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Synthesis of optically active α-monobenzoyl glycerol by asymmetric transesterification of glycerol

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Abstract

Efficient asymmetric synthesis of optically active α-monobenzoyl glycerol (α-MBG) was examined by enzymatic transesterification of vinylbenzoate with glycerol in organic solvents. Optical purity and absolute configuration of the synthesized α-MBG could be easily determined with HPLC after derivatization to solketal benzoate by reacting with camphorsulfonic acid and acetone dimethylacetal. 1,4-Dioxane was selected to be the most suitable solvent in which glycerol is soluble. Although almost enzymes lost their activity in 1,4-dioxane, lipases immobilized on macroporous resin catalyzed the synthesis of optically active α-MBG. By using the carrier-fixed CHIRAZYME L-2, the reaction conditions, such as substrate concentrations, reaction temperature, and acyl donor, were optimized. Under the optimized condition, (*R*)-α-MBG having 54% e.e. was synthesized with 94% yield on a multigram-scale, and its optical purity was brought up to over 95% by one recrystallization.

Keywords: Lipase; Screening; Transesterification; Glycerol; Optically active; α-monobenzoyl glycerol

1. Introduction

Optically active α-monoacyl glycerol is known to be a useful starting material for the preparation of chiral drugs, such as β-blockers [1], platelet activating factor (PAF) [2], (*S*)-carnitine, and γ-amino-β-hydroxybutyric acid (GABOB) [3]. Although optically active α-monoacyl glycerols are commonly prepared by chemical synthesis from the chiral pool, such as D-mannitol [4], L-serine, and L-ascorbic acid [5], there is currently a great interest in the use of enzymes for their synthesis [6,7]. The enzymatic methods include: (1) stereoselective acylation of prochiral glycerol derivatives whose β-hydroxyl group is protected; and (2) kinetic resolution of racemic α-monoacyl glycerol derivatives. Although the product can be obtained quantitatively by the former method, the preparation of the substrate requires many steps [8]. On the other hand, the latter method is shorter than the former one, but the maximum

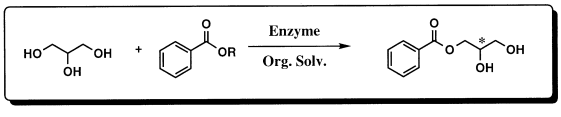

Fig. 1. Enzymatic asymmetric transesterification of glycerol with benzoate derivatives in organic solvents.

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yield is 50% [9]. We therefore examined a novel and efficient method of synthesizing optically active α-monobenzoylglycerol (α-MBG) by an enzymatic transesterification of glycerol with benzoate derivatives in organic solvents. In this process, optically active α-MBG can be theoretically obtained in one step and in a quantitative yield (Fig. 1). Here we describe screening commercial hydrolases that would catalyze the transesterification of glycerol with vinyl benzoate in organic solvents, the optimization of conditions for the transesterification reaction, and its application to a multigram scale synthesis of optically active α-MBG.

2. Materials and methods

2.1. Analytical methods

^{1}H and ^{13}C-NMR spectra were recorded by JEOL LA-400 spectrometer with tetramethylsilane as an internal standard. The infrared (IR) spectra were recorded on a Shimadzu FTIR-8100 infrared spectrophotometer. Mass spectra were recorded on a JEOL JMS-AX500 mass spectrometer. Melting point was determined on a Yanaco MP-J3 (Yanagimoto) hot plate micro melting point apparatus. Optical rotations were measured on a Horiba SEPA-300 polarimeter.

2.2. Materials

HPLC columns ODS-80Ts and Chiralcel OJ were purchased from Tosoh (Tokyo, Japan) and Daicel Chem. Ind. (Tokyo, Japan), respectively. Thin layer chromatography used was Merck silica gel plates Kieselgel 60 F_{254} from Kanto Chem. (Tokyo, Japan). All other chemicals were from commercial sources and used without further purification.

