

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 September 2007 (07.09.2007)

PCT

(10) International Publication Number
WO 2007/100227 A1

(51) International Patent Classification:
C07D 307/32 (2006.01)

(21) International Application Number:
PCT/KR2007/001052

(22) International Filing Date: 2 March 2007 (02.03.2007)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2006-0019944 2 March 2006 (02.03.2006) KR

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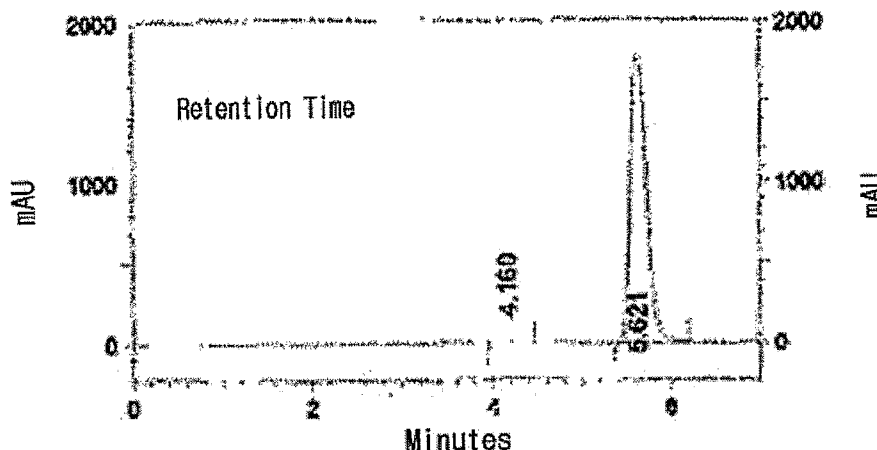
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,

[Continued on next page]

(54) Title: METHOD FOR PREPARING (S)-3-HYDROXY-GAMMA-BUTYROLACTONE USING HYDROLASE



2:238nm, 8nm			
Name	Retention Time	Area	Area Percent
Benzoic acid	4.160	64601	0.28
BBL	5.621	23157191	99.72

(57) Abstract: The present invention relates to a method for preparing S-HGB ((S)-3-hydroxy- γ -butyrolactone) using hydrolase, and more particularly to a method for preparing S-HGB in a high purity by hydrolyzing S-BBL ((S)- β -benzoyloxy- γ -butyrolactone) in the presence of hydrolase. According to the present invention, the S-HGB having an optical purity can be obtained in a high yield under simple process conditions without requiring reaction conditions of high pressure and high temperature or complex operation conditions by hydrolyzing S-BBL with hydrolase.

WO 2007/100227 A1



FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,
PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *with international search report*

Description

METHOD FOR PREPARING (S)-3-HYDROXY- γ -BUTYROLACTONE USING HYDROLASE

Technical Field

- [1] The present invention relates to a method for preparing high-purity (S)-3-hydroxy- γ -butyrolactone (S-HGB) by hydrolyzing (S)- β -benzoyloxy- γ -butyrolactone (S-BBL).

[2]

Background Art

- [3] S-HGB ((S)-3-hydroxy- γ -butyrolactone) is a chiral compound having asymmetric carbon atoms, and has been used in the various fields of applications. For example, the S-HGB is used as an intermediate for manufacturing Lipitor[®] (Atorvastatin, Pfizer) and Zyvox[®] (Linezolid, Pharmacia & Upjohn), and used also as an ingredient, L-Carnitine, of food additives.
- [4] The S-HGB synthesis methods have been reported in a large number of the patents and literatures. For example, it was reported that L-malic acid is selectively reduced in the presence of catalyst, in particular the presence of sodium borohydride and metal catalyst in the case of US Patent No.5,808,107 and US Patent No. 6,429,319, respectively. However, these methods have problems that their operation conditions are very complicated and they requires expensive apparatuses since the methods are continuously carried out at high temperature and high pressure (50-550 °C, 15-5,500 psig).
- [5] The synthesis of S-HGB using a reduction reaction of β -ketoester with an enzyme or catalyst (*J. Am. Chem. Soc.*, 105:5925, 1983) has problems that the S-HGB is yielded in a low optical purity and an expensive metal catalyst is used.
- [6] Also, there has been also reported a method for producing S-HGB using a de-halogenation reaction with a microorganism (*Enterobacter Sp.* DS-S-75) (Suzuki *et al.*, *Enzyme Microb. Technol.*, 24:13, 1999), but the method has problems that the S-HGB is obtained in a low yield and optical purity.
- [7] There are some reports that S-HGB is synthesized using an oxidation reaction using inexpensive soluble carbohydrate as a starting material (US Patent No. 5,292,939; US Patent No. 5,374,773; and US Patent No. 6,124,122), but these methods have a disadvantage that the S-HGB is obtained in a low yield since they are exothermic and use harmful compounds such as sulfuric acid, and it is difficult to separate and purify the final product.
- [8] As described above, the conventional production methods for S-HGB have problems that their process is complicated, the obtained S-HGB has a low optical

