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Two-step Biocatalytic Resolution of *rac*-Primary Alcohol for Obtaining Each Isomeric Intermediate of Xanthorrhizol

Jung In Pyo,[†,‡] Ryung Wha Kim,[†] Azam Sharif Mohammed Shafioul,[†] Chiman Song,[†] Chan Seong Cheong,[†,*] and Kwan Soo Kim[‡]

[†]*Chemical Kinomics Research Center, Future Convergence Research Division, Korea Institute of Science and Technology, Seoul 136-791, Korea.* [*]*E-mail: c2496@kist.re.kr*
[‡]*Department of Chemistry, Yonsei University, Shinchon dong, Seoul 136-702, Korea*
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From many kinds of natural products, interesting compounds have been extracted for human health and exhibited various biological activities,[1] such as anticancer, antifungal, anti-oxidant, *etc*. They have been used as either raw mixtures or modified single ingredients for increased effectiveness. In addition, natural products have been used as lead compounds for new drug discovery programs.[2] Phenolic sesquiterpene compounds have attracted much interest for their broad biological activities.[3] Among them, xanthorrhizol has been investigated in the search for new biological activities. It was first isolated by Kochendoerfer[4] from rhizomes of *Curcuma xanthorrhiza Roxb.* and exhibited antibacterial and antifungal activities. It has one chiral center, thus (*R*)- and (*S*)-isomer can be generated as in Figure 1.

Antibacterial activity is shown by (*R*)-xanthorrhizol which is a naturally occurring isomer. Owing to its broad pharmacological activities, several research groups have synthesized it as racemic or an enantiomerically pure compound. Of these, 3-methoxy-4-methyl acetophenone was employed as starting material and reacted with the corresponding homoprenyl bromide.[5] Meyers *et al*.[6] asymmetrically synthesized (*S*)-xanthorrhizol and analogues employing oxazolines as a chiral auxiliary. On the other hand, there is a report concerning chemoenzymatic transformation.[7] In this report, they used baker's yeast to reduce α,β-unsaturated aldehyde asymmetrically. Additionally, other reports were published to synthesize similar structures, such as curcuphenol[8] and heliannuol D.[9] Recently, Serra S. reported a result about resolution of substituted 2-aryl-propanols.[10] This report is a comprehensive study about resolution of various substrates with lipases and includes a xanthorrhizol intermediate analogue. In spite of these reports, there is no one which gave both enantiomers as a pure state. Thus, we focused on the development of a method obtaining both enantiomers using a biocatalyst. As reported previously in patent,[11] the primary alcohol **2**, 2-(3-methoxy-4-methylphenyl)propan-1-ol, was selected as a substrate for resolution as Figure 1.

In order to prepare it, the commercially available compound **3**, methyl 3-methoxy-3-methyl benzoate, was transformed to **2** through 3-steps as Scheme 1. The compound **3** was reacted with the Grignard reagent, CH_3MgBr in THF, to give the carbinol **4**. The compound **4** was carefully dehydrated to give **5** under the concentrated sulfuric acidic condition, otherwise transformed to the indane cyclic structure.[12] Lastly, the resulting double bond was hydroborated with borane-SMe_2 complex and oxidized with alkaline H_2O_2 to give the compound **2**. To obtain the optically pure each isomer of the compound **2**, we used hydrolysis or esterification reaction, which is dependent on the substrate physical property.

At first, to get a suitable enzyme for this reaction, various enzymes were screened and their results are shown in Table 1. For hydrolysis, the compound **2** was acetylated as the substrate as Scheme 2. Its reaction condition was under pH 7

