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Efficient Lipase-Catalyzed Synthesis of Chiral Glycerol Derivatives

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Efficient asymmetric syntheses of glycerol derivatives were achieved by lipase-catalyzed transesterification. 2-*O*-Substituted glycerol was enzymatically esterified with acetic acid or acetate in an organic medium to afford an optically active monoester. In particular, lipase-catalyzed transesterification with vinyl acetate or phenyl acetate gave high chemical and optical yields.

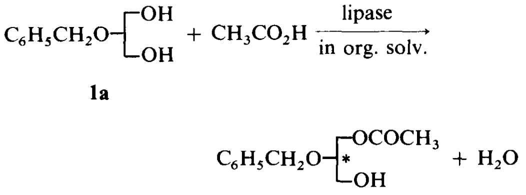
Keywords asymmetric synthesis; glycerol derivative; lipase; transesterification; vinyl acetate

Enzymic reactions are becoming accepted as routine procedures in organic synthesis.1) Such synthetic technology has been utilized by the pharmaceutical industry.1b,2) The most useful enzymes for organic synthesis are those which accept a broad structural range of substrates and are commercially available and inexpensive. One such group of enzymes is the lipases. However, the solvent system in enzymic reactions is also an important factor for organic chemists. Recently, some enzymes have been reported to act well as asymmetric catalysts even in organic solvents.3)

From these points of view, we have investigated the utilities of enzymes for the asymmetric synthesis of useful chiral building blocks. In this paper, we show that the lipase-catalyzed synthesis of chiral glycerol derivatives, which are useful as starting materials for the preparation of many chiral drugs,4) can be achieved in some organic solvents or in a non-solvent system5)

In 1986, Schneider and his co-workers6) reported that (*R*)-1-*O*-acetyl-2-*O*-benzylglycerol was obtained in good chemical and optical yield by enzymic hydrolysis of prochiral 2-*O*-benzylglycerol diacetate in an aqueous medium. The disadvantages of this procedure are the difficulty in the recovery of the enzyme for reuse and the need to maintain an optimal pH during the reaction course. In order to overcome these problems, we employed an enzymic reaction in an organic medium. First, the esterification of 2-*O*-benzylglycerol with carboxylic acid was examined in an organic solvent.

As preliminary experiments suggested that the esterification proceeded with lipase P (from *Pseudomonas fluorescens*), lipase B (from *Pseudomonas fragi*), and lipase LP (from *Chromobacterium viscosum*) in 1,1,1-trichloroethane (TCE) and mixed solvents containing TCE, we examined the effects of each lipase and solvent system on the chemical and optical yields under controlled conditions (Table I).



1a

2a

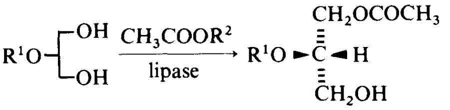
In all cases, these lipases enantiotopically catalyzed the esterifications to afford (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol in good to moderate optical yields. These results indicated

TABLE I. Lipase-Catalyzed Esterification of 2-*O*-Benzylglycerol (**1a**) with Acetic Acid a)

Entry	Lipase	Solvent (Ratio)	React. time (h)	C.Y. (%)	O.Y. b) (%ee)
1	Lipase P	TCE	2	61	68
2	Lipase B	TCE	2	58	36
3	Lipase LP	TCE	2	45	74
4	Lipase P	*n*-Hexane : TCE (1 : 4)	2	52	60
5	Lipase B	*n*-Hexane : TCE (1 : 4)	2	50	52
6	Lipase LP	*n*-Hexane : TCE (1 : 4)	2	38	26
7	Lipase P	Acetone : TCE (1 : 9)	2	51	70
8	Lipase B	Acetone : TCE (1 : 9)	2	52	60
9	Lipase LP	Acetone : TCE (1 : 9)	2	32	74

a) The reaction were carried out with **1a** (1 mmol), acetic acid (1.5 mmol), lipase (7000—8000 units), and a solvent (10 ml) at 25 °C. *b)* Optical yields were determined by HPLC analysis using a column packed with LKB Enantio Pack. The absolute configuration was *S* in every case.

