http://dx.doi.org/10.5012/bkcs.2012.33.2.409

Lipase Catalyzed Kinetic Resolution of *rac*-2-(3-Methoxy-4-methylphenyl) propan-1-ol and *rac*-2-(3-Hydroxy-4-methylphenyl)propyl propanoate for *S*-(+)-Xanthorrhizol

Azam Sharif Mohammed Shafioul[†,‡] and Chan Seong Cheong[‡,*]

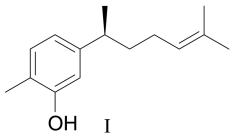
[†]*University of Science & Technology, Daejeon, South Korea*
[‡]*Biomolecules Function Research Center, Future Convergence Research Division, Korea Institute of Science and Technology, Seoul 136-791, Korea. *E-mail: c2496@kist.re.kr*
Received November 1, 2011, Accepted November 23, 2011

Xanthorrhizol is a bisabolane type of natural sesquiterpene, the major component of essential oils of *Curcuma xanthorrhiza.* 2-(3-Methoxy-4-methylphenyl)propan-1-ol and 2-(3-hydroxy-4-methyl phenyl)propan-1-ol could be essential building block for enantioselective synthesis of xanthorrhizol. Enantioselective (c = 53%, E = 80 ± 3) for *R*-(+)-2-(3-hydroxy-4-methylphenyl) propan-1-ol and (c = 58%, E = 27 ± 1) for *R*-(+)-2-(3-methoxy-4-methylphenyl) propan-1-ol resolution processes were developed *via* lipase-catalyzed reaction. We found lipase *Aspergillus oryzae* (AOL) and *Porcine pancreas* (PPL) are selective to transesterification and hydrolysis in organic and aqueous phase. Modified demethylated substrate is appropriate for enantioselective hydrolysis reaction without any additives. Enantiopure chiral alcohol was crystallized from ethyl acetate/ *n*-hexane co-solvent system. Gram scale resolved chiral intermediate will facilitate the synthesis of the unnatural *S*-(+)-xanthorrhizol, the corresponding isomer of the natural one.

Key Words : Lipase, Sesquiterpene, Xanthorrhizol, Transesterification, Hydrolysis

Introduction

R-(−)-Xanthorrhizol is a bisabolane type of natural sesquiterpene containing a stereogenic centre at the benzylic position. *R*-(−)-Enantiomer was isolated from the natural sources, as constituent of rhizomes of *Curcuma xanthorrhiza*,[1] and from the Mexican medicinal plant *Iostephane heterophylla*.[2] Among several naturally occurring bisabolane types of sesquiterpenes, *R*-(−)-xanthorrhizol has a variety of activity like *anti*-cancer,[3,4] anti-bacterial,[5] *anti*-metastatic,[6] *anti*-oxidant and *anti*-inflammatory.[7] Including others total synthesis of xanthorrhizol was also reported recently.[8] The synthetic procedures toward its enantiomers have not been fully explored and each enantiomer shows different biological activity frequently. Due to improved pharmacological potential and industrial demand enantioselective synthesis of xanthorrhizol became promising.[3-7] Enantioselective synthesis of *S*-(+)-xanthorrhizol (I) has been reported earlier through multiple steps.[9] This is also a potential starting material for the synthesis of some other bisabolane-types of sesquiterpenoids.[10] 2-(3-Methoxy-4-methylphenyl)-propan-1-ol (II) and 2-(3-hydroxy-4-methyl phenyl)propan-1-ol (III) are suitable starting material for xanthorrhizol and could be synthesized from commercially available methyl-3-methoxy-4-methylbenzoate. Its (*R*)-isomer is required in enantiopure state to get *S*-(+)-xanthorrhizol (Figure 1). Lipase-catalyzed kinetic resolution is an efficient technique for the synthesis of enantiomerically enriched compounds[11] and could produce enantioselective isomers for each xanthorrhizol synthesis. But 2-phenyl-propan-1-ol showed low enantioselectivity (E < 10) towards lipase.[12] Some devoted attempt like using bulky acyl group[13] and chiral acyl donor[14] resulted improvement. Basically moving the aryl group closer or further away from 3 position of the chiral center resulted in low enantioselectivity.[15] Meanwhile only lipases from *Pseudomonas cepacia* (PCL) and *Porcine pancreas* (PPL) are known to efficiently resolve those primary alcohols and their esters.[16] Recently substituent's effect on aromatic ring was studied focusing on those reported potential lipases.[17] To find out appropriate one for our substrate, transesterification and hydrolysis were studied intensively using various lipases. During the process of hydrolysis reaction we faced low substrate solubility in aqueous buffer solution. It made longer reaction time and low enantioselectivity. To solve this problem, substrate modification was focused. If the compound II is demethylated, generated phenol derivative solubility might be increased in aqueous buffer solution. Thus the demethylated compound III was used as substrate in enzymatic hydrolysis and its reaction condition was optimized. Modified *rac*-(+)-2-(3-hydroxy-4-methylphenyl)propyl propanoate (V) showed an appropriate substrate for enzymatic hydrolysis reaction without any additives.