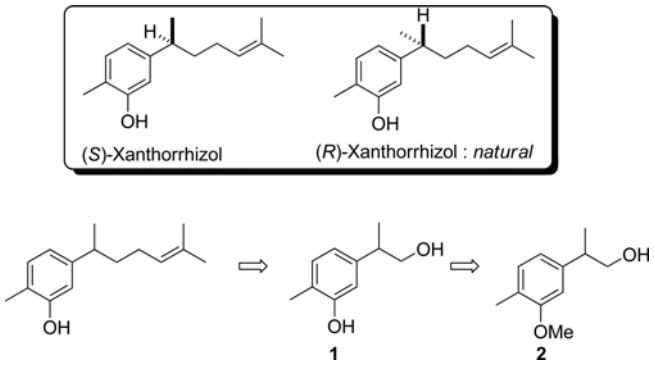
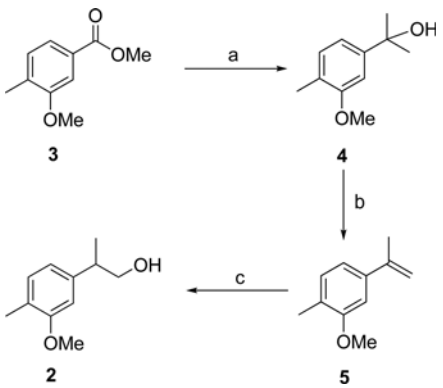
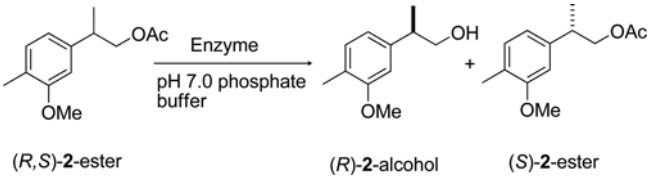


Figure 1. Xanthorrhizol and primary alcohol **1**-**2**.



Scheme 1. Synthesis of compound **2**: (a) CH_3MgBr/THF (b) conc. H_2SO_4/THF (c) BH_3-SMe_2/THF; H_2O_2, NaOH.



Scheme 2. Hydrolysis of (*R*,*S*)-**2**-ester using enzyme.

phosphate aqueous buffer solution at 30 °C. The reaction progress was monitored by HPLC on a chiral column CHIRALCEL OD (250 × 4.6 mm), and enantioselectivity was determined for each reaction step. Among these results, *Porcine pancreas* lipase (PPL), *Burkholderia cepacia* lipase (LAH), *Aspergillus oryzae* lipase (LAO), *Bacillus subtilis* lipase (BLS-01), *Rhizopus oryzae* esterase (ERO) moderately showed an ability to separate the ester substrate **2** to each enantiomer. In view of the conversion rate, PPL, LAH and BLS-01 were favorable. But in spite of the relatively low reaction rate, LAO and ERO were suitable examples to attain each pure enantiomer. Between the two lipases, LAO was superior over ERO and selected as the final catalyst for this work.

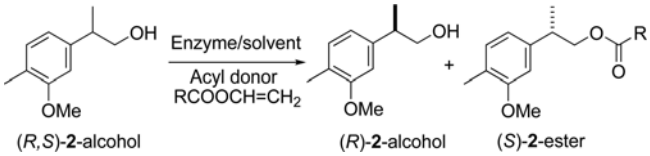
For transesterification, LAO was used according to the hydrolysis results as Scheme 3. In this reaction, an acyl donor is a crucial reagent as reported elsewhere. Thus, several acyl donors were reacted under the general reaction condition, and their results are shown as Table 2.

When the effect of the chain length of the acyl donor on the enantioselectivity was tested, the four acyl donors were effective, except for the vinyl butylate. But considering the conversion and enantiomeric purity of each isomer, vinyl propionate and laurate were more favorable candidates. Between the two reagents, vinyl propionate was the best one

Table 1. Resolution of (*R*,*S*)-**2**-ester by enzymatic hydrolysis

Enzyme	Enzyme (mg)[a]	Time (h)	Conversion (%)	(*S*)-**2**-ester (% ee)	(*R*)-**2**-alcohol (% ee)
PLE	5	0.5	70	3	4
PPL	25	8	40	19	51
LAH	5	10	60	41	57
LAO	10	36	21	5	83
PFL(fluka)	10	7	66	37	23
PFL	20	50.5	33	9	41
BLS-01	30	9.5	21	1	51
Proteinase bacterial Type XXIV	10	31	41	22	39
LBT	5	2.5	93	21	7
LRO	20	34	20	4	46
Lipase alcaligines sp.	10	24	83	52	19
ERO	10	34	13	1	53