purity, and the reagents, which are dangerous in handling and harmful to environments, should be used. Accordingly, there are limitations on their commercialization since the complex processes results in the reduction in S-HGB yield.

[9] Meanwhile, in US Patent No. 5,928,933, it was reported that only one of forty-four protease, lipase and esterase enzymes shows an optical purity of 95 % when a method for kinetically resolving enantiomers in the selective hydrolysis using an enzyme is selected for the purpose of producing a chiral compound having asymmetric carbon atoms, and then their reaction specificities against the forty-four enzymes are confirmed using enantioselectivity of the hydrolase. In the vitamin precursor synthesis using the regioselectivity of enzymes (Gotor *et al.*, *J. Org. Chem.* 67:1266, 2002; and EP 1,239,045), there is reported a method in which only one of two esters which are present in one molecule is hydrolyzed with hydrolase.

[10] Also, Japanese Patent Publication No. 2003-299496 discloses a method for producing S-HGB using microorganism-derived esterase, but the method has difficulty in obtaining S-HGB in a high yield since the S-HGB is produced by reducing 4-halo-3-oxo butane acid ester through a two-step process.

[11]

Disclosure of Invention

Technical Problem

[12] Accordingly, the inventors have made comprehensive studies on the method for producing S-HGB, and therefore they found that S-HGB having a high optical purity may be obtained in a high yield while suppressing side reactions by hydrolyzing S-BBL with hydrolase. As a result, the present invention is completed according to the above findings.

[13] Accordingly, it is an object of the present invention to provide a method for preparing S-HGB having a high optical purity (ee >99.5 %) by hydrolyzing S-BBL in the presence of hydrolase.

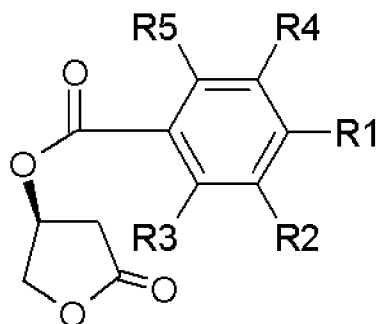
[14]

Technical Solution

[15] In order to accomplish the above object, the present invention provides a method for preparing (S)-3-hydroxy- γ -butyrolactone (S-HGB), wherein the S-HGB is obtained by hydrolyzing (S)- β -benzoyloxy- γ -butyrolactone (S-BBL) represented by the following Formula 1 in the presence of hydrolase:

[16] [Formula 1]

[17]



[18] in the Formula 1,

[19] R1, R2, R3, R4 and R5 are independently one selected from the group consisting of hydrogen, (C1-C10)alkyl, (C5-C6)cycloalkyl, fluoro(C1-C10)alkyl, OR', aryl, aryl(C1-C10)alkyl, NO₂, NR'R'', C(O)R', CO₂R', C(O)NR'R'', N(R'')C(O)R', N(R'')CO₂R', N(R'')C(O)NR'R'', S(O)_mNR'R'', S(O)_mR', CN and N(R'')S(O)_mR', provided that two adjacent Rs among the groups R1, R2, R3, R4 and R5 may share carbon atoms to form a fused aromatic or cycloalkene ring,

[20] the R' and R'' are each independently one selected from the group consisting of hydrogen, (C1-C10)alkyl, aryl and aryl(C1-C10)alkyl, provided that, if the R' and R'' are attached to the same nitrogen atom, they may bind to each other to form a 5-, 6- or 7-membered ring containing 1 to 3 heteroatoms selected from the group consisting of N, O and S, and

[21] m is an integer from 0 to 2.