S-(+)-xanthorrhizol

Figure 1. Structure of *S*-(+)-xanthorrhizol (I).

Experimental Section

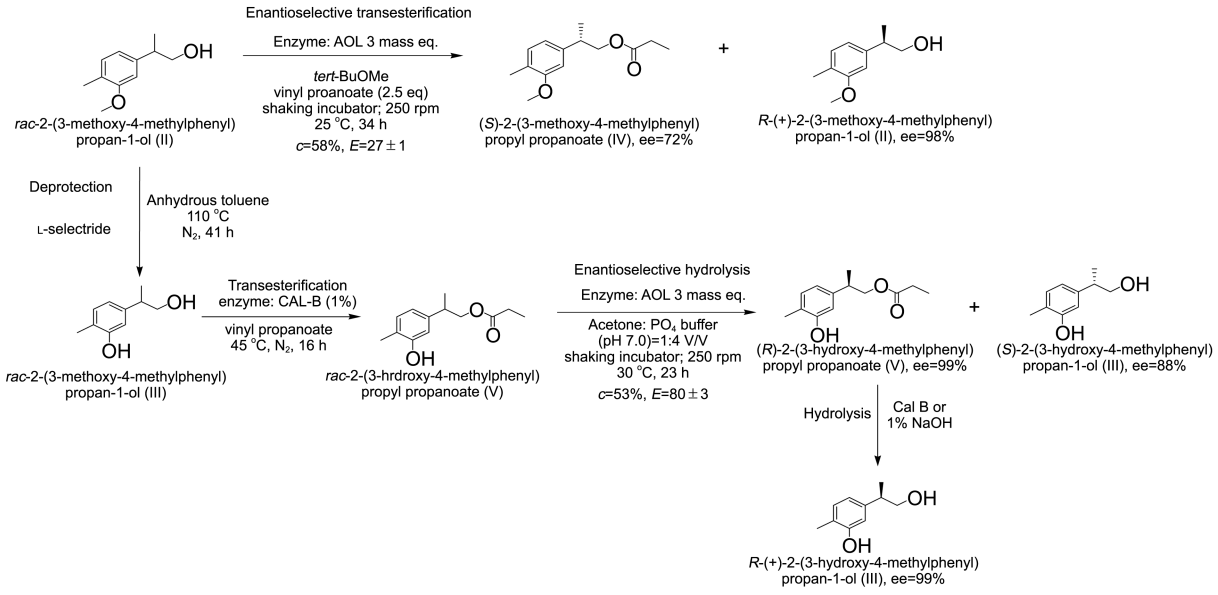
Materials and Analytical Instruments. Lipase *Aspergillus*

oryzae (AOL), *Porcine pancreas* (PPL) and *Candida antarctica* B (CAL-B, Novozym 435) were used with activity 2 U/mg, 20 U/mg and 120 U/mg respectively. Enzymes were collected from Fluka, Amano, Chirazyme and Novozymes. Vinyl propanoate, methyl 3-methoxy-4-methyl benzoate, phosphate buffer pH 7.0 and other reagents were purchase from TCI and Sigma-Aldrich with sufficient purity. Shaking incubator: Vision Scientific Co. Ltd. Chiral Columns: Chiralpak AS-H; Manufacturer: Daicel chemical Ind. Ltd. Japan and (*R*,*R*)-Whelk-01; Manufacturer: Regis Technologies Inc. USA. The methyl protected *rac*-alcohol namely, 2-(3-methoxy-4-methylphenyl) propan-1-ol (II) was synthesized according to the patent.[18] Solubility was measured with Nephelostar instrument from BMG Lab Tech. ^{1}H and ^{13}C NMR were recorded by 400 and 100 MHz, respectively, with a Varian 400 Mercury plus instrument using $CDCl_3$ as solvent and TMS as internal standard. Elemental analysis was done with Flash 2000 (Thermo Scientific) and Fisons EA 1108 instruments. Specific optical rotation was measured in $CHCl_3$ using Autopol III polarimeter from Rudolph research analytical.