PLE: pig liver esterase; PPL: *Procine pancreatic* lipase; LAH: *Burkholderia cepacia* lipase; LAO: *Aspergillus oryzae* lipase; BLS-01: *Bacillus subtilis* K2168; PFL: *pseudomonas fluorescence* lipase; LBT: *Bacillus thermoleovarans* lipase; LRO: *Rhizopus oryzae* lipase; ERO: *Rhizopus oryzae* esterase; [a]mass equivalent to substrate



Scheme 3. Transesterification of (*R*,*S*)-**2**-alcohol using enzyme.

Table 2. Effects of different acyl donors on the transesterification of primary alcohol using the lipase from *Aspergillus oryzae*

Acyl donor	Enzyme (mg)[a]	Time (h)	Conversion (%)	(*R*)-alcohol (% ee)	(*S*)-ester (% ee)
Vinyl acetate	20	3	25	28	85
Vinyl propionate	20	3	52	86	86
Vinyl butylate	20	24	8	16	29
Vinyl hexanoate	20	4	47	79	77
Vinyl laurate	20	3	59	92	79

[a]mass equivalent to substrate

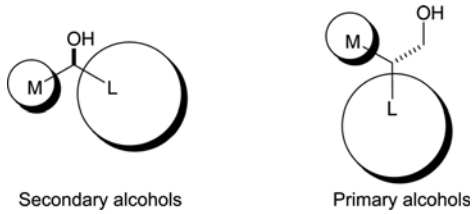
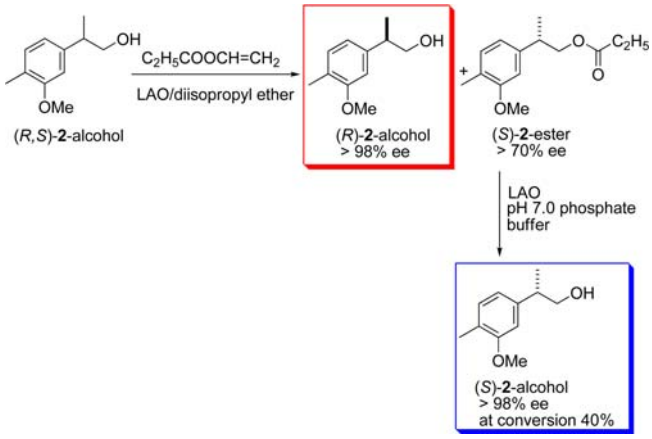


Figure 2. Empirical model.

with respect to processability and molecular economy.

When (*R*,*S*)-**2**-alcohol reacted with LAO, (*S*)-**2**-alcohol was esterified to the corresponding (*S*)-**2**-ester. This result proved (*S*)-form was a fast reacting isomer and followed an empirical model.[13]

It implied the shape of the favored enantiomer of chiral secondary alcohols and extended to primary alcohols using PCL. Between these two groups, the outcome of stereochemistry was inverted as shown below. In the optimization of resolution condition, the solvent and reaction temperature are critical factors. Therefore, the reaction temperature was under 30 °C and various solvents were tested.



Scheme 4. (*R*)- and (*S*)-**2**-alcohol obtained by double lipase resolutions.