[22] In the present invention, it may be characterized in that the hydrolysis of S-BBL is carried out in a hydrophilic solvent or a two-phase system of a hydrophilic solvent-hydrophobic organic solvent. As the hydrophilic solvent, it is preferred to use at least one selected from the group consisting of water, lower alcohol including methanol, ethanol, propan-2-ol, etc., acetic acid, acetone, ethylacetate, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, tetrahydrofuran, dioxane, dimethylformamide and dimethylsulfoxide, and as the hydrophobic organic solvent, it is preferred to use at least one selected from the group consisting of isopropyl ether, tert-butyl methyl ether (TBME), chloroform, dichloromethane, carbon tetrachloride, hexane, toluene and cyclohexane.

[23] In the present invention, it may be characterized in that the hydrolase is selected from the group consisting of lipase, proteinase and esterase, and the hydrolase is particularly preferably *Candida rugosa*-derived lipase, *Alcaligenes sp.*-derived lipase or their mixture.

[24] The present invention also provides a method for preparing high-purity S-HGB, the method comprising (a) preparing S-HGB by hydrolyzing S-BBL in the presence of hydrolase: (b) removing enzymes from the hydrolysate of the step (a) and removing

benzoic acid, which is present as a by-product in the resultant hydrolysate, by extracting the benzoic acid with a hydrophobic organic solvent; and (c) extracting the benzoic acid-free hydrolysate of the step (b) with a hydrophilic organic solvent at 0 °C or below to obtain S-HGB.

[25] In the method, it may be characterized in that the enzyme of the step (b) is removed using a filtration method. Also, as the hydrophobic organic solvent for removing benzoic acid in the step (b), it is preferred to use at least one selected from the group consisting of isopropyl ether, tert-butyl methyl ether (TBME), chloroform, dichloromethane, carbon tetrachloride, hexane, toluene and cyclohexane. As the hydrophilic organic solvent for extracting S-HGB of the step (c), it is preferred to use at least one selected from the group consisting of lower alcohol including methanol, ethanol and propan-2-ol, acetic acid, acetone, ethylacetate, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, tetrahydrofuran, dioxane, dimethylformamide and dimethylsulfoxide.

[26]

[27] Hereinafter, the present invention will be described in more detail, as follows.

[28] At first, the method of the present invention includes a step of preparing S-BBL represented by the Formula 1. The S-BBL represented by the Formula 1 may be prepared using the following method, but the present invention is not limited to the following exemplary method for preparing S-BBL. For example, (S)- β -benzoyloxy- γ -butyrolactone in which R1 to R5 in the S-BBL represented by the Formula 1 are all hydrogen may be synthesized by synthesizing S-BSA ((S)-2-benzoyloxy-succinic anhydride) from benzoil chloride and L-malic acid and reacting the S-BSA with $\text{ZnCl}_2/\text{KBH}_4$. The S-BBL synthesis method is described in detail in Korean Patent Publication No. 2002-0073751.

[29] Next, the S-BBL is hydrolyzed with a hydrolase powder or an immobilized enzyme in a hydrophilic solvent or a two-phase system of hydrophilic solvent-hydrophobic organic solvent at constant pH and temperature to prepare S-HGB. At this time, the used hydrophilic solvent is preferably at least one selected from the group consisting of water, lower alcohol including methanol, ethanol and propan-2-ol, acetic acid, acetone, ethylacetate, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, tetrahydrofuran, dioxane, dimethylformamide and dimethylsulfoxide, and the used hydrophobic organic solvent is preferably at least one selected from the group consisting of isopropyl ether, tert-butyl methyl ether (TBME), chloroform, dichloromethane, carbon tetrachloride, hexane, toluene and cyclohexane. The organic solvent is more preferably at least one selected from the group consisting of TBME, cyclohexane, hexane and mixtures thereof, which may stably sustain activity of the enzyme.