2.3. Enzymes

Commercial lipases were purchased from Fluka Chemie AG (Buchs, Switzerland), Kanto Chemicals (Tokyo, Japan), Seikagaku Kogyo (Tokyo, Japan), Sigma (St. Louis, USA), and Toyo Jozo (Shizuoka, Japan), and were kindly presented as a gift from Amano Pharmaceutical (Nagoya, Japan) and Roche Diagnostics K.K. (Tokyo, Japan).

2.4. Chemical synthesis of α-monoacylglycerols

Authentic α-monoacylglycerols were synthesized from solketal and acyl chloride in the manner as described by Yodo et al. [10].

2.5. Measurement of α-MBG yield

The yield of α-MBG was quantitatively measured by HPLC (Shimadzu) using ODS-80Ts column at a flow rate of 1.0 ml/min with 30% CH_3CN as a solvent by monitoring at 254 nm.

2.6. Analysis of optical purity and absolute configuration of α-MBG

The enzymatically prepared α-MBG was converted to solketal benzoate by the following method [11]. To a 10 μl of the reaction mixture were added 10 μl of 10 mM 1,4-dioxane solution of (+)-10-camphorsulfonic acid and 10 μl of 20% acetone dimethylacetal in ethylacetate. The mixture was then incubated at 30°C for 1 h. Hexane (1 ml) and $NaHCO_3$ (2 mg) were added to the mixture and centrifuged ($12\,000 \times g$, 3 min). The supernatant obtained was analyzed by HPLC at 254 nm using CHIRALCEL OJ column at a flow rate of 0.3 ml/min with hexane/iPrOH = 95/5 as a solvent. (*S*)-Solketal benzoate and its (*R*)-isomer were eluted with their retention times of about 26 and 28 min, respectively.

2.7. Immobilization of lipases on a macroporous resin

One hundred mg of lipase was dissolved in 25 ml of distilled water and to this was added 400 mg of macroporous resin (DIAION HPA

25, Mitsubishi Chemicals, Tokyo, Japan). The mixture was gently shaken at 100 rpm at 10°C for 3–24 h. The resins were collected by filtration and dried under reduced pressure over silica-gel for 8 h at room temperature.

2.8. General reaction condition for the transesterification of glycerol

For each enzyme, 100 mg was added to a reaction mixture (2 ml), which consisted of 100 mM each of glycerol and vinyl benzoate in 1,4-dioxane, and the mixture was shaken at 170 rpm for 1–7 days at 30°C.

2.9. Multigram scale synthesis of (R)-α-MBG by the transesterification of glycerol with carrier-fixed CHIRAZYME L-2

To a 1,4-dioxane solution (1 l) containing of 9.2 g (100 mmol) of glycerol and 22.6 g (100 mmol) of benzoic anhydride, 15 g of carrier-fixed CHIRAZYME L-2 (CHIRAZYME L-2, c.-f., C-2, lyo) was added, and the mixture was stirred for 24 h at 15°C. After a removal of the enzyme by filtration, the reaction mixture was evaporated under reduced pressure and the residue was partitioned between saturated brine and ethyl acetate (100 ml each), and extracted with ethyl acetate for 3 times. The combined organic layer was washed with 5% $NaHCO_3$ solution and saturated brine, and dried over anhydrous Na_2SO_4. Purification by short column chromatography on silica-gel (ethylacetate) afforded 18.4 g of (*R*)-α-MBG (94% yield, 54% e.e.) as a colorless oil. The α-MBG was dissolved in 2 l of hexane/iPrOH (95/5) at 65°C and to this was added a trace amount of optically pure (*R*)-α-MBG. The solution was left at room temperature for 1 h, and the precipitate formed was filtered, washed with hexane/iPrOH (95/5), and dried under reduced pressure over silica-gel. (*R*)-α-MBG was obtained as colorless needles in a yield of 6.65 g (35.2%) whose optical purity was 95.4% e.e. ^{1}H-NMR (400 MHz, DMSO-d_6), δ ppm 8.00–7.98 (m, 2H), 7.67–7.63 (m,1H), 7.54–7.50 (m, 2H), 5.02 (d, 1H, $J = 5.4$), 4.70 (t, 1H, $J = 5.6$), 4.30 (dd, 1H, $J = 3.9, 11.2$), 4.16 (dd, 1H, $J = 6.3, 11.2$), 3.81–3.77 (m, 1H), 3.46–3.43 (m, 2H): ^{13}C-NMR (100 MHz, DMSO-d_6), δ ppm 167.0, 133.3, 129.8, 129.7, 129.6, 70.3, 65.6, 63.5: FAB-Mass *m/z*: 77 (rel. int. 89.2%), 93 (59.8%), 105 (100.0%), 106 (100.0%), 123 (71.7%), 179 (100.0%), 197 (100.0%, $M + H$), 198 (94.1%, $M + 2H$): m.p. 66–67°C [lit. for (*S*)-α-MBG 65–66°C [10]]: $[\alpha]_D^{23} + 18.8°$ (c = 1.00, EtOH) [lit. for (*S*)-α-MBG $[\alpha]_D^{23} - 19.0°$ [10]]: IR (KBr), 3600–3100, 3050, 1700, 1300, 690 cm^{-1}.