Table 3. Double resolved results from using the first resolved (*S*)-**2**-ester

Substrate	Time (h)	Conversion (%)	(*S*)-**2**-ester (% ee)	(*S*)-**2**-alcohol (% ee)
(*S*)-**2**-ester 500 mg (> 70% ee)	4	9	62	> 98
	5	15	62	> 98
	6	24	62	> 98
	7	38	62	> 98

Generally, in a nonpolar solvent such as methyl *t*-butyl ether, diisopropyl ether, *n*-hexane, or methylene chloride, the resolved results were high. Therefore, we used diisopropyl ether as a transesterification reaction medium in the other reactions. On the other hand, to get high resolved results, additives such as pyridine, Et_3N, $NaHCO_3$, molecular sieves 4 Å were tested to remove the generated acetaldehyde as a byproduct. For the basic condition, the (*R*)-**2**-alcohol was resolved to an almost pure enantiomer. As mentioned above, a fast reacting isomer was (*S*)-form. Thus, it was presumed that a low ee value (*S*)-form ester would be resolved to a pure form by hydrolysis using the same enzyme LAO. This type of double enzymatic reaction has previously been reported.[14] In that report, they used different enzymes and studied their regioselectivity. Remained (*R*)-**2**-alcohol and reacted (*S*)-**2**-ester were resolved to 98% and 71% ee at 58% conversion in diisopropyl ether. This ester (71% ee), which was separated from the mixture, was subjected to a hydrolysis reaction as previously described. These overall reactions and results were presented in Scheme 4. Under the condition of diisopropyl ether and a conversion at 58%, (*R*)-**2**-alcohol and (*S*)-**2**-ester was obtained as up to 98% and 71% ee by transesterification, respectively. To obtain (*S*)-2-alcohol at up to 98% ee as well, this obtained (*S*)-2-ester (> 70% ee) should be used as a substrate of hydrolysis using the same lipase, LAO. In this reaction, the conversion of transesterification should be controlled within 40% as in Table 3; otherwise the ee value of the required (*S*)-2-ester was below 70%. In this case, it was difficult to obtain the pure (*S*)-**2**-alcohol.

In conclusion, the key intermediate (*R*,*S*)-**2**-alcohol for xanthorrhizol was resolved by the biocatalytic process. Among many hydrolases, a lipase from *Aspergillus oryzae* (LAO) worked well for the substrate. Transesterification and hydrolysis reaction were performed for resolution of (*R*)-**2**-alcohol and (*S*)-**2**-alcohol, respectively. According to these results, it was shown that the fast reacting isomer for LAO was (*S*)-**2**-ester in transesterification reaction and that both enantiomers were obtained through double enzymatic transformations.

Experimental

Hydrolysis. To a solution of substrate (10 mg) in aqueous pH 7 phosphate solution (1 mL, 10% acetone) was added an enzyme (mass equivalent) at 30 °C. The reaction mixture was shaken at 254 rpm and aliquots were taken in due time. These were filtered off and analyzed using the condition of the above HPLC.

Transesterification. To a solution of substrate (500 mg) in selected solvent (50 mL) was added vinyl propionate acyl donor (1.5 equivalent) at 30 °C. The reaction mixture was stirred at the same temperature and aliquots were taken at regular time intervals. Its analytical results were obtained by the same procedure like above.

Two-step Enzymatic Resolution. To a solution of substrate (500 mg) and LAO (500 mg) in diisopropyl ether (50 mL) was added a vinyl acyl donor (1.5 equivalent) at 30 °C. The reaction mixture was shaken at 254 rpm and monitored by HPLC at regular time intervals. At 65% conversion, the reaction mixture was quenched with a saturated aqueous NH_4Cl solution and extracted with *n*-hexane. The organic phase was concentrated in vacuo, and the crude product was purified by flash column chromatography to give the reacted (*S*)-**2**-ester (414 mg) and remained (*R*)-**2**-alcohol (175 mg).

To a solution of this (*S*)-**2**-ester (414 mg) in aqueous pH 7 phosphate solution (50 mL, 10% acetone) was added LAO (420 mg) at 30 °C. The reaction mixture was stirred for 6 hours (conversion 38%) and quenched with a saturated aqueous NH_4Cl solution. After the same work-up procedure and flash column chromatography, remained (*S*)-**2**-ester (62% ee) and reacted (*R*)-**2**-alcohol (> 98% ee) were obtained, respectively.

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