TABLE II. Asymmetric Synthesis of (*S*)-1-*O*-Acetyl-2-*O*-substituted Glycerol by Lipase-Catalyzed Transesterification a)

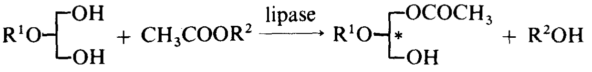


Entry	R^1	R^2	Lipase	Reaction time (h)	Conv. (%)	C.Y. (%)	O.Y. b) (%ee)
10	$PhCH_2$	C_2H_5	Lipase P	3	26	20	90
11	$PhCH_2$	C_4H_9	Lipase P	3	29	23	90
12	$PhCH_2$	iso-C_4H_9	Lipase P	3	5	4	92
13	$PhCH_2$	$PhCH_2$	Lipase P	3	32	28	88
14	$PhCH_2$	Ph	Lipase P	3	92	88	90
15	$PhCH_2$	$CH_2=CH$	Lipase P	3	100	92	90
16	$PhCH_2$	$CH_2=CH$ c)	Lipase P	3.5	100	92	92
17	$PhCH_2$	$CH_2=CH$ d)	Lipase P	4	100	92	94
18	$PhCH_2$	$CH_2=CH$	Lipase B	3	98	82	71
19	$PhCH_2$	$CH_2=CH$	Lipase LP	4	12	10	84
20	$PhCH_2$	$CH_2=CH$	Lipase MY	4	95	60	6
21	$PhCH_2$	$CH_2=CH$	Lipase OF	4	25	20	20
22	CH_3	$CH_2=CH$	Lipase P	2.5	100	85	70
23	CH_3	$CH_2=CH$	Lipase B	1.5	100	72	48
24	CH_3	$CH_2=CH$	Lipase LP	2	100	88	92
25	C_2H_5	$CH_2=CH$	Lipase P	3	100	90	90
26	C_2H_5	$CH_2=CH$	Lipase B	2	100	84	61
27	C_2H_5	$CH_2=CH$	Lipase LP	4	100	87	89

a) The reactions were carried out with a substrate (50 mmol), acetic acid ester (100 mmol), and lipase (7000—8000 units) at 25 °C unless otherwise noted. *b)* Optical yields were determined by HPLC analysis using a column packed with LKB Enantio Pack (entries 10—21) and with Chiralcel OB after benzoylation of the hydroxy group (entries 22—27). *c)* Reaction temperature, 17 °C. *d)* Reaction temperature, 8 °C.

that the lipase-catalyzed esterification could proceed in an organic medium, and the enzyme could be easily removed by usual filtration. However, in this system the reverse reaction seems to occur gradually owing to the formation of water as the reaction proceeds. Encouraged by the potential usefulness of this method, we next attempted to find conditions that would give satisfactory yield and optical purity.

We found that the transesterification between a glycerol derivative and a carboxylic acid ester was catalyzed by lipase even in an non-solvent system. When a mixture of 2-*O*-benzylglycerol, ethyl acetate, and lipase P was stirred vigorously at 25 °C for 3 h, (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol was obtained in 20% yield and its optical purity was 90% enantiomer excess (ee). After further experiments as summarized in Table II, a high yield (92%) of the product with high optical purity (90%ee) was achieved by the use of vinyl acetate under the same conditions.



1 2

R^1: **a**, $PhCH_2$, **b**, CH_3, **c**, C_2H_5

Figure 1 shows that the monoester is formed with a sufficiently rapid velocity and the substrate is almost completely consumed after 3 h, although slow formation of the diester is also observed. Besides the system of vinyl acetate and lipase P, that of phenyl acetate and lipase B also gave the satisfactory results (entries 14, 18). The optical purity of the product was improved slightly by lowering the reaction temperature (entries 16, 17).