Enantioselective Enzymatic Transesterification Reaction of *rac*-2-(3-Methoxy-4-methylphenyl) propan-1-ol (II). *rac*-2-(3-Methoxy-4-methylphenyl)propan-1-ol (II) (10 g, 55.5 mmol) and vinyl propanoate (15 mL) were taken in *tert*-BuOMe (480 mL) according to Scheme 1. Water (3 mL) and AOL (30 g) were added to the reaction mixture and placed in a shaking incubator at 25 °C and 250 rpm. 20 μL sample was drawn at different time point. Collected sample was evaporated under reduced pressure and diluted with 30 μL of respective mobile phase and filtered through 0.45 μm syringe filter. 10 μL samples were analyzed by HPLC. After 34 h the reaction mixture was filtered with the suction filter and enzyme was washed with solvent then evaporated under reduced pressure. Finally 4.0 g of the remaining *R*-(+)-2-(3-methoxy-4-methylphenyl)propan-1-ol (II) was separated by flash column chromatography using *n*-hexane: ethyl acetate = 50:1 as eluent system with 40% yield. Yellowish liquid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 7.6 Hz, 1H), 6.74 (dd, J = 7.5, 1.5 Hz, 1H), 6.70 (d, J = 1.2 Hz, 1H), 3.83 (s, 3H), 3.71-3.68 (m, 2H), 2.94-2.90 (m, 1H), 2.19 (s, 3H), 2.04 (s, 1H), 1.27 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.9, 142.5, 130.8, 124.9, 118.9, 109.4, 68.8, 55.3, 42.5, 17.7, 15.8; ee = 98%; $[\alpha]^{20}$ = +13.1 (c = 1.0, $CHCl_3$). Anal. calcd. for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95; O, 17.75. Found: C, 73.9; H, 9.5; O, 18.1.

Synthesis of *rac*-2-(3-Hydroxy-4-methylphenyl)propan-1-ol (III). *rac*-2-(3-Methoxy-4-methylphenyl)propan-1-ol (II) (1.4 g, 7.8 mmol) and anhydrous toluene (12 mL) were taken in a 3-neck flask and placed under N_2 at room temp (Scheme 1). L-selectride (31 mL, 1 M solution in tetrahydrofuran) was added slowly to the stirred solution. The reaction mixture was stirred for 41 h at 110 °C. The reaction progress was checked by TLC with hexanes: ethyl acetate = 1:1 eluent system. Reaction mixture was then quenched at room temp using 10% HCl to the neutral point. Aqueous layer was extracted 3 times with ethyl acetate then washed with brine. The organic part was concentrated under reduced pressure. Target phenolic alcohol III was purified through flash silica gel column using *n*-hexane: ethyl acetate = 10:1 as eluent system. Finally 1.28 g of *rac*-2-(3-hydroxy-4-methylphenyl)propan-1-ol (III) was recrystallized from the mixture of ethyl acetate/*n*-hexane as white crystalline solid with 99% yield. White crystalline solid, mp 75-76 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, J = 7.6 Hz, 1H), 6.72 (dd, J = 7.6, 1.5 Hz, 1H), 6.65 (d, J = 1.1 Hz, 1H), 5.28 (br s, 1H), 3.71-3.63 (m, 2H), 2.91-2.83 (m, 1H), 2.22 (s, 3H), 1.68 (br s, 1H), 1.23 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 142.8, 131.2, 122.2, 119.5, 114.1, 68.7, 41.9, 17.6, 15.4. Anal. calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49; O, 19.25. Found: C, 72.4; H, 8.8; O, 18.8.

CAL-B Catalyzed Synthesis of *rac*-2-(3-Hydroxy-4-



Scheme 1. Chemo-enzymatic processes for obtaining each moiety.