3. Results and discussion

3.1. Determination of an optical purity and an absolute configuration of α-MBG

Since the stereoisomers of α-MBG could not be separated on chiral HPLC columns at any conditions, we investigated to establish a simple method to determine an optical purity and an absolute configuration of α-MBG by its derivatization. It was found that α-MBG was easily transformed to its 1,2-isopropylidene derivative (solketal benzoate) by reacting with acetone dimethylacetal and (+)-camphorsulfonic acid at 30°C [11]. Their stereoisomers could be easily separated using a Chiralcel OJ column. We therefore determined a stereoselectivity of the enzyme reaction by the method throughout this study.

3.2. Estimation of organic solvent suitable for the transesterification reaction

Glycerol was highly insoluble in hydrophobic organic solvents, such as CH_2Cl_2, cyclohexane, toluene, CCl_4, $CHCl_3$, iPr_2O, Et_2O, nitrobenzene, *t*-butylacetate, *t*-butyl methyl ether, and isooctane, in which the reaction mixture became biphasic. We found that hydrophilic solvents,

such as CH_3CN, DMF, pyridine, 1,4-dioxane, acetone, THF, and Et_3N solubilized glycerol well, and we selected 1,4-dioxane as a most suitable solvent for the reaction from its ease to manipulate.

3.3. Enzymatic transesterification reaction by commercially available enzymes

We chose vinyl benzoate as an acyl donor for the transesterification reaction. This reagent was screened against 40 commercially available lipases and hydrolases. Although almost enzymes did not exhibit the transesterification activity due to the inactivation in 1,4-dioxane, lipases such as Lipozyme and carrier-fixed CHIRAZYME L-2 (c.-f., C-2, lyo), which already immobilized on resins, catalyzed the reaction. We assumed that immobilization might increase the enzyme stability towards 1,4-dioxane. The commercial enzymes were immobilized on macroporous resin [12] by absorption. Immobilization yield was determined by measuring the residual protein concentration in the supernatant of the immobilization mixture at 280 nm. The yields for lipases from *Pseudomonas cepacia* (PS) and *Pseudomonas* sp. (Toyozyme LIP) were around 80%, those for lipases from *Aspergillus niger*, *Candida lipolytica*, *Humicola* sp. (L-8), *Pseudomonas fluorescens* (AK), *Aspergillus niger* (A), *Mucor javanicus* (M), *Geotricum candidum* (GC-20), *Penicillium aurantiogriseum* (G), *Penicillium. roqueforti* (*R*), and *Mucor* sp. (M-AP) were 50–60%. Those for lipases from *Rhizopus arrhizus*, *Penicillium roqueforti*, *Candida rugosa*, *Candida rugosa* (L-3), *Pseudomonas* sp. (L-4), *Mucor javanicus*, *Rhizopus* sp. (D), *Rhizopus* sp. (F-AP15), and porcine pancreas were 30–40%. Those for proteases from animal pancreas (Pancreatin F), and *Rhizopus niveus* (Nealase F) were also around 30%. Those for lipases from *Rhizopus delemar*, *Burkholderia* sp. (L-1), *Pseudomonas* sp. (L-6), *Bacillus subtilis* (N), and *Aspergillus* sp. (PZ-6) were 10–20%. On the other hand, lipases from wheat germ and *Humicola langinosa* (CE), esterase (AC 409), and pancreatin were not immobilized. The immobilized lipases were examined for the transesterification reaction. Among them, 14 enzymes catalyzed the reaction and gave α-MBG (Table 1). As expected, we could find out the transesterification activity in the commercial enzymes by immobilization but enantiomeric excess of the obtained α-MBG were low (up to 32%) compared to that of α-MBG synthesized by carrier-fixed CHIRAZYME L-2 (60%).