The enzymic discrimination of an enantiotopic group in this transesterification was recognized with 2-*O*-methyl and 2-*O*-ethylglycerol (entries 22—27). It is of interest that lipase LP functioned better than lipase P in the case of 2-*O*-methylglycerol (entry 24).

The advantages of this procedure for asymmetric synthesis of glycerol derivatives are as follows. 1) Chemical and optical yields are generally high. 2) The enzyme can be removed by routine filtration and the product is obtained simply by concentration of the filtrate, without extraction procedures. Therefore, the procedure seems to be suitable for large-scale production. 3) Optically active 2-*O*-substituted glycerol derivatives appear to be useful chiral building blocks. The 2-*O*-benzyl derivative is particularly noteworthy because the benzyl group can be easily removed by catalytic hydrogenolysis.[4]

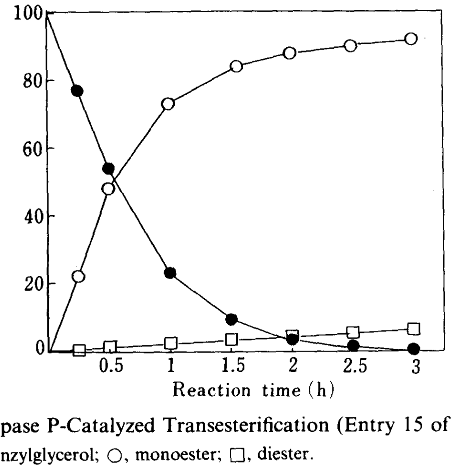


Fig. 1. Lipase P-Catalyzed Transesterification (Entry 15 of Table II)
●, 2-*O*-benzylglycerol; ○, monoester; □, diester.

Experimental

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JNM FX90Q FT-NMR spectrometer using tetramethylsilane (in $CDCl_3$) as an internal standard. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra and mass spectra (MS) were recorded on a JASCO IR-810 IR spectrometer and a JEOL JMS D-100 mass spectrometer, respectively. Optical rotations were determined with JASCO DIP-140 digital polarimeter. High-performance liquid chromatography (HPLC) was carried out with a JASCO TRI ROTAR-V (ultraviolet detection) equipped with a column packed with LKB Enantio Pack (8 mM phosphate buffer containing 0.1 M NaCl) or Chiralcel OB (2-propanol/hexane). Column chromatography was carried out on silica gel (Kiesel gel-60, 70—230 mesh, Merck). Thin-layer chromatography was used to monitor the reaction and to ascertain the purity of the reaction products. The spots were visualized by spraying with a 10% solution of phosphomolybdic acid in ethanol and heating. The following lipases were used; lipase P (Amano Seiyaku Co., Ltd.), lipase B (Sapporo Breweries Ltd.), lipase LP (Toyo Jozo Co., Ltd.), lipases MY and OF (from *Candida cylindracea*, Meito Sangyo Co., Ltd.).

2-*O*-Alkylglycerol 2-*O*-Benzyl-, 2-*O*-methyl-, and 2-*O*-ethylglycerol were prepared according to the method reported by Baggett and his co-workers.[7]

2-*O*-Benzylglycerol: bp 180 °C (bath) (3 mmHg). IR (neat) cm^{-1}: 3380 (OH). ^{1}H-NMR (5% $CDCl_3$ solution) δ: 3.20 (2H, s, 2 × OH), 3.43—3.70 (5H, m, CH_2CHCH_2), 4.53 (2H, s, CH_2Ph), 7.20 (5H, s, C_6H_5).

2-*O*-Methylglycerol: bp 120 °C (bath) (3 mmHg). IR (neat) cm^{-1}: 3380 (OH). ^{1}H-NMR (5% $CDCl_3$ solution) δ : 3.19 (2H, s, 2 × OH), 3.39 (3H, s, OCH_3), 3.45 (1H, t, $J=4.2$ Hz, CH), 3.64 (2H, d, $J=4.2$ Hz, 2 × CH_2O).