methylphenyl)propyl Propanoate (V). *rac*-2-(3-Hydroxy-4-methylphenyl)propan-1-ol (III) (13 g, 77.9 mmol) was dissolved in vinyl propanoate (9.3 mL) in a 3-neck flask and placed under N_2 atmosphere at room temp (Scheme 1). CAL-B (1.3 g) was added to the solution. The temp of the reaction mixture was raised to 45 °C and maintained for 16 h. The reaction progress was checked by TLC with *n*-hexane:ethyl acetate = 4:1 eluent system. Excess vinyl propanoate was removed by distillation from the reaction mixture. The target ester V was purified through flash silica gel column using *n*-hexane: ethyl acetate = 20:1 eluent system. Finally 17 g of *rac*-2-(3-hydroxy-4-methylphenyl)-propyl propanoate (V) was obtained with 99% yield. The colorless liquid was confirmed by ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, J = 7.6 Hz, 1H), 6.69 (dd, J = 7.6, 1.6 Hz, 1H), 6.65 (d, J = 1.5 Hz, 1H), 5.83 (s, 1H), 4.20-4.07 (m, 2H), 3.04-2.96 (m, 1H), 2.31 (q, J = 7.6 Hz, 2H), 2.21 (s, 3H), 2.05 (s, 1H), 1.25 (d, J = 4.2 Hz, 3H), 1.10 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 154.2, 142.3, 130.9, 122.3, 119.3, 113.8, 69.5, 38.5, 27.7, 21.1, 15.4, 9.1. Anal. calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16; O, 21.59. Found: C, 70.2; H, 8.3; O, 21.1.

Screening of Enzymes for Enantioselective Hydrolysis of *rac*-2-(3-Hydroxy-4-methylphenyl)propyl Propanoate (V). The following lipase enzymes were screened for hydrolysis reaction under similar conditions as described in Scheme 1: *Candida antarctica* (CAL-B, Novozym 435), *Schweineleber* sp. (PLE), *Candida rugosa* (CRL), *Aspergillus niger* (LAN), *Aspergilus* sp. (Acylase), *Pseudomonas fluorescens* (LAK), *Pseudomonas cepacia* (PCL), *Burkholderia cepacia* (LAH), *Aspergillus oryzae* (AOL) and *Porcine pancreas* (PPL). Out of those 10 types of enzyme, AOL and PPL were found to be selective to the hydrolysis reaction. Screening results are provided in Table 1.

Enantioselective Enzymic Hydrolysis of *rac*-2-(3-Hydroxy-4-methylphenyl)propyl Propanoate (V). To the solution of *rac*-propanoate ester V (1 g, 4.5 mmol) in acetone (10 mL), phosphate buffer pH 7.0 (40 mL) was added and homozinized for 20 min using vortex mixture. AOL (3 mass equivalents) was added to the mixture and placed in a shaking incubator at 30 °C and 250 rpm (Scheme 1). At different time intervals, 20 μL of sample was collected in a separatory funnel and extracted with 2 mL $CHCl_3$. It was then filtered through anhydrous $MgSO_4$ and the solvent was removed in rotary evaporator. The residue was diluted with 30 μL of mobile phase and filtered through 0.45 μm syringe filter. 10 μL samples were injected in to the HPLC system. After 23 h of reaction (Figure 2), the mixture was extracted using $CHCl_3$ and treated with anhydrous $MgSO_4$ and evaporated to dryness. Finally 431 mg of the remaining pure (*R*)-ester V was obtained with 43.1% yield by column chromatography using *n*-hexane: ethyl acetate = 50:1 as eluent system.

Table 1. Conversions, enantiomeric excess and enantioselectivity of enzyme catalyzed hydrolysis reactions of *rac*-substrate V[a]

Enzyme	Time (h)	c (%)[b]	ee_s (%)[c]	ee_p (%)[c]	E[b]
Cal B	4	4	3	64	6
PLE	6	29	5	13	1
CRL	4	47	16	18	2
LAN	13	40	20	31	2
Acylase	13	64	23	13	2
LAK	5	49	17	18	2
PCL	4	75	25	8	1
LAH	3	69	29	13	2
AOL	19	53	99	87	80
PPL	19	67	99	50	14

[a]Reaction condition: 10 mg *rac*-substrate V, enzyme 30 mg, a mixture of acetone: phosphate buffer (pH 7.0) = 1:4 was shaken at 30 °C and 250 rpm. Here c: conversion, E: enantioselectivity, ee_s: enantiomeric excess of starting material, ee_p: enantiomeric excess of product. [b]Determined from the equation described in enantioselective HPLC analysis section. [c]Determined by HPLC using (*R*,*R*)-Whelk-01, 250 × 4.6 mm, 5 μm column, mobile phase: *n*-hexane: ethanol: diethyl amine = 97:03:0.05% v/v with flow rate 0.4 mL/min at rt.