- [30] After the production of the S-HGB is completed, the used enzyme is removed by filtration, and a by-product, benzoic acid, produced along with the S-HGB is extracted with a hydrophobic organic solvent. At this time, the remaining S-HGB in an aqueous solution is collected by evaporation of the solvent and extraction with the hydrophilic (polar) organic solvent. The used hydrophilic solvent and hydrophobic organic solvent are the same solvent used in the hydrolyzation.
- [31] The S-HGB, collected as described above, has a very high optical purity (for example, ee >99.5 %). Particularly, the immobilized enzyme has an advantage that it may be collected from the reaction products through the filtration and re-used.
- [32] The hydrolase used in the present invention may be selected from at least one selected from the group consisting of lipase, proteinase and esterase, and the enzyme may be in a form of powder or an aqueous solution. In some cases, the enzyme immobilized on a carrier may also be used. As the method for immobilizing an enzyme, various methods in which an enzyme is attached to a polymer carrier or an inorganic carrier such as celite are known as apparent to those skilled in the art.
- [33] An effective amount of the enzyme is varied since the amount of the used enzyme may be determined according to a variety of factors such as reaction temperature, pH, amount of reactants, reaction time, etc., and the effective amount of the enzyme preferably ranges from about 0.1 to 100 % by weight, based on the weight of a substrate. In this case, a reaction time is extended if the amount of the used enzyme is too small, while the use of the excessive enzyme causes inadequate problems in the reaction process such as low economical efficiency and difficulty in the enzyme separation if the amount of the enzyme is too large.
- [34] There is no particular limitation on reaction conditions using the hydrolase, but the reaction conditions is preferably a temperature of 0~60 °C and a pH value of 3~12 so as to optimize the enzyme reaction. The more preferred reaction temperature ranges from 30 to 50 °C. As described above, the enzyme reaction may be carried out in an aqueous solution (a hydrophilic solvent), and a two-phase system of an organic solvent-aqueous solution (a hydrophilic solvent) mixture including a small amount of an organic solvent may be used to enhance solubility of the substrate and reduce an inhibitory effect of products on the enzyme.
- [35] Concentration of the substrate used for the enzyme reaction may also be varied according to a variety of reaction factors, and the concentration of the substrate preferably ranges from 500 mM to 1 M so as to optimize the enzyme reaction, but the substrate may be used in a higher concentration. In the case of the reaction in the high concentration, an ester compound is not easily dissolved at the beginning of the reaction, but the reaction is accelerated by contact of the enzyme with the ester compound dissolved in water. In order to increase a reaction rate of the enzyme and

maintain activity of the enzyme, the reaction may be carried out in a solvent containing the minimum amount of water and an organic solvent as a main component.

[36] The optimum conditions of the enzyme reaction may be determined in consideration of other various reaction factors, in addition to the factors as described above.

[37] In order to prepare S-HGB in a high purity according to the present invention, the S-HGB prepared in the hydrolyzation is extracted at a low temperature of 0 °C or below if it is extracted with an organic solvent. This is why the extraction at a low temperature can prevent the S-HGB from being deteriorated.

[38]

Brief Description of the Drawings

[39] FIG. 1 is a diagram showing a chromatographic analysis profile of a purity of S-BBL prepared according to one embodiment of the present invention.

[40] FIG. 2 is a diagram showing a chromatographic analysis profile of a S-BBL hydrolysate using a free enzyme (A: 1.5 hours, B: 2 hours).

[41] FIG. 3 is a diagram showing a chromatographic analysis profile of a S-BBL hydrolysate using an immobilized enzyme (A: 38 hours, B: 60 hours).

[42]

Best Mode for Carrying Out the Invention

[43] Hereinafter, preferred embodiments of the present invention will be described in detail referring to the accompanying drawings. However, the description proposed herein is just a preferable example for the purpose of illustrations only, not intended to limit the scope of the invention, so it should be understood that other equivalents and modifications could be made thereto without departing from the spirit and scope of the invention. The preferred embodiments of the present invention will be described in detail for the purpose of better understandings, as apparent to those skilled in the art.

[44]

Example 1. Synthesis of S-BBL

[45] 1.5M benzoil chloride and 0.5M L-malic acid were reacted at 60 °C for 10 hours, and washed with TBME to prepare S-BSA ((S)-2-benzoyloxy-succinic anhydride). One equivalent of S-BSA and 0.5 equivalent of $\text{Zn}(\text{BH}_4)_2$ were added to THF (tetrahydrofuran), reacted at a constant temperature of 25 °C for 5 hours while stirring, and then THF was evaporated from the resultant mixture and the mixture was extracted with dichloromethane to prepare S-BBL.

