Whole-Cell Biotransformation in a Biphasic Aqueous-Organic Solutions. Preparation of Optically Active *cis*-1,3-Dibenzyl-2-oxoimidazolidinedicarboxylic Acid Monoester as a Chiral Precursor of (+)-Biotin

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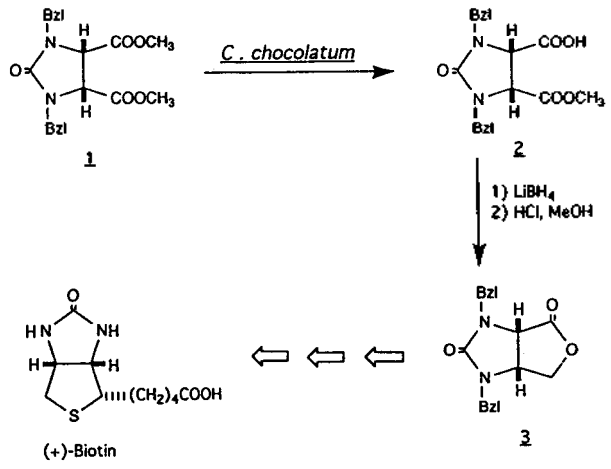
A life essential molecule biotin, *i.e.* vitamin B in the human nutrition, plays an important role on the various enzymatic reactions as a cofactor. The important biological functions in human nutrition has influenced the synthesis of optically active compound. The biotin consists of relatively simple 2-alkyl-3,4-diaminothiophane. Since Goldberg *et al.*[1] synthesized (+)-biotin *via* 14-steps involving an optical resolution of intermediate, the great number of synthetic approaches were reported toward racemic biotin as well as (+)-biotin.[2-4] The use of biological system (enzymes or micro-organisms) to prepare optically active intermediates is widespread and very efficient. Enzymatic hydrolysis of dicarboxylic esters generally applied to obtain as optically active monoester. The one of the examples is the use of a pig liver esterase (PLE).[5] The chemical yield of enzymic hydrolysis and optical purity of product is moderate (Scheme 1). The monoester is reduced with $LiBH_4$ and then cyclized to check the optical purity. The whole-cell biotransformation is well established in conventional aqueous system. However, many substrates are too hydrophobic to react with enzymes during aqueous whole cell bioconversions. These problems could be overcome if organic solvents were used in a whole cell biotransformation. The one of possible drawbacks is the deactivation of whole cells in organic solvents. To the best of our knowledge only one whole cell (*Chromobacterium chocolatum*) bioconversion of dimethyl *cis*-1,3-dibenzyl-2-oxoimidazolidine-4,5-dicarboxylate were reported.[6] In our lab, the reported procedure was followed up but it was very difficult to reach the reported yield. One of the possible reasons was the solubility of dicarboxylic ester in the aqueous reaction system. The organic solvent have commonly been used for enzyme-catalysed reactions.[7] Here we wish to report the characteristics and the substituent effect of *cis*-1,3-dibenzyl-2-oxo-imidazolidine-4,5-dicarboxylates on the biotransformation in two phase system of aqueous and organic media as well as the effects of dicarboxylic ester derivatives.



Scheme 1.

Materials

^{1}H NMR spectra were recorded on a Varian Gemini 2000 (200 MHz) spectrometer. Chemical shifts are given in parts per millions (ppm) relative to $(CH_3)_4Si$ as internal standard. The *Chromobacterium chocolatum* was purchased from Institute for Fermentation, Osaka(Japan). All chemicals and solvents for the synthetic reaction and fermentaion were of reagent grade and were used without further purification unless otherwise noted.

General Procedue

Loopful of spores of *Chromobacterium chocolatum* (IFO-3758) from a sporulated agar slant was inoculated in 500 mL Erlenmeyer flask containing 100 mL of the seed medium consisting of polypepton 1%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, (pH=7). The fermentaion was carried out at 28 °C, 250 rpm for 2 days. Of the seed culture, 10 mL was used to inoculate 100 mL of sterilized production medium in a 500 mL flask. The total volume of production medium was 1.1 liter. Inoculated production culture were again grown for 24hr at 28 °C with rotary shaking at 300 rpm. The cells were collected with centrifugation (5000 rpm, 30 minutes, 4 °C). The collected cells were dissolved in 0.1 M phosphate buffer (pH=7.0, 200 mL) and isooctane (80 mL). A 200 mg of dimethyl *cis*-1,3-dibenzyl-2-oxoimidazolidine-4,5-dicarboxylate was added every hour until the total amount of substrate was reached the 2 grams. The mixture was shaken at 25 °C, 200 rpm for 7 days. The reaction mixture was filtered through a layer of celite and the pH of filtrate was adjusted 10 with 1N NaOH and then extracted with ethyl acetate (3×30 mL). The organic layer contained the starting materials. The pH of aqueous layer was adjusted to 2 with 2N sulfuric acid and then extacted with ethyl acetate (3×30 mL). The organic layer was dried with $MgSO_4$ (anhydrous), concentrated under reduced pressure to give monoester 2 (yield=84%).