Enantioselective HPLC Analysis. Enantioselectivity was analyzed at different time point by Younglin binary gradient HPLC system equipped with UV detector at 230 nm and conditions mentioned below. Chiralpak AS-H, 250 × 4.6 mm, 5 μm column, mobile phase: *n*-hexane: IPA = 95:05%

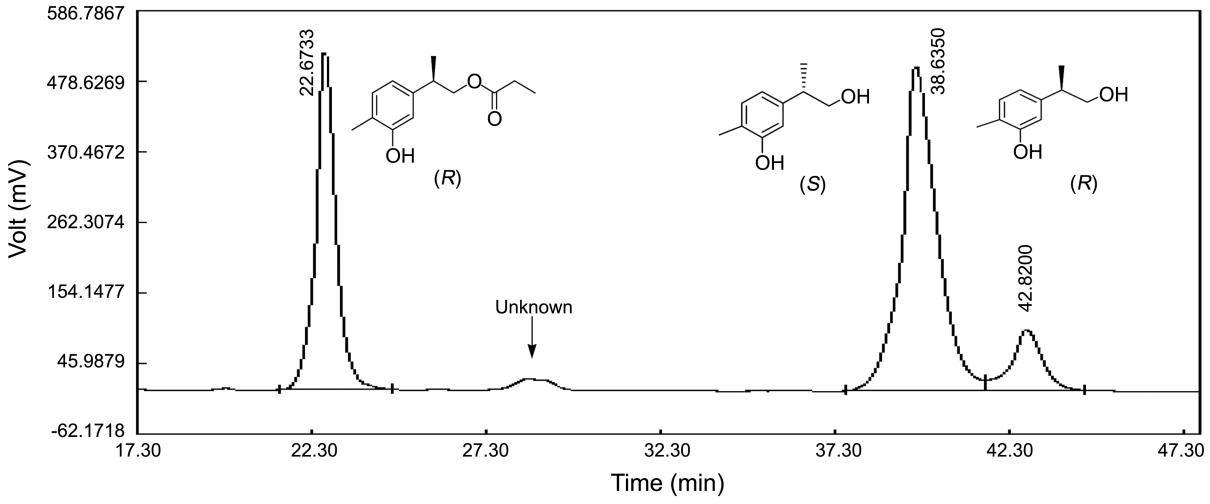


Figure 2. HPLC chromatogram for (*R*)-ester, (*S*)- and (*R*)-alcohol respectively after 23 h of enantioselective hydrolysis reaction using AOL.

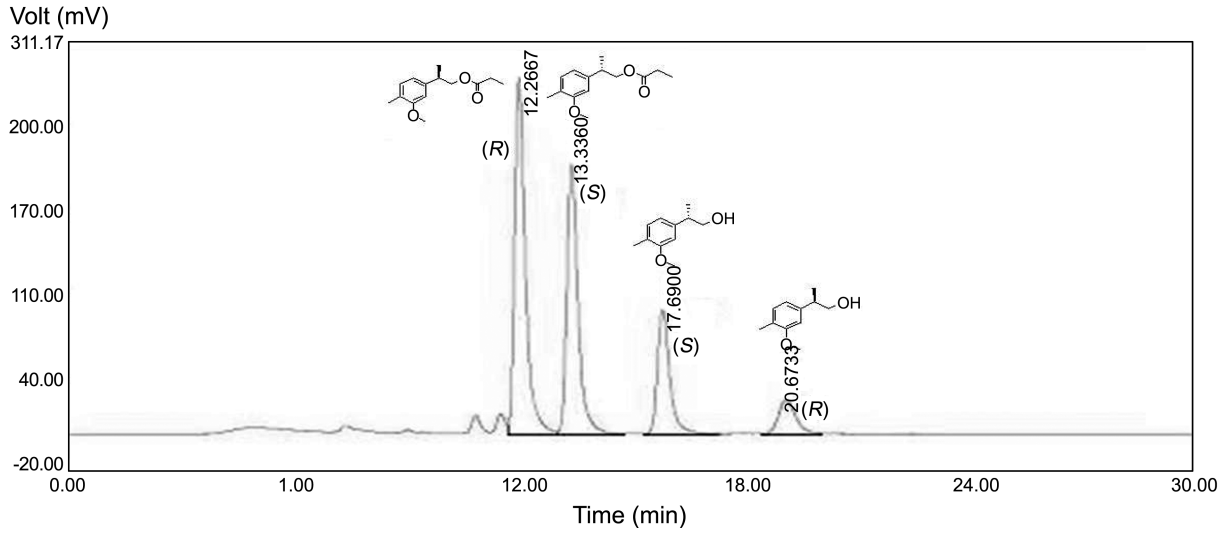


Figure 3. HPLC chromatogram of (*R*)- and (*S*)-ester, (*S*)- and (*R*)-alcohol, respectively.