[47] FIG. 1 shows an HPLC analysis profile of the prepared S-BBL. As shown in FIG. 1, a retention time of the S-BBL was 5.6 minutes, and its purity was 99.7 %. Accordingly, it was revealed that the S-BBL obtained according to the method has a

purity of 99 % or more.

[48] Conditions of the HPLC analysis is as follows: acetonitrile:water=60:40 (v/v), C18 Capcell Pak, 1ml/min, 238nm UV).

[49]

[50] Example 2. Screening of S-BBL Hydrolase

[51] 10 mg of S-BBL and 17 mg of enzyme powder were added to a mixture solution of 250 ul of Tris-HCl buffer (0.1M, pH 7.0) and 200 ul of acetone, reacted at 37 °C for 24 hours and 48 hours, and then the resultant test samples were taken and hydrolyzed, and then the resultant product was analyzed under the HPLC condition of the Example 1 (benzoic acid: 4.1 min, S-BBL: 5.3 min). The benzoic acid was a hydrolysate by-product, and the S-BBL was a starting material which remains unhydrolyzed. The results are shown in FIGs. 2 and 3.

[52] FIGs. 2 and 3 are diagrams showing chromatographic analysis profiles of S-BBL hydrolysates according to the reaction time, the hydrolysates being obtained by using a free enzyme and an immobilized enzyme, respectively. FIG. 2 (A) and (B) show analysis profiles of the S-BBL hydrolysates when the reaction times are 1.5 hours and 2 hours, respectively, and FIG. 3 (A) and (B) show analysis profiles of the S-BBL hydrolysates when the reaction times are 38 hours and 60 hours, respectively.

[53] At this time, the generated S-HGB in the reaction product was observed through its development in an iodine chamber since it was not observed under an ultraviolet (UV).

[54] The kinds of and conversions by the enzymes used in this Example were listed in the following Table 1. The S-BBL was converted into the S-HGB by various kinds of hydrolases, and the conversion is the most excellent when *Candida rugosa*- and *Alcaligenes sp.*-derived lipases are used.

[55] Table 1

Enzyme	Source	Conversion (%)	
		Day 1	Days 2

Lipase	Lipase PS	<i>Burkholderia cepacia</i>	16	22
	Novozym 525L	<i>Candida antarctica</i>	20	29
	Lipase OF	<i>Candida rugosa</i>	100	-
	Lipase PL	<i>Alcaligenes</i> sp.	100	-
	Lipase PS-D	<i>Burkholderia cepacia</i>	13	18
	Lipase PS-C	<i>Burkholderia cepacia</i>	-	56
	Lipase MY	<i>Candida rugosa</i>	49.5	23
	Lipase AYS	<i>Candida rugosa</i>	66	81
	Lipozyme	<i>Humicola lanuginosa</i>	17	28
	Lipase (Sigma type VII)	<i>Candida rugosa</i> .	47	59
	Lipoprotein lipase (Toyobo)	<i>Pseudomonas</i> sp.	44	50
	Lipase AH	<i>Burkholderia cepacia</i>	31	46
	Novozym 525L	<i>Candida antarctica</i>	20	29
Proteinase	Protease PS	<i>Bacillus</i> sp.	23	32
	Neutral Proteinase (Toyobo)	<i>Bacillus</i>	12	16
Esterase	PLE-AL amano	<i>Porcine liver</i>	21	33
	PLE-Roche	<i>Porcine liver</i>	28	34
Others	Alcalase 0.6L	<i>Bacillus licheniformis</i>	35	48
	Alcalase (0.6L)	<i>Bacillus licheniformis</i>	21	26
	Acylase Amano	<i>Aspergillus melleus</i>	47	59
	PGA-450 (Roche)	<i>E. coli</i>	61	83

[56]

[57]

Example 3. Effect of Organic Solvent on Enzyme Reaction

[58]

10 mg of S-BBL and 10 mg of Lipase OF enzyme powder were added to a mixture solution of 100 ul of Tris-HCl buffer (0.1M, pH 7.0) and 300 ul of acetonitrile, acetone and TBME, reacted at 37 °C for 2 hours and 6 hours while stirring at a rotary speed of 200 rpm, and then the resultant test samples were taken in a dose of 20 ul and diluted with 980 ul of acetonitrile, and then the resultant product was analyzed under the HPLC conditions of Example 1. The results are listed in the following Table 2.

