for complexation gas chromatography was made by the method of Schurig [12].

Analytical instruments

Gas chromatography was carried out on a Shimazu GC-9A system (Kyoto, Japan) attached with a 1-m PEG 20M-HP packed column. The column temperature was 160 °C and carrier gas was nitrogen. Complexation gas chromatography was conducted on a Shimazu GC-14A system (Kyoto, Japan) attached with our home-made capillary column (coated with bis-3-(heptafluorobutyryl)-1R-camphorates of Co(II) in SE-54; using the dynamic method). The column temperature was 40 °C, carrier gas was nitrogen, the split ratio was 1/50, and detection method was FID. HPLC was carried out on a Shimazu LC-6A system (Kyoto, Japan) equipped with a reverse phase column of C18-Si (Zorbax ODS, 4.6 mm × 25 cm; 5 μ; made by Du Pont Co.). Detection was done at 254 nm. Optical rotation was measured on a Jasco Dip-360 high speed automatic digital polarimeter (Tokyo, Japan). ^{1}H-NMR spectra were measured on Jeol JX-260 and GX-500 (Tokyo, Japan) spectrometers.

RESULTS

Reaction in the bioreactor

Table 1 summarizes results of a series of continuous batches. (RS)-2,3-Dichloro-1-propanol in each batch-reaction was degraded by the immobilized cells and the degradation was stopped at 50%. The average reaction time required 63–83 h/batch. The immobilized cells beads were well contained by the palanquin. The culture filtrate was removed through bottom exit of the bioreactor and the synthetic medium for next batch was added through the top entrance. The next reaction was carried out under

TABLE 1

Operation results of the bioreactor

Experiment run No.	Total tested days	Total tested batches	Reaction time (h/batch)		
			Minimum	Maximum	Average
1	32	10	48	116	77
2	38	11	56	148	83
3	41	15	46	96	66
4	50	19	48	98	63

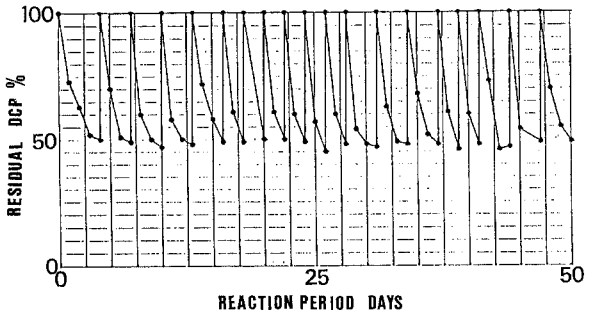

Fig. 2. Time course of continuous batches with the bioreactor. -●-, residual 2,3-dichloro-1-propanol(%) in the bioreactor.

the same conditions. Fig. 2 shows the best result (experimental run No. 4). Nineteen continuous batches were examined for 50 days, still, the degradation activity was not decreased.

Purification of residual 2,3-dichloro-1-propanol

80 l of filtrate (1 batch) were applied onto a charcoal column (10 × 150 cm) equilibrated with water. The residual 2,3-dichloro-1-propanol was absorbed onto the charcoal. The column was washed with two volumes of water, and eluted with 30 l of acetone. Fractions of 2,3-dichloro-1-propanol (30 l) were evaporated to 1.5 l at 40 °C in vacuo. Sodium chloride was added to the condensate until the oil of 2,3-dichloro-1-propanol floated on water. The oil was extracted with 500 ml of diethyl ether, dried with anhydrous $MgSO_4$, and distilled with a Wittomer distillation column in vacuo. 2,3-Dichloro-1-propanol (109 g) was fractionated at 75–76 °C (20 mmHg). The chemical purity was 99.9% according to gas chromatography. The specific rotation was $[\alpha]_D^{25}$–10.5 (c = 1.36 in CH_2Cl_2).

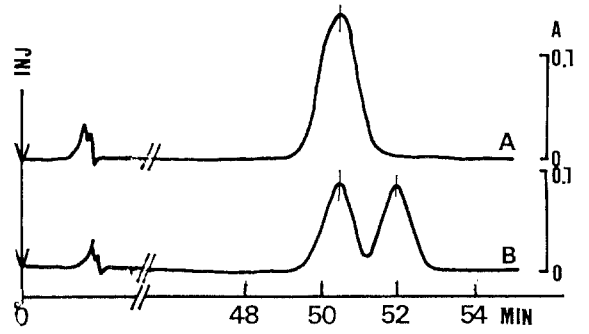

Fig. 3. Estimation of product chiral purity by HPLC. Five μl of each sample (about 0.1% in MeOH) were subjected to HPLC. Column, Zorbax Ods (Du Pont, 5 μl) 2.4 × 250 mm; eluent, MeOH-0.1 M phosphate buffer (pH 6.8) (65 : 35 v/v); flow rate 1.0 ml/min; temperature, ambient; detection UV at 260 nm. Samples: A. (R)-MTPA ester of (S)-2,3-dichloro-1-propanol, B. (R)-MTPA ester of (RS)-2,3-dichloro-1-propanol.