v/v with flow rate 0.4 mL/min at room temp for transesterification. Figure 3 shows the resolved ester and alcohol, respectively. (*R*,*R*)-Whelk-01, 250 × 4.6 mm, 5 μm column, mobile Phase:*n*-hexane:ethanol:diethyl amine = 97:03:0.05% v/v with flow rate 0.4 mL/min at room temp for hydrolysis. The *E* value was calculated from the ee_s and ee_p values at different conversion, *c* (%) using the following equation [19]:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]};\ c = \frac{ee_s}{ee_s+ee_p}$$

Experimental ee_s, ee_p, conversion *c* (%) and enantioselectivity (*E*) are given in Table 2.

Hydrolysis of *R*-2-(3-Hydroxy-4-methylphenyl)propyl propanoate (V). (*R*)-Ester V (220 mg, 0.90 mmol) was taken in acetone (2.2 mL) and phosphate buffer pH 7.0 (4.4 mL) and then homozinized for 5 min using a vortex mixture. CAL-B (220 mg) was added to the mixture and placed in a shaking incubator at 30 °C and 250 rpm. After total hydrolysis of ester at 96 h, the reaction mixture was filtered to remove enzymes then evaporated under reduced pressure to remove acetone. Then the mixture was extracted 3 times with ethyl acetate and the organic layer was washed with brine solution. The organic layer was accumulated and treated with anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure after filtration. *R*-(+)-2-(3-Hydroxy-4-methylphenyl) propan-1-ol (III) was purified with flash column chromatography using *n*-hexane:ethyl acetate = 1:1 eluent system with 98% yield. 10 μL samples with a concentration of 20 μg/μL in mobile phase were then injected to the HPLC to evaluate ee value of *R*-(+)-2-(3-hydroxy-4-methylphenyl)propan-1-ol (III): White crystals, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, *J* = 7.6 Hz, 1H), 6.71 (d, *J* = 7.6 Hz, 1H), 6.64 (s, 1H), 5.78 (br s, 1H), 3.71-3.62 (m, 2H), 2.88-2.84 (m, 1H), 2.21 (s, 3H), 1.85 (br s, 1H), 1.22 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2, 142.7, 131.2, 122.4, 119.3, 114.1, 68.7, 41.9, 17.6, 15.4; ee = 99%; $[\alpha]^{20}$= +21.1 (c = 1.0, $CHCl_3$). Anal. calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49; O, 19.25. Found: C, 72.4; H, 8.7; O, 18.9.

Results and Discussion

During our previous experiment,[18] we found that AOL enzyme worked well to resolve compound II. The compound II was efficiently resolved by enzymatic transesterification in *tert*-BuOMe solvent using AOL with enantioselectivity, *E* = 27 ± 1 and enantiomeric excess (ee_s = 98% and ee_p = 72%) at 58% conversion as shown in Table 2. About 0.6% water was added to maintain the structure and flexibility of enzyme in non polar solvent.[20]

However during hydrolysis reaction of its *rac*-ester IV, some problems arose because of its hydrophobic nature. This made the reaction mixture inhomogeneous and delayed the reaction rate. To overcome these problems, some addi-

Table 2. Conversion, enantiomeric excess and enantioselectivity for enzyme catalyzed transesterification[a] and hydrolysis[b] reaction

Enzyme	Time (h)	wt. (Substrate)	*c* (%)[e]	ee_s (%)	ee_p (%)	*E*[e]
AOL	34	10 g (II)[a]	58	98[c]	72[c]	27 ± 1
AOL	23	1 g (V)[b]	53	99[d]	88[d]	80 ± 3
Bi-phase reaction (*n*-hexane:phosphate buffer pH 7.0 = 2:4)						
AOL	48	0.1 g (V)[b]	58	99[d]	72[d]	31 ± 3