[59]

Table 2

Organic Solvent	Conversion (%)	
	2 hrs	6 hrs

TBME	100	-
Acetone	44	73
Acetonitrile	< 1	< 1

[60] As a result, it was revealed that the reaction solvent of the enzyme used for preparing S-HGB is more preferably non-polar TBME than acetone or acetonitrile having polarity since the non-polar TBME may stably maintain activity of the enzyme, as listed in the Table 2.

[61]

[62] Example 4. Change in Relative Amount of Enzyme and S-BBL

[63] The method of Example 3 was repeated to analyze a final product, except that the final product was analyzed every 30 minutes with varying amounts of the Lipase OF and the substrate S-BBL. The results are listed in the following Table 3.

[64] Table 3

S-BBL : Lipase OF	Conversion (%)	
	30 Min	60 Min
20:1	41.3	65.8
20:2	64.4	75
20:3	87.5	100
20:4	100	-

[65] As a result, it was revealed that the conversion of S-BBL to S-HGB is increased to a certain level with increase in an amount of the enzyme when the substrate is used in an equivalent amount, as listed in the Table 3.

[66]

[67] Example 5. Immobilization and Re-use of Enzyme

[68] Amberlite® XAD-7, which was purchased from Rohm & Haas, was used as poly-methacrylate-based cross-linked polymer particle having a mesh size of 20~60. 1 g of Lipase OF (Meito Sagyo, Japan) was dissolved at a room temperature in 50 mL of 1M phosphate buffer (pH 6.0), 0.5 g and 1 g of XAD-7 was added, respectively, to 2.5 mL of a solution (equivalent to 50 mg of the enzyme), and then mixed while stirring overnight to bind the XAD-7 to the substrate. The bound enzyme was collected by filtration, and then activity of the enzyme in the supernatant was measured. As a result, an XAD-7-bound resin showed an activity of 5 %, compared to an XAD-7-free Lipase OF.

[69] The enzyme-bound resin was put into 5 mL of 0.1M phosphate buffer (pH 7.0), and 25 ul of 25% glutaraldehyde solution was added, and then treated at a room

temperature for 6 hours. After the filtration, 1.5 g of S-BBL was added to a mixture of 7.5 mL of water and 7.5 mL of TBME, and then reacted at 35 °C while stirring. The immobilized enzyme was re-used by repeating a method in which an immobilized enzyme is collected by filtering the reaction solution, and 1.5 g of S-BBL was added to a mixture of 7.5 mL of water and 7.5 mL of TBME and reacted again at 35 °C while stirring. The results are listed in the following Table 4.

[70] Table 4

Use Count	Reaction Time (hr)	Conversion (%)	
		0.5g XAD-7	1g XAD-7
1	20	95	86
2	21	91	88
3	20	89	79
4	38	99	96.3
5	22	71	72
6	27	81	83
7	23	68	50

[71] As listed in Table 4, it was revealed that the activity of the enzyme is not lowered even after the enzyme was re-used 6 times.

[72]

[73] Example 6. Separation and Purification of S-HGB

[74] 1 kg of Lipase OF was immobilized onto 5 kg of XAD-7 in the same manner as in Example 5, added to a mixture solution of 100 L of water and 100 L of TBME, and then 20.5 kg of S-BBL was added and reacted for 11 hours, and a TBME fraction was removed.

[75] 20.5 kg of S-BBL and 100 L of TBME were newly added to the reaction solution from which the TBME fraction was removed, reacted for 12 hours, and then a TBME fraction was removed. Also, 20.5 kg of S-BBL and 100 L of TBME were additionally added to the reaction solution, reacted for 14 hours, and then a TBME fraction was removed. Subsequently, an immobilized enzyme was removed by filtering an aqueous solution fraction, and the resultant reaction product was washed with TBME to remove a by-product, benzoic acid, and then a reaction solution, obtained by washing the reaction product three times with 100 ml of a polar solvent, ethyl acetate, at 0 °C or below, was distilled at 30 °C under a reduced pressure.

[76] The reaction product was extracted using the method as described above to obtain 24.3 kg of S-HGB having a purity of 99 %.

