Enzymatic Resolution of *trans*-2,3-Dihydro-3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (*trans*-Flavanon-3-ol) by Lipase

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trans-2,3-Dihydro-3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (*trans*-flavanon-3-ol) was resolved to acetate of (2*S*,3*S*)-(-)-*trans*-flavanon-3-ol and (2*R*,3*R*)-(-)-*trans*-flavanon-3-ol by an enzymatic transesterification with vinyl acetate in the presence of *Pseudomonas cepacia* lipase.

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Various methods of the synthesis of *trans*-2,3-dihydro-3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (*trans*-flavanon-3-ol) and derivatives have been described in literature during recent years [1]. *trans*-Flavanon-3-ols are widely distributed in plants, and are interesting compounds with respect to the existence of optically active compounds such as fustin (3,7,3',4'-tetrahydroxyflavanone) [2], strobobanksin (3,5,7-trihydroxy-6-methylflavanone) [3], pinobanksin (3,5,7-trihydroxyflavanone) [4], aromadendrin (3,5,7,4'-tetrahydroxyflavanone) [5], Deodarin (3,5,6,3',4'-pentahydroxy-8-methylflavanone) [6], alpinone (3,5-dihydroxy-7-methoxyflavanone) [7], taxifolin (3,5,7,3',4'-pentahydroxyflavanone) [5], and dihydrorobinetin (3,7,3',4',5'-pentahydroxyflavanone) [8]. Furthermore, Takahashi and coworkers [9] reported the enantioselective synthesis of enantiomers of *trans*-flavanon-3-ol **1** from the chiral epoxychalcone which was obtained by the asymmetric epoxydation of 2'-methoxychalcone. Racemic **1** was also separated into their enantiomers by hplc on chiralpack OT(+) column [10]. On the other hand, the use of enzymes as chiral catalysts for the preparation of chiral compounds of synthetic value is well documented [11]. In particular, hydrolytic enzymes which can be used in organic solvents as well as in aqueous solution are attractive [12], and there have been many reports describing the asymmetric and enantioselective synthesis of optical active alcohols and esters by lipase- and esterase-catalyzed transesterification in organic solvents. In this paper, we wish to report the enantioselective transesterification of racemic **1** with *Pseudomonas cepacia* lipase (lipase PS) using vinyl acetate as acyl donor.

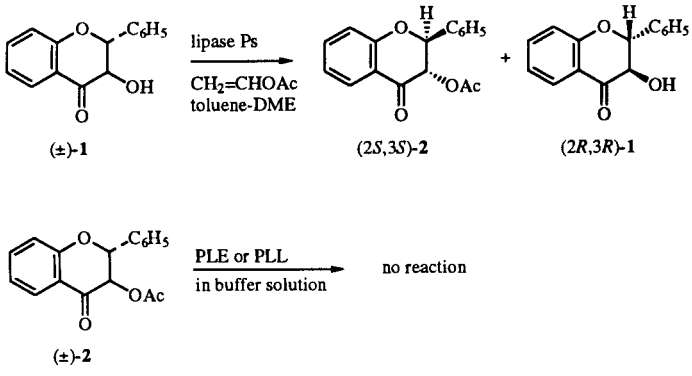
Results and Discussion.

In an initial attempt, enzymatic hydrolysis of *trans*-3-acetoxy-2,3-dihydro-2-phenyl-4*H*-1-benzopyran-4-one (*trans*-flavanon-3-ol acetate) (**2**) was performed *via* incubation using pig liver esterase (PLE) or porcine pancreatic lipase (PPL, Sigma Type II) in phosphate buffer solution at room temperature for 10 days, however, the lipases exhibited very low catalytic activities in the hydrolysis of acetate **2**, and did not afford the enzymatically hydrolyzed products. Whereas, lipase-catalyzed esterification reactions in organic solvents are often more enantioselective than the corresponding hydrolytic reactions in water [13]. For these reasons, we turned our attention to the transesterification of (±)-**1** using lipases.