3.4. Reaction condition for the enzymatic transesterification by carrier-fixed CHIRAZYME L-2

Based on the results, carrier-fixed CHIRAZYME L-2 was found to be the most suitable catalyst for the transesterification of glycerol with vinyl benzoate in 1,4-dioxane. To optimize the reaction, substrate concentrations and reaction temperature were varied. Fig. 2A shows the effects of glycerol concentrations (10–150 mM) on the reaction. At any glycerol concentration, α-MBG was formed in proportion to the substrate concentrations, but the yield was decreased when the glycerol concentration was over 150 mM. As shown in Fig. 2B, the presence of vinyl benzoate over 150 mM also decreased the yield of α-MBG. We therefore set the concentrations of acyl donor and acceptor to below 150 mM.

Fig. 3 shows effects of temperature on the reaction. The enzyme activity increased with the reaction temperature, but the enantiomeric excess of the α-MBG decreased at higher temperatures in a time dependent manner. To elucidate the possible mechanism of the decrease in the e.e. of the (*R*)-α-MBG, the chiral monoester was incubated with the CHIRAZYME L-2 in 1,4-dioxane. The enantiomeric excess of the (*R*)-α-MBG decreased in a time-dependent manner and the decreasing rate was proportional to the enzyme concentration and reaction temperature (data not shown). We assumed that the racemization was due to a chemical reaction and

Table 1
Enzymatic transesterification of glycerol by commercial lipases and immobilized lipases on macroporous resin

Lipase from (lipase name)	Manufactured by	Yield (%)	Optical purity (% e.e.)	Absolute config.
Commercial lipase				
Candida antharctica (CHIRAZYME)[a]	Roche[b]	62	60	*R*
Mucor miehei (Lipozyme)	Toyo Jozo[c]	87	15	*R*
Immobilized lipase				
Pseudomonas sp. (PS)	Amano[d]	100	37	*R*
Rhizopus sp. (D)	Amano[d]	10	32	*S*
Pseudomonas sp. (L-4)	Roche[b]	6.2	27	*R*
Pseudomonas sp. (L-6)	Roche[b]	17	24	*R*
Rhizopus sp. (F-AP15)	Amano[d]	17	24	*S*
Mucor sp. (M-AP)	Amano[d]	4.8	18	*S*
Pseudomonas fluorescens (AK)	Amano[d]	13	11	*R*
Rhizopus delemar	Seikagaku[e]	7.6	11	*S*
Pseudomonas sp. (Toyozyme LIP)	Toyo Jozo[c]	30	11	*S*
Aspergillus sp. (PZ-6)	Amano[d]	11	5.4	*R*
Humicola sp. (L-8)	Roche[b]	68	4.2	*S*
Pseudomonas cepacia (PS)	Amano[d]	14	3.9	*R*
Burkholderia sp. (L-I)	Roche[b]	18	2.3	*R*
Penicillium aurantiogriseum (G)	Amano[d]	13	2.3	*S*

[a] CHIRAZYME L-2, c-f., C-2, lyo.
[b] Roche Diagnostics K.K. (Tokyo, Japan).
[c] Toyo Jozo (Shizuoka, Japan).
[d] Amano Pharmaceutical (Nagoya, Japan).
[e] Seikagaku Kogyo (Tokyo, Japan).

not an enzyme catalysis since the same result was seen when heat-inactivated the CHIRAZYME L-2 (150°C for 2 h), was used. It is well known that monoacyl groups attached to 1,3-diols are very easy to rearrange under weakly acidic or basic conditions [11]. Since the resins of the CHIRAZYME L-2 had basic functional group itself, the reason of the chemical racem-