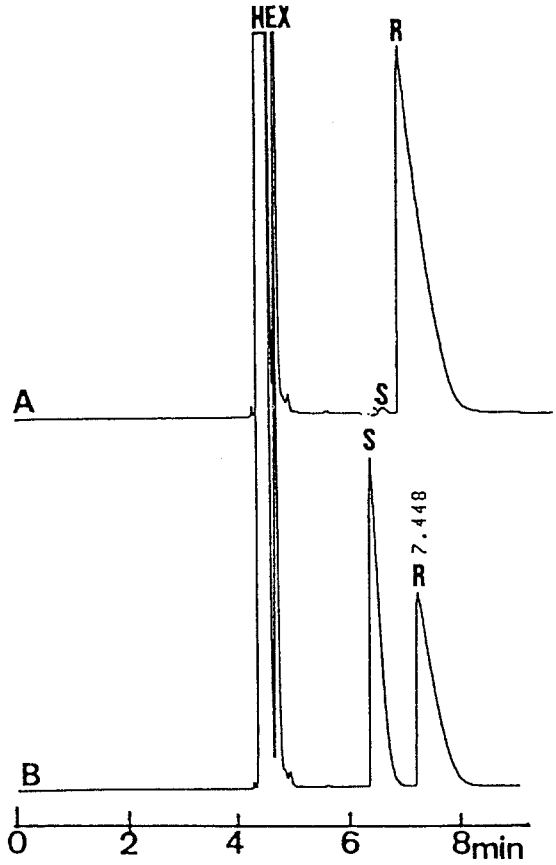

Fig. 4. Estimation of optical purity of (R)-epichlorohydrin by complexation gas chromatography. Samples: A. (R)-epichlorohydrin, B. (RS)-epichlorohydrin; Symbols: HEX, Hexane; S, (S)-epichlorohydrin; R, (R)-epichlorohydrin. Analysis conditions. column, 0.25 mm × 30 m (coated with bis-3-(heptafuluorobutyryl)-1R-camphorates of cobalt (II) in SE-54 by dynamic method); sample 0.6 μl of 5% (v/v) hexane solution, column temperature, 40 °C; injection temperature and detector temperature (FID). 150 °C; carrier gas (nitrogen), 1 ml/min; split ratio, 1/50.

Chiral purity

The (R)-MTPA ester of (RS)- and purified 2,3-dichloro-1-propanol were analyzed by HPLC. Two peaks were observed for (R)-MTPA-(RS)-2,3-dichloro-1-propanoate at 50.5 min and 52.0 min. However, only one peak could be seen for (R)-MTPA-product at 50.5 min as shown in Fig. 3. The prepared dichloro-*N*-phenyl-carbamate from the product gave white crystals having a 165 °C (mp.), $[\alpha]_D^{25}$–16.4 (c = 1, in MeOH) (lit., $[\alpha]_D^{25}$–14.5 in 90% e.e.) [8]. Acetate of the product gave a colorless oil having $[\alpha]_D^{25}$–18.3 (c = 1.2, in MeOH), (lit., $[\alpha]_D^{25}$–16.5, in 90% e.e.) [8]. (R)-MTPA and (S)-MTPA esters of the product were analyzed with using a ^{1}H-NMR (500 MHz) spectrometer. Each methylene proton signal at the C2 position of the derivatives illustrated no overlapping.

These results indicated the chiral purity of the purified 2,3-dichloro-1-propanol was 100% e.e., and the configuration was (S)-form.

Preparation of (R)-epichlorohydrin

(R)-Epichlorohydrin was obtained from the (S)-2,3-dichloro-1-propanol via treatment with aqueous NaOH with a yield of 74%. The specific rotation was $[\alpha]_D^{23}$ −34.3 (c = 1.2, in MeOH). This specific rotation agreed with the highest literature value (lit., $[\alpha]_D^{23}$ −34.3) [5]. The chiral purity could be determined as 99.3% e.e. through an examination of the ^{1}H-NMR spectrum using Eu(hfc)$_3$, and complexation gas chromatography (Fig. 4).

It is shown here for the first time that highly pure optically active (R)-epichlorohydrin can be prepared from (S)-2,3-dichloro-1-propanol.

DISCUSSION

Generally, the resolution of oily substances is very difficult since crystallization can not be carried out. Examples of the preparation of chiral alcohols by microbial reduction or stereospecific hydrolases are widely known [13, 14], although the chiral center atom itself in most cases has an hydroxyl (OH) group. In the case of an OH group apart from the chiral center, as with 2,3-dichloro-1-propanol, effective resolution is difficult. Cambou et al. and Iriuchijima et al. attempted the resolution of 2,3-dichloro-1-propanol using stereospecific esterase or lipase, but the desired optical purity was not obtained. Since there were no data for optically active 2,3-dichloro-1-propanol, it was necessary to prove the chiral purity by various analyses. Here, optically pure (S)-2,3-dichloro-1-propanol was successfully obtained by removing the (R)-isomer through bacterial assimilation.

Racemic epichlorohydrin is usually made by epoxidation in aqueous Ca(OH)$_2$ on an industrial scale, but we found the method accelerated the racemization because of by-product formation from 1,3-dichloro-2-propanol. In the report of Cambou et al., it was stated that the racemization was due to the temperature during distillation [9], but we believe that racemization of the epichlorohydrin may be due to chloride ion. We performed the conversion from (S)-2,3-dichloro-1-propanol to (R)-epichlorohydrin successfully using aqueous NaOH. Since the e.e. value was only slightly decreased, we confirmed that there was no problem in the performing epoxidation in aqueous NaOH and the subsequent distillation.

Although this method has the disadvantage that one isomer is lost, it is still quite effective. (RS)-2,3-Dichloro-1-propanol can be produced very economically in the petrochemical industry, so this method may be of value. The optically active epichlorohydrin is useful as a C3 chiral synthon for various pharmaceuticals, agrochemicals and ferro-electric liquid crystals. The present method is thus shown to serve as a new process for manufacturing optically active (S)-2,3-dichloro-1-propanol and (R)-epichlorohydrin. Chiral epichlorohydrin may become a readily available chiral synthon through this process. In fact, (R)-epichlorohydrin so prepared has been used in some studies recently [15–17]. We are now investigating a more efficient reactor system for industrial scale manufacturing.

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