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A NOVEL METHOD FOR THE GENERATION OF (R)- AND (S)-3-CHLORO-1,2-PROPANEDIOL BY STEREOSPECIFIC DEHALOGENATING BACTERIA AND THEIR USE IN THE PREPARATION OF (R)- AND (S)-GLYCIDOL

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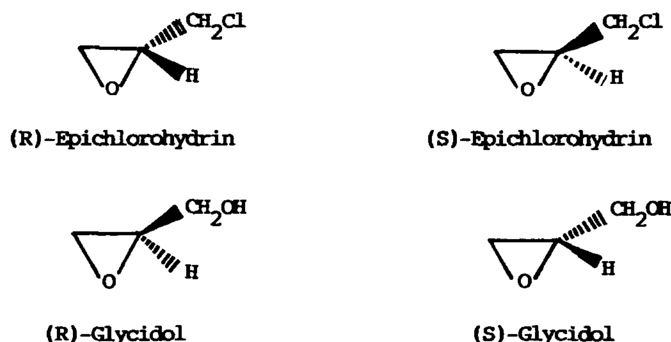
Summary: A novel and effective method for the preparation of highly pure optically active (R)- and (S)-3-chloro-1,2-propanediol (99.5% ee and 99.4% ee, respectively) was established based on stereospecific dehalogenation and assimilation with bacteria. From these intermediates highly pure optically active (R)- and (S)-glycidol (99.3% ee and 99.4% ee, respectively) were prepared.

Optically active glycidol (GLD) is a very important C3 chiral building block for chiral pharmaceuticals such as beta-adrenergic blockers[1,2] and cardiovascular drugs.[3,4] A synthesis starting from D-mannitol was reported by Fisher *et al.* in 1942,[5] however, it has not proven practical due to racemization of (S)-glycerol-1,2-acetonide that was found to occur during distillation.[6]

Recently, Sharpless *et al.* established a method for the preparation of optically active glycidol which was based on the asymmetric epoxidation of allyl alcohol in the presence of the catalysts of titanium (IV) isoproxide and diisopropyl (+)- or (-)-tartrate (Sharpless Oxidation).[1,7,8] This method is quite good but has the disadvantage that the enantiomer excess of the formed optically active GLD is low (91% ee).[9] Optically active 3-chloro-1,2-propanediol (CPD) is also an important chiral C3 building block and a precursor of optically active glycidol. Their asymmetric synthesis from methyl-6-chloro-6-deoxy-D-glucopyranoside[10] and D-mannitol[11] has been reported. However, a more simple, effective, and practical preparation method was needed for obtaining optically active GLD and CPD.



For a number of years, we have conducted biological resolutions to produce highly pure optically active C3 building blocks such as (R)- and (S)-epichlorohydrin.[12,13] Recently we resolved (R)- and (S)-CPD from the racemate by microbial resolution and prepared highly pure (R)- and (S)-GLD. This method described herein is simple and very effective.

This letter describes stereospecific CPD dehalogenating and assimilating bacteria newly isolated from soil, the resolution and isolation of highly pure (R)- and (S)-CPD using the bacteria, and their use in the preparation of highly pure (R)- and (S)-GLD. The results are summarized in Figure 1.

Stereospecific CPD assimilating microorganisms were screened as follows: An enrichment culture was made in a synthetic medium containing (RS)-CPD as the sole source of carbon. The grown bacterial stereospecificity of assimilation was assessed using (R)- and (S)-CPD made from (R)- and (S)-epichlorohydrin.[12,13] Three strains for each enantiomer were isolated. All (R)- and (S)-CPD assimilating bacteria belonged to *Alcaligenes* sp.[14] and *Pseudomonas* sp.[15], respectively. In each cultivation, CPD was dehalogenated, converted to glycerin via glycidol, and assimilated by the bacteria. The stereospecific dehalogenating activity for CPD is discussed first.

A typical preparation of (R)- and (S)-CPD is as follows: A bacterial culture was inoculated into a 2.5 L of synthetic medium containing 1% (v/v) (RS)-CPD, 0.5% (w/v) $(NH_4)_2SO_4$, 0.02% (w/v) K_2HPO_4, 0.02 % (w/v) $Na_2HPO_4 \cdot 12H_2O$, 0.04% (w/v) $NaH_2PO_4 \cdot 2H_2O$, 0.05% (w/v) $MgSO_4 \cdot 7H_2O$, 0.001% (w/v) $FeSO_4 \cdot 7H_2O$, 0.0001% (w/v) $CuSO_4 \cdot 5H_2O$, and 0.0001% (w/v) $MnSO_4 \cdot 5H_2O$. Cultivation was subsequently carried out at 30°C for 48 h under aerobic conditions (seed[16] volume, 2% (v/v); agitation,500 rpm; aeration, 0.5 L/min, pH was maintained at 6.8 with 1N aq. NaOH). (S)- or (R)-CPD was assimilated preferentially with the release of chloride ions, and unassimilatable (R)- or (S)-CPD was present in the culture. After cultivation, the broth was centrifuged (10,000x*g*, for 10 min) to remove grown cells, and condensed to a syrup at 40°C *in vacuo*. This syrup of (R)- or (S)-CPD was extracted with ethylacetate and distilled *in vacuo*. (bp 80°C/4 mmHg, average recovery yield was 44.7%). Optical puritiy of the purified (R)- and (S)-CPD was estimated to be greater than 99.5% ee by HPLC analysis of the tosylated derivatives (3-tosyloxy-1-chloro-2-propanol).[17] 200 L scale cultivation could be conducted in a similar manner.[18]

Conversion to GLD was carried out according to the procedure of Marple *et al.*[19] as follows: To an ice cooled solution of (R)- or (S)-CPD in 2-propanol (5 eq) was added dropwise aq. NaOH (1.0 eq; 50% w/v). After stirring in an ice bath for 40 min (>90% conversion), the solution was filtered, evaporated at 40°C *in vacuo*, and (R)- or (S)-GLD was distilled with a Wittomer distillation column *in vacuo*. (bp 40°C/4 mmHg; 45 % yield). The optical purity of (R)- and (S)-GLD was estimated to be graeter than 99.3% ee and 99.4% ee, respectively, by gas chromatography.[9,20] The recovery yield of GLD from the distillation was low but could be improved by scaling-up.