The results of enzymatic transesterification of (±)-**1** with lipases using vinyl esters as acyl donor are summarized in Table 1. In the enzyme-mediated transesterification with *porcine pancreatic* lipase (PPL, Type II) or lipase from *Candida cylindracea* (lipase MY, 62,000 Ug^{-1}) using vinyl acetate as acyl donor, the alcohol (±)-**1** was not esterified even though the substrate was allowed to react for 10 days (entries 2 and 3). The transesterification of (±)-**1** with lipase PS using vinyl acetate as acylating agent, however, proceeded smoothly with high degree of enantioselectivity, to afford (2*S*,3*S*)-(-)-*trans*-3-acetoxy-2,3-dihydro-2-phenyl-4*H*-1-benzopyran-4-one ((2*S*,3*S*)-**2**) and (2*R*,3*R*)-(-)-*trans*-2,3-dihydro-3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one ((2*R*,3*R*)-**1**) (entry 1). For example, in the presence of 4Å molecular sieve powder, the alcohol (±)-**1** and vinyl acetate in the mixture of toluene and DME (2:1) were incubated with lipase PS at 22-23° with stirring. After 5 days, the reaction was terminated by filtration of the enzyme, (2*S*,3*S*)-**2** (96% ee) and (2*R*,3*R*)-**1** (59% ee) were isolated in 15% and 42% yields, respectively. The absolute configuration of (2*S*,3*S*)-**2** and (2*R*,3*R*)-**1** was established by comparision of optical rotation with literature data. The optical purities (ee) of (2*S*,3*S*)-**2** and (2*R*,3*R*)-**1** were determined by chiral hplc analysis with Daicel chiralcel OG column. The transesterification of (±)-**1** with lipase PS using vinyl butanoate, however, proceeded to the no formation of the butylated product even though the substrate was allowed to react 10 days (entry 4).

Recently, Burgess and Jennings [14] proposed a rule for predicting the fast-reacting enantiomer in the resolution of unsaturated alcohols mediated by lipase from *Pseuomonas sp.* (Figure 1), and suggested a strategy for improving the efficiency of resolutions catalyzed by the

Scheme 1



enzyme. In addition, Burgess and Jennings [14], and Naemura and coworkers [15] proposed independently a cubic-spaced active site model for lipase from *Pseudomonas sp.* or lipase YS which predict the fast-reacting enantiomer of the alcohol on the basis of the sizes of the substituents at the chiral centre of the substrate. Naemura and coworkers [15] assumed that the hydroxy group being acylated is always positioned at the catalytic site and the hydrophobic moieties of the substrate are located at the back of the large hydrophobic pocket. In the enzymatic transesterification of (±)-**1**, C_2-phenyl group is significantly larger than C_4-carbonyl group, and the stereospecificity of lipase PS for alcohol **1** is interpreted straightforwardly as shown in the binding orientation (Figure 2) in which the "large group" on the chiral center occupies the wide HL_r site.

Figure 1. Simple model for predicting which substrates will be resolved effectively *via* biocatalytic acylations mediated by lipase from *Pseuomonas sp.*, and the sense of the enantioselection.

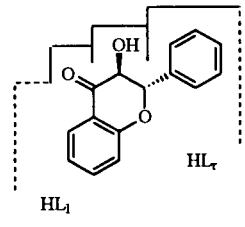


Figure 2. Top perspective view of the active site model. HL_l and HL_r = left and right site of large hydrophobic binding site, respectively.

EXPERIMENTAL

All melting points were taken with a Gallenkamp melting points apparatus and are uncorrected. The ir spectra were recorded on a Hitachi 260-10 spectrometer, ^1H and ^{13}C nmr spectra were obtained with a Hitachi R-90H spectrometer in deuteriochloroform, using tetramethylsilane as internal standard. Mass spectra were run on a JMS-AX505(JEOL) mass spectrometer. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. The enantiomeric excess value (ee) of the products was determined by chiral HPLC analysis with a Daicel chiralcel OG column (4.6 mm x 25 cm) with hexane-propan-2-ol (50:1) as mobile phase at a flow rate of 0.4 cm^3 min^{-1}; detection, uv at 220 nm.

(±)-*trans*-2,3-Dihydro-3-hydroxy-2-phenyl-4*H*-1-benzopyran-4-one (**1**) was prepared from 2'-hydroxychalcone as described in the literature [16]. (±)-*trans*-3-Acetoxy-2,3-dihydro-2-phenyl-4*H*-1-benzopyran-4-one (**2**) was prepared from **1** as described in the literature [17]. *Pseudomonas cepacia* lipase (lipase PS) and lipase from Candida Cylindracea (lipase MY, 62,000 u g^{-1}) were purchased from Amano Pharmaceutical Co. and from Meito Sangyo Co., respectively. Porcine pancreatic lipase (PPL, Type II) and porcine liver esterase were obtained from Sigma Chemical Co. Enzymatic transesterification of (+)-**1** with lipase PS using vinyl esters (entries 1 and 4).

To a solution of (±)-**1** (480 mg, 2 mmoles) in a mixture of toluene and dimethoxyethane (DME) (2:1, 20 ml) was successively added lipase PS (480 mg), 4-Å molecular sieve powder (480 mg) and vinyl acetate (172 mg, 2 mmoles) and the mixture stirred vigorously at 22-23° with monitoring tlc.