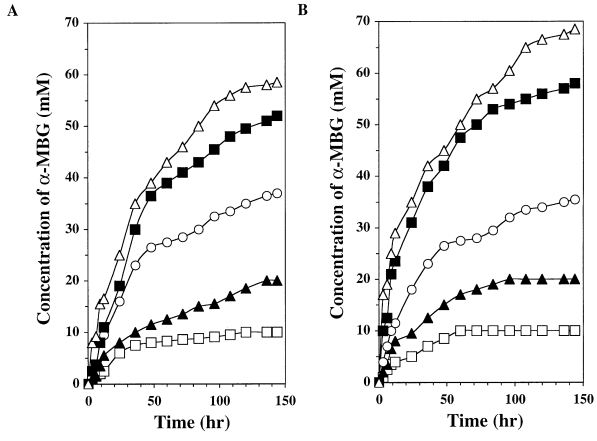

Fig. 2. Effect of (A) glycerol and (B) vinyl benzoate concentration on the transesterification by carrier-fixed CHIRAZYME L-2. (A) The reaction mixture (2 ml) contained 10–150 mM of glycerol, 100 mM of vinyl benzoate, and 10 mg of the enzyme in 1,4-dioxane, was incubated at 30°C with shaking at 170 rpm. (B) The reaction was carried out as described above except that 10–150 mM of vinyl benzoate, and 100 mM of glycerol were used as acyl donor and acceptor, respectively. The varied concentration of the substrates were; 10 mM (□); 20 mM (▲); 50 mM (○); 100 mM (■); 150 mM (△).

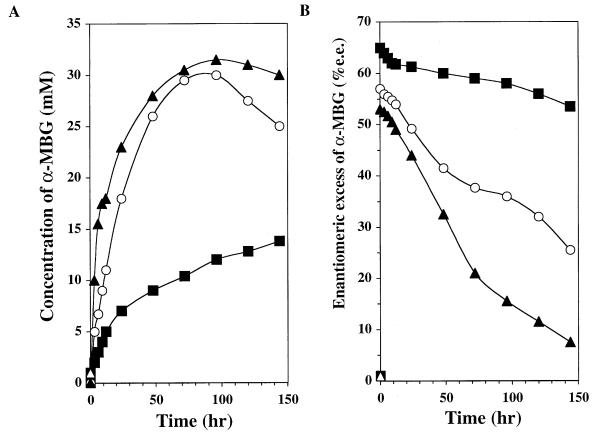

Fig. 3. Effect of temperature on the transesterification by carrier-fixed CHIRAZYME L-2. (A) Yield and (B) enantiomeric excess of (*R*)-α-MBG. The reaction mixture (2 ml) consisted of 100 mM of glycerol, 100 mM of vinyl benzoate, and 10 mg of the enzyme in 1,4-dioxane, was incubated at 15°C (■), 30°C (○), or 45°C (▲) with shaking at 170 rpm.

ization of optically active α-MBG produced by the enzyme reaction was probably due to the 1,3-rearrangement of the benzoyl group of the chiral α-MBG to racemic α-MBG catalyzed by the resins. The problem will be solved by using the enzymes that are immobilized on resins and have no functional groups. Although the enzyme activity was lower than that at 30°C, the chemical racemization was suppressed at low temperature. Therefore, we set the reaction temperature to 15°C.

One hundred mM of various acyl donors, such as methyl benzoate, benzoic anhydride, phenyl benzoate, 2-chloroethyl benzoate, trifluoromethyl benzoate, vinyl pivalate, vinyl butyrate, and vinyl caproate were added to the standard reaction mixture in place of vinyl benzoate and the reactions were incubated at 15°C. Table 2 shows the yields and optical purities of the corresponding monoacyl glycerols produced by the transesterification reaction. Methyl benzoate was also accepted as an acyl donor, but relative activity was very low. The reason could be as follows: a by-product methanol might competitively interfered with glycerol, resulting in a decrease of α-MBG formation. On the other hand, phenyl benzoate, 2-chloroethyl benzoate and trifluoromethyl benzoate were inert as acyl donors. It was considered that phenyl benzoate might not be accepted as a substrate due to its bulky ester group. However, the latter two benzoate derivatives, which having more small ester group, did not act as substrates. The details of the reasons were not clear. Among alkanoic acid vinyl esters tested, only vinyl pivalate acted as an acyl donor but the relative activity was low. Among of them, benzoic anhydride was selected as the best acyl donor for the reaction, since the reaction rate was markedly increased when it was used as an acyl donor.