[a]Reaction condition: *rac*-substrate II, vinyl propanoate (2.5 eq.), AOL Enzyme (3 mass equivalent), *tert*-BuOMe was shaken at 25 °C and 250 rpm. [b]Reaction condition: *rac*-substrate V, AOL Enzyme (3 mass eq.), a mixture of acetone: phosphate buffer (pH 7.0) = 1:4 was shaken at 30 °C and 250 rpm. Here *c*: conversion, *E*: enantioselectivity, ee_s: enantiomeric excess of starting material, ee_p: enantiomeric excess of product. [c]Determined by HPLC using Chiralpak AS-H, 250 × 4.6 mm, 5 μm column, mobile phase: *n*-hexane: IPA = 95:05% v/v with flow rate 0.4 mL/min at RT. [d]Determined by HPLC using (R,R)-Whelk-01, 250 × 4.6 mm, 5 μm column, mobile Phase: *n*-hexane: ethanol: diethyl amine = 97:03:0.05% v/v with flow rate 0.4 mL/min at rt. Values of *c*, ee_s and ee_p are the average of four determinations. [e]Determined from the equation described in enantioselective HPLC analysis section with average of *c* and ee_s of three determination.

Table 3. Solubility of the methyl protected and deprotected alcohol and propanoate ester

rac-compound	Solubility (g/L)[a]	Type
II	2.5	Slightly soluble
III	7.1	Slightly soluble
IV	0.052	Partially soluble
V	0.089	Partially soluble

[a]Solubility was measured with Nephelostar instrument from BMG Lab Tech. Values are the average of three determinations.

tives such as surfactants were used to increase solubility in the aqueous phase. However this modification made the enzymes inactive. Here the critical issue is substrate solubility in aqueous phase. The solubility of the *rac*-ester IV was too low and it aggregated in aqueous phase, which prolonged the reaction time. The comparison of solubility of the compound II and III is shown in Table 3. As expected, the deprotected *rac*-2-(3-hydroxy-4-methylphenyl) propan-1-ol (III) and *rac*-2-(3-hydroxy-4-methylphenyl)propyl propanoate (V) were more soluble in aqueous phase than the corresponding protected ones. The solubility of deprotected phenolic alcohol III was three times higher than that of methyl protected alcohol. Similarly the solubility of deprotected propanoate ester V was almost twice to that of methyl protected compound. Furthermore, to improve solubility and homogeneity of the reaction mixture we employed acetone/phosphate buffer instead of aqueous phase alone, which facilitated hydrolysis reaction. Other co-solvent system was also studied but the reported condition found best. Based on those data, the ester of the compound III was used for hydrolysis reaction. Among the different kinds of commercially available lipase, AOL and PPL showed enantioselectivity to the substrate. Either enzymes could be used for the resolution process, but AOL was more favorable with higher *E*. However AOL was not used in the earlier studies[12-17] to resolve similar compounds. For the methyl protected compound of the corresponding II, (*S*)-isomer was fast reacting isomer, thus (*R*)-alcohol was obtained as remaining substrate during transesterification. For hydrolysis, we obtained similar results which prove a correlation between the experiments as enzyme was AOL for both cases. The docking pattern of substrate in AOL and PPL enzymes might be similar.

rac-Ester V was synthesized using CAL-B from *rac*-alcohol III through transesterification process (Scheme 1). CAL-B was able to produce primary ester whereas acid catalyzed reaction produced both primary and phenolic esters. During HPLC analysis elution was (*R*)- and (*S*)-ester then (*S*)- and (*R*)-alcohol (Figure 3) respectively throughout our study. Remaining (*R*)-ester V was hydrolyzed (Scheme 1) using 1% NaOH as well as enzymatically to obtain the corresponding (*R*)-alcohol III. After hydrolysis (*R*)-alcohol III was checked by HPLC and found no trace of (*S*)-isomer which may prove that the unknown peak of Figure 2 is not due to (*S*)-ester V. In this hydrolysis reaction, the enzymatic procedure was more favorable than the chemical one in view of cleaner reaction and higher yield. Finally the compound V was efficiently resolved by enzymatic hydrolysis in acetone/phosphate buffer using AOL with enantioselectivity, $E = 80 \pm 3$ and enantiomeric excess ($ee_s = 99\%$ and $ee_p = 88\%$) at 53% conversion (Table 2), similarly blank test was done to confirm the enzymatic reaction and almost no conversion was obtained after 144 h. For bi-phase reaction in *n*-hexane and phosphate buffer, the substrate ester still remained in the organic phase although its solubility increased in aqueous phase which extended reaction time to 48 h. Determined *E* value at different time point was crosschecked using the alternative method 2 of Chen *et al*.[21] and method 3 of Rakels *et al*.[22] described by Straathof *et al*.[19] and found same.