The reaction ceased at approximately 50% conversion by filtration of the enzyme (5 days) and the filtrate was evaporated to dryness under reduced pressure. The reacted ester (2*S*,3*S*)-(-)-**2** and the unesterified alcohol (2*R*,3*R*)-**1** were separated by column chromatography on silica gel with benzene-ethyl acetate (20:1) as eluent.

The first eluate afforded (2*S*,3*S*)-**2** (82 mg, 15% yield) as a colorless oil; $[\alpha]_D^{23}$ = -20.9° (c = 0.79, dichloromethane) (ee = 96%); ir (neat): 1750 (-C=O), 1700 (-$OCOCH_3$), 760, 740, 690 cm^{-1} (Ar-H); ^1H-nmr: δ 1.97 (3H, s, -$OCOCH_3$), 5.40 (1H, d, C_2-*H*, J = 12 Hz), 5.81 (1H, d, C_3-*H*, J = 12 Hz), 7.01-7.22 (3H, m, Ar-*H*) 7.38-7.64 (5H, m, Ar-*H*), 7.90 ppm (1H, d-d, C_5-*H*, J = 9 and 1.5 Hz); ^{13}C-nmr: δ 20.2, 73.9, 81.7, 117.8, 119.7, 122.1, 127.3, 127.5, 128.5, 129.3, 135.4, 136.4, 160.8, 168.9, 188.1 ppm; ms: m/z 282 (M^+).

Anal. Calcd. for $C_{17}H_{14}O_4$: C, 72.33; H, 4.99. Found: C, 72.18; H, 4.85.

The absolute configuration was established by comparison of the optical data with those reported [9] [colorless oil: $[\alpha]_{589}^{19}$ = -12.5° (c = 0.47, dichloromethane, ee = 60%)].

The second eluate with benzene-ethyl acetate afforded (2*R*,3*R*)-(-)-**1** as colorless crystals, mp 189-190°; $[\alpha]_D^{23}$ = -15.9° (c = 0.5, dichloromethane), (ee = 59%); ir (potassium bromide): 3550 (-OH), 1690 cm^{-1} (-C=O); ^1H-nmr: δ 3.75 (1H, s, OH), 4.55 (1H, d, C_3-*H*, J = 12.5 Hz), 5.13 (1H, d, C_2-*H*, J = 12.5 Hz), 7.00-7.21 (3H, m, Ar-H), 7.35-7.70 (5H, m, Ar-H), 7.87 (1H, d-d, C_5-H, J = 9 and 1.5 Hz) ppm; ^{13}C-nmr: δ 73.6, 83.8, 118.0, 118.5, 122.0, 127.2, 127.4, 128.5, 129.1, 136.3, 136.7, 161.6, 194.0 ppm; ms: m/z 240 (M^+).

Anal. Calcd. for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 74.78; H, 4.92.

The absolute configuration of (2*R*,3*R*)-**1** was established by comparison of the optical data with those reported [9] [colorless

crystals, mp 170-173°; $[\alpha]_{589}^{22}$ = -7.5° (c = 0.4, dichloromethane, ee = 52%)].

Enzymatic Hydrolysis of Acetate **2** using Lipases.

Potassium dihydrogenphosphate buffer (0.2 *M*, *p*H 7.6, 7.0 ml, 1 M = 1 mol dm^{-3}), 2 wt% aqueous poly(vinyl alcohol) solution (7.5 ml), and PLE or PPL (800 mg) were added successively to a solution of acetate **2** (282 mg, 1 mmole) in diisopropyl ether (15 ml).

The mixture was stirred at 23° with monitoring by tlc analysis (silica gel, benzene). However, the acetate **2** was unhydrolysed even though the substrate was allowed to react for 10 days.

Table 1

Enzymatic Resolution of (±)-**1** using Lipases

Entry	Enzyme [a]	Acyl donor [b]	Time (day)	Conversion [c]	(2*S*,3*S*)-**2** Yield	ee	(2*R*,3*R*)-**1** Yield	ee	E [d]
1	PS	A	5	48%	15%	96%	42%	59%	89
2	PPL	A	5	0%	0%	-	88%	0%	-
3	MY	A	10	0%	0%	-	85%	0%	-
4	PS	B	6	0%	0%	-	90%	0%	-

[a] PS = lipase from *Pseudimonas cepacia* (lipase PS). PPL = porcine pancreatic lipase (PPL). MY = lipase from *Candida Cylindracea* (lipase MY). [b] A = vinyl acetate, B = vinyl butanoate. [c] Determined by hplc (benzene-ethyl acetate). [d] Enantiomeric ratio [18]:

$E = \ln [1-c(1 + ee_p)]/[1-c(1-ee_p)]$, Where $c = ee_p/ee_s + ee_p$, ee_p, ee% of esterified product **2**; ee_s, ee% of unesterified alcohol **1**.

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