Based on the results, the optimum reaction condition was established as follows: 100 mM each of glycerol and benzoic anhydride was

Table 2
Effect of acyl donors on the transesterification reaction
The reaction mixture (2 ml) contained 100 mM of glycerol, 100 mM of acyl donor, and 30 mg of carrier-fixed CHIRAZYME L-2 in 1,4-dioxane, was incubated at 15°C with shaking at 170 rpm

Acyl donor	Reaction time (h)	Yield (%)	Optical purity (% e.e.)
Vinyl benzoate	72	39.6	58.6
Methyl benzoate	120	16.8	56.1
Benzoic anhydride	72	91.9	50.1
Vinyl pivalate	72	19.0[a]	Not determined

[a]The yield of α-monopivaloyl glycerol was measured by HPLC at 215 nm.

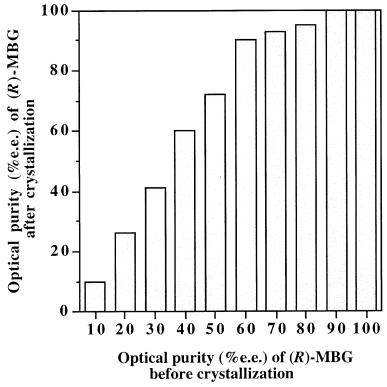

Fig. 4. Preferential crystallization of (*R*)-α-MBG having various enantiomeric excess. (*R*)-α-MBG having various enantiomeric excess was prepared by mixing (*R*) and (*S*)-α-MBG. The monoester (10 mg) was dissolved in 2 ml of hexane/iPrOH (95/5) at 65°C and to this was added a trace amount of (*R*)-α-MBG at room temperature. The solution was left at −20°C for 1 day, and the precipitate formed was filtered and washed with the same cold solvent. The obtained monoesters were derived from solketal benzoate with CSA/dimethoxypropane and their optical purity values were measured.

incubated with 15 mg/ml of carrier-fixed CHIRAZYME L-2 in 1,4-dioxane at 15°C.

3.5. Preferential crystallization of (R)-α-MBG

Since the optical purity of (*R*)-α-MBG synthesized by the enzyme reaction was still low (∼60% e.e.), increasing its optical purity by a preferential crystallization [13,14] was investigated. We have screened crystallization conditions for α-MBG and found good crystals may be recovered from hexane/iPrOH (95/5). Samples of (*R*)-α-MBGs of varying enantiomeric excess (0–100% e.e.) were prepared and recrystallized from the solvent system and their optical purities measured. As shown in Fig. 4, (*R*)-α-MBGs having 20–90% e.e. were markedly increased in their optical purities by one recrystallization procedure.

3.6. Multigram scale–synthesis of (R)-α-MBG by the transesterification with carrier-fixed CHIRAZYME L-2

A multigram scale–synthesis (100 mmol scale) of (*R*)-α-MBG by the transesterification reaction under the optimized conditions was examined. Reaction was completed within 24 h. After passing through short column chromatography, the reaction product was obtained in 94% yield (18.4 g) and its structure was confirmed to be (*R*)-α-MBG (54% e.e.) by its NMR, IR, and mass spectrum and HPLC analysis. Its optical purity was elevated to 95% by one recrystallization from 2-l of hexane/2-propanol (95/5).

In this study, we describe a novel efficient method of preparing (*R*)-α-MBG by the transesterification of glycerol with benzoate derivatives in the hydrophilic organic solvent, such as 1,4-dioxane. Among the various commercial hydrolases tested, carrier-fixed CHIRAZYME L-2 exhibited the most suitable results. Under the optimized condition, (*R*)-α-MBG having >95% e.e. was synthesized in one step.

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