The produced alcohol was more soluble in the aqueous phase and separated very easily from the unreacted ester compound. (*R*)-alcohol III was precipitated as pure form from the mixture of ester using ethyl acetate and *n*-hexane co-solvent system which seems promising for the scale-up production. These chiral intermediates would play an important role for synthesis of valuable unnatural sesquiterpenes. Finally *R*-(+)-2-(3-methoxy-4-methylphenyl)propan-1-ol (II) and *R*-(+)-2-(3-hydroxy-4-methylphenyl)propan-1-ol (III) were used to synthesize *S*-(+)-xanthorrhizol (I) according to the process described in [18]. We will communicate soon the elaborate process to synthesize *S*-(+)-xanthorrhizol (I).

Conclusions

Enantioselective enzymatic transesterification and hydrolysis were developed for *rac*-2-(3-methoxy-4-methylphenyl)propan-1-ol (II) and *rac*-2-(3-hydroxy-4-methylphenyl)-propyl propanoate (V) respectively. The simple separation technique of alcohol from ester will facilitate synthesis of chiral intermediates like unnatural synthetic *S*-(+)-xanthorrhizol (I) and its analogues to get more optimized biological properties.

Acknowledgments. This work was financially supported by the Ministry of Knowledge Economy and Korea Institute of Science and Technology, Korea.

References

1. (a) Rimpler, H.; Hansel, R.; Kochendoerfer, L. *Z. Naturforsch Teil B* **1970**, *25*(9), 995. (b) John, T. K.; Krisna Rao, G. S. *Indian. J. Chem.* **1985**, *24B*(1), 35.
2. Aguilar, M. I.; Delgado, G.; Bye, R.; Linares, E. *Phytochemistry* **1993**, *33*(5), 1161.
3. Aggarwal, B. B.; Kumar, A.; Bharti, A. C. *Anticancer Res.* **2003**, *23*, 363.
4. Pillai I, G. R.; Srivastava I, A. S.; Hassanein, T. I.; Chauhan, D. P.; Carrier, E. *Cancer Lett.* **2004**, *208*, 163.
5. Hwang, J. K.; Shim, J. S.; Baek, N. I.; Pyun, Y. R. *Planta Med.* **2000**, *66*(2), 196.
6. Choi, M. A.; Kim, S. H.; Chung, W. Y.; Hwang, J. K.; Park, K. K. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 210.
7. Lim, C. S.; Jin, D. Q.; Mok, H.; Oh, S. J.; Lee, J. U.; Hwang, J. K.; Ha, I.; Han, J. S. *J. Neurosci. Res.* **2005**, *82*(6), 831.
8. Montiel, L. E.; Zepeda, L. G.; Tamariz, J. *Helv. Chim. Acta* **2010**, *93*, 1261.

9. Fuganti, C.; Serra, S. *J. Chem. Soc., Perkin Trans 1* **2000**, *22*, 3758.
10. Sirat, H. M.; Hong, N. M.; Jauri, M. H. *Tetrahedron Lett.* **2007**, *48*, 457.
11. Ghanem, A. *Tetrahedron.* **2007**, *63*, 1721.
12. Barth, S.; Effenberger, F. *Tetrahedron: Asymmetry* **1993**, *4*, 823.
13. Goto, M.; Kawasaki, M.; Kometani, T. *J. Mol. Catal. B: Enzym.* **2000**, *9*, 245.
14. Hirose, K.; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. *Tetrahedron: Asymmetry* **2000**, *11*, 1199.
15. Nordin, O.; Nguyen, B. V.; Vorde, C.; Hedenstrom, E.; Hogberg, H. E. *J. Chem. Soc., Perkin Trans 1* **2000**, 367.
16. Kazlauskas, R. J.; Bornscheuer, U. T. Biotechnology: Biotransformations I; Kelly, D. R., Ed.; Weinheim, Wiley-VCH: 1998; 2nd ed.; Vol. 8a, p 37.
17. Serra, S. *Tetrahedron: Asymmetry* **2011**, *22*, 619.
18. Cheong, C. S.; Ku, I. H.; Kim, R. H.; Kim, U. J.; Ban, J. G. *Repub. Korea* 2009; p 20, Application: KR 2007-0108998.
19. Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559.
20. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Weinheim, Wiley-VCH: 1999; p 47.
21. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
22. Rakels, J. L. L.; Straathof, A. J. J.; Heijnen, J. J. A. *Enzyme Microb. Technol.* **1993**, *15*, 1051.