2-*O*-Ethylglycerol: bp 115 °C (bath) (4 mmHg). IR (neat) cm^{-1}: 3380 (OH). ^{1}H-NMR (5% $CDCl_3$ solution) δ: 1.20 (3H, t, $J=6.2$ Hz, CH_3), 3.40—3.80 (9H, m, 2 × OH, 3 × CH_2O, CH).

General Procedure for Lipase-Catalyzed Esterification of 2-*O*-Benzylglycerol with Acetic Acid A mixture of 2-*O*-benzylglycerol (183 mg, 1 mmol), acetic acid (90 mg, 1.5 mmol), lipase (7000—8000 units), and a solvent (10 ml) was shaken at 25 °C for 2 h. After removal of the lipase by filtration, the filtrate was concentrated under reduced pressure. The residue was chromatographed on a short silica gel column (methanol : dichloromethane = 1 : 40) to give (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol. Its ethanol solution was always dextrorotatory and the optical purity was determined by HPLC analysis. IR (neat) cm^{-1}: 3380 (OH), 1745 (CO). ^{1}H-NMR (5% $CDCl_3$ solution) δ: 2.04 (3H, s, $COCH_3$), 3.53—3.70 (4H, m, $HOCH_2CH$), 4.15 (2H, d, $J=5.5$ Hz, CH_2OCO), 4.58 (1H, s, OCH_2Ph), 7.24 (5H, s, C_6H_5). MS *m/z*: 224 (M^+). Yields of the product are listed in Table I.

General Procedure for Lipase-Catalyzed Transesterification A mixture of substrate (50 mmol), acetic and ester (100 mmol), and lipase (7000—8000 unit) was shaken at 25 °C (except in entries 16 and 17). After removal of the lipase by filtration, the filtrate was concentrated under reduced prossure. the residue was chromatographed on a short silica gel column (methanol : dichloromethane = 1 : 40) to give 2-*O*-substituted 1-*O*-acetylglycerol. An ethanol solution of each product was dextrorotatory and the optical purity was determined by HPLC analysis. The reaction times and yields are given in Table II.

(*S*)-1-*O*-Acetyl-2-*O*-benzylglycerol (**2a**): The spectral data are described above. $[\alpha]_D^{20}$ +13.4° ($c=2.0$, EtOH) (obtained in entry 17).[8]

(*S*)-1-*O*-Acetyl-2-*O*-methylglycerol (**2b**): IR (neat) cm^{-1}: 3370 (OH), 1740 (CO). ^{1}H-NMR (5% $CDCl_3$ solution) δ: 2.02 (3H, s, CH_3CO), 3.31—3.63 (4H, m, $HOCH_2CH$), 3.40 (3H, s, OCH_3), 4.09 (2H, d, $J=5.4$ Hz, CH_2OCO). MS *m/z*: 148 (M^+). $[\alpha]_D^{21}$ +6.2° ($c=2.0$, EtOH) (obtained in entry 24).[9]

(*S*)-1-*O*-Acetyl-2-*O*-ethylglycerol (**2c**): IR (neat) cm^{-1}: 3370 (OH), 1740 (CO). ^{1}H-NMR (5% $CDCl_3$ solution) δ: 1.21 (3H, t, $J=6.0$ Hz, CH_3), 2.17 (3H, s, CH_3CO), 3.47—3.81 (6H, m, $HOCH_2CHOCH_2$), 4.20 (2H, d, $J=5.4$ Hz, CH_2OCO). MS *m/z*: 162 (M^+). $[\alpha]_D^{21}$ +10.6° ($c=1.0$, EtOH) (obtained in entry 25).[9]

References and Notes

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8) $[\alpha]_D^{21}$ -13.2 ($c=3$, EtOH) for (R)-isomer (ref. 6).

9) The absolute configuration was determined by conversion into (R)-2-methoxy-(or ethoxy)-3-methoxy-1-propanol, which was also derived from (S)-1-O-acetyl-2-O-benzylglycerol (**2a**).