Our method of biological resolution similar to that employed by Pasteur in the resolution of racemic tartaric acid by fungi assimilation, is quite effective and simple. (RS)-CPD is produced economically from propylene via epichlorohydrin using petroleum chemicals, and it should be possible to use highly pure optically active (R)- and (S)-CPD and (R)- and (S)-GLD

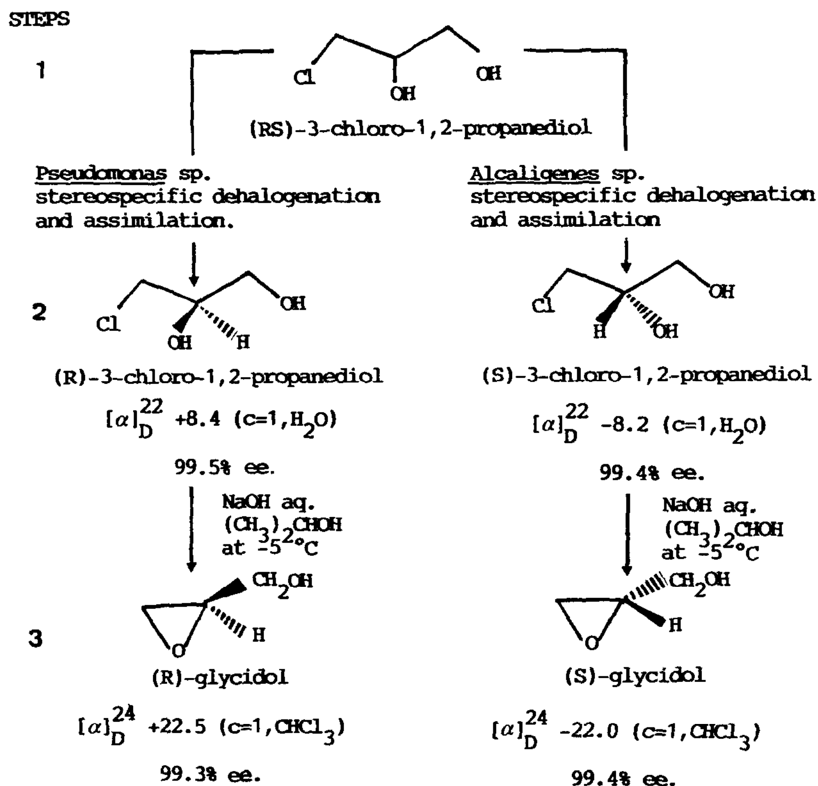


Figure 1: Method for Preparing (R)- and (S)-3-chloro-1,2-propanediol, (R)- and (S)-glycidol

Steps 1 to 2: (RS)-3-chloro-1,2-propanediol was dehalogenated stereospecifically, converted to glycerin via glycidol, and assimilated by the bacteria; steps 2 to 3: conversion ratio was >90% although, distillation yield was low. This could be improved by scaling-up.

for easily obtaining C3 chiral building blocks using our method. More detailed research and the establishment of a pilot-plant are presently in progress.

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14. These three strains belong to genus of ***Alcaligenes***, ***Alcaligenes*** sp. DS-S-7G, ***Alcaligenes*** sp. DS-S-8S, and ***Alcaligenes*** sp. DS-S-1C and were deposited with Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, respectively.
15. These three strains belong to genus of ***Pseudomonas***, ***Pseudomonas*** sp. DS-K-2D1, ***Pseudomonas*** sp. DS-K-9D1, and ***Pseudomonas*** sp. DS-K-14A4 and were deposited as described above.
16. The seed culture was cultivated in an Erlenmeyer flask containing 1% (w/v) Polypeptone, 1% (w/v) yeast extracts, 1% (w/v) glycerin, pH 7.0, at 30 °C for 18-24 h. A similar result will be obtained with any strain described above.
17. HPLC analysis was carried out with a CHIRALCEL OC (0.46 X 25 cm, DAICEL Co., Ltd.; Japan) Conditions: eluent, hexane:2-propanol=95:5 (v/v); sample, 1 μL of 0.5% (w/v) 3-tosyloxy-1-chloro-2-propanol ethanol solution; flow rate 1.0 mL/min; detection, absorbance at 235 nm. Under these conditions, the (R)-form eluted at 90.8 min, and (S)-form eluted at 102.9 min.
18. In a large scale cultivation, residual (R)- or (S)-CPD were absorbed on a charcoal column, eluted with acetone, and the acetone and water were evaporated at 40 °C *in vacuo*. This procedure proved effective for removing the water.
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20. The analysis was carried out with a CHIRALDEX^{R}-A-PH capillary column (0.25 mm X 30 m; astec, Inc. NJ, USA). Conditions: sample, 1 μL of 2% (v/v) GLD 2-propanol solution; carrier gas, nitrogen; flow rate, 0.9 mL/min; split ratio; 1/200; column temp., 45 °C; detector temp., 150 °C; detection, FID. Under these conditions, (R)-GLD was detected at 58.4 min and (S)-GLD at 60.7 min.