^{1}H NMR (200 MHz, $CDCl_3$) δ 3.68 (s, 3H) 4.08 (d, *J*=5.0 Hz, 1H) 4.15 (s, 2H) 4.17 (d, *J*=5.0 Hz, 1H) 5.03 (d, *J*=11 Hz, 1H) 5.13 (d, *J*=11 Hz, 1H) 6.7 (bs, 1H) 7.3 (m, 10H)

To check the optical purity, the monoester 2 (240 mg 0.65 mmol) in THF (5 mL) was added into the solution of $LiBH_4$

Table 1. Degree of hydrolysis in aqueous-organic solution

Solvent[a]	Monoester yield (%)[b,c]	$[\alpha]^{20}_D$ ($CDCl_3$)
Control	29.5%	+60
Acetonitrile	6.9%	
Benzene	35.1%	
Carbon Tetrachloride	63.6%	+59
Cyclohexane	83.1%	+58
n-Hexane	68.5%	+60
Isooctane	82.8%	+59
Metanol	12.7%	
Acetone	4.2%	

[a] pH 8.0 phosphate buffer-28% organic solvent. [b] Yield was obtained after 7 days of incubation period. [c] Pure and isolated yield.

(43 mg, 1.98 mmol) in the dry THF (20 mL) at room temperature. The reaction mixture was refluxed overnight under N_2 gas. The solvent was evaporated under reduced pressure and the residue was dissolved in methanol (10 mL) and added con. HCl (0.1 g). The reaction mixture was refluxed for 2 hours for lactonization. The solvent was evaporated under reduced pressure and then dissolved in ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ (anhydrous) and then concentrated. The lactone (3) was purified by flash chromatography (Silicagel, n-hexane : ethyl acetate=1 : 1). The yield was 93%. $[\alpha]^{20}_D$= +58° (c, 1, $CHCl_3$; ee=99).{lit.[2c] $[\alpha]^{23}_D$= +58.3° (c, 1, benzene)}

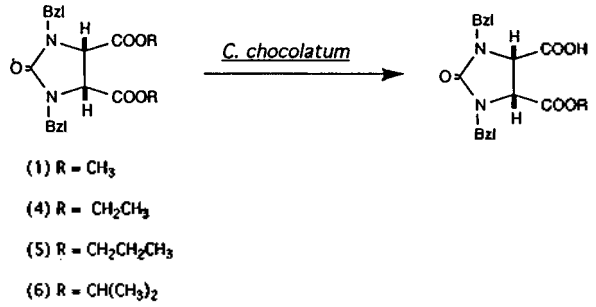
^{1}HMR (200 MHz, $CDCl_3$) δ 3.92 (d, *J*=8.0 Hz, 1H) 4.1 (q, *J*=4 Hz, 1H) 4.15 (s, 2H) 4.3 (d, *J*=5.8 Hz, 1H) 4.4 (d, *J*=5.5 Hz, 1H) 4.63 (d, *J*=15.2 Hz, 1H) 5.04 (d, *J*=15.0 Hz, 1H) 7.3 (m, 10H).

Results and Discussion

Aiming to get the optimum condition of hydrolysis of dialkyl carboxylate, we have investigated the solvents systematically from polar to nonpolar ones with various alkyl derivatives. Isooctane, cyclohexane and n-hexane are best solvents for the hydrolysis of dimethyl ester (1). The highest biotransformation yield and the highest optical purity were obtained when isooctane or cyclohexane was used as an organic solvent in two phase system. For example, methanol and acetone were fairly good for the dissolution of substrate in aqueous system. However the conversion yield was very low. It is not easy to explain why the hydrolysis of dimethyl ester (1) was much better in branched or cyclic hydrocarbon solvent than in polar one. One of the possible reasons is that the extracellular bacterial lipase could be denaturated or the surface of microorganism could be destroyed in polar solvents.[8]

As shown in the experimental section, the feeding amount of substrate was controlled carefully because the amount of substrate affected the yield. The whole cell biotransformations were conducted with the various amount of dimethyl ester. When the 200 mg of dimethyl ester was fed to the bacteria every hour until the total amount was reached to 2 gram. Under this condition with isooctane as a solvent, the product 2 was obtained in 84% yield with 99% of enantiomeric excess (e.e.). (Table 1) The same reaction were carried with different esters of ethyl, n-propyl and isopropyl (Scheme 2).

Iriuchijima *et al.*[5] showed that the hydrolysis of the dipropyl ester (5) with pig liver esterase affored 85% of the monoester with 75% of enantiomeric excess. In case of pig liver esterase, the dipropyl ester (5) was enzymatically hydrolyzed with higher selectivity then the dimethyl ester (1). Even though dimethyl ester was converted into product [(4S, 5R)-3] with high chemical yield as well as the optical purity, only 1-3% of monoester was obtained from the hydrolysis of other diesters such as ethyl, n-propyl and isopropyl under the same condition. This observation may arise from the narrow substrate specificity of the lipase in *C. chocolatum*. Purificaion and gene cloning of the lipase from *C. chocolatum* are currently under investigation.



(1) R = CH_3

(4) R = CH_2CH_3

(5) R = $CH_2CH_2CH_3$

(6) R = $CH(CH_3)_2$

Scheme 2.

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