[77] As described above, the specific embodiments of the present invention has been described in detail. However, the description proposed herein is just a preferable example for the purpose of illustrations only, not intended to limit the scope of the invention, so it should be understood that other equivalents and modifications could be made thereto without departing from the spirit and scope of the invention, as apparent to those skilled in the art.

[78]

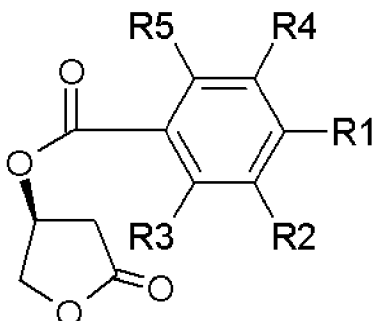
Industrial Applicability

[79] According to the present invention, the S-HGB ((S)-3-hydroxy- γ -butyrolactone) having an optical purity can be obtained in a high yield under simple process conditions without requiring reaction conditions of high pressure and high temperature or complex operation conditions by hydrolyzing S-BBL ((S)- β -benzoyloxy- γ -butyrolactone) with hydrolase.

Claims

- [1] A method for preparing (S)-3-hydroxy- γ -butyrolactone (S-HGB), wherein the S-HGB is obtained by hydrolyzing (S)- β -benzoyloxy- γ -butyrolactone (S-BBL) represented by the following Formula 1 in the presence of hydrolase:

[Formula 1]



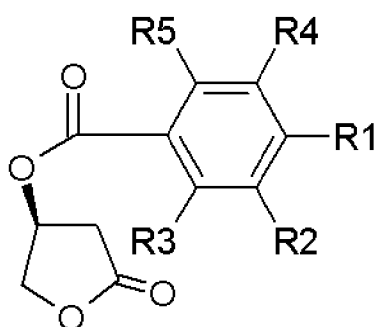
In the Formula 1,

R1, R2, R3, R4 and R5 are each independently one selected from the group consisting of hydrogen, (C1-C10)alkyl, (C5-C6)cycloalkyl, fluoro(C1-C10)alkyl, OR', aryl, aryl(C1-C10)alkyl, NO₂, NR'R'', C(O)R', CO₂R', C(O)NR'R'', N(R'')C(O)R', N(R'')CO₂R', N(R'')C(O)NR'R'', S(O)_mNR'R'', S(O)_mR', CN and N(R'')S(O)_mR', provided that two adjacent Rs among the groups R1, R2, R3, R4 and R5 may share carbon atoms to form a fused aromatic or cycloalkene ring, the R' and R'' are each independently one selected from the group consisting of hydrogen, (C1-C10)alkyl, aryl and aryl(C1-C10)alkyl, provided that, if the R' and R'' are attached to the same nitrogen atom, they may bind to each other to form a 5-, 6- or 7-membered ring containing 1 to 3 heteroatoms selected from the group consisting of N, O and S, and m is an integer from 0 to 2.

- [2] The method according to claim 1, wherein the hydrolysis of S-BBL is carried out in a hydrophilic solvent or a two-phase system of a hydrophilic solvent-hydrophobic organic solvent.
- [3] The method according to claim 2, wherein the hydrophilic solvent is at least one selected from the group consisting of water, lower alcohol, acetic acid, acetone, ethylacetate, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, tetrahydrofuran, dioxane, dimethylformamide and dimethylsulfoxide.
- [4] The method according to claim 2, wherein the hydrophobic organic solvent is at least one selected from the group consisting of isopropyl ether, tert-butyl methyl ether (TBME), chloroform, dichloromethane, carbon tetrachloride, hexane,

toluene and cyclohexane.

- [5] The method according to claim 1, wherein the hydrolase is at least one selected from the group consisting of lipase, proteinase and esterase.
- [6] The method according to claim 5, wherein the hydrolase is *Candida rugosa*-derived lipase, *Alcaligenes sp.*-derived lipase or their mixture.
- [7] The method according to claim 1, wherein the hydrolase is an immobilized enzyme.
- [8] A method for preparing high-purity (S)-3-hydroxy- γ -butyrolactone (S-HGB), the method comprising:
 (a) preparing S-HGB by hydrolyzing (S)- β -benzoyloxy- γ -butyrolactone (S-BBL) represented by the following Formula 1 in the presence of hydrolase:
 [Formula 1]



in the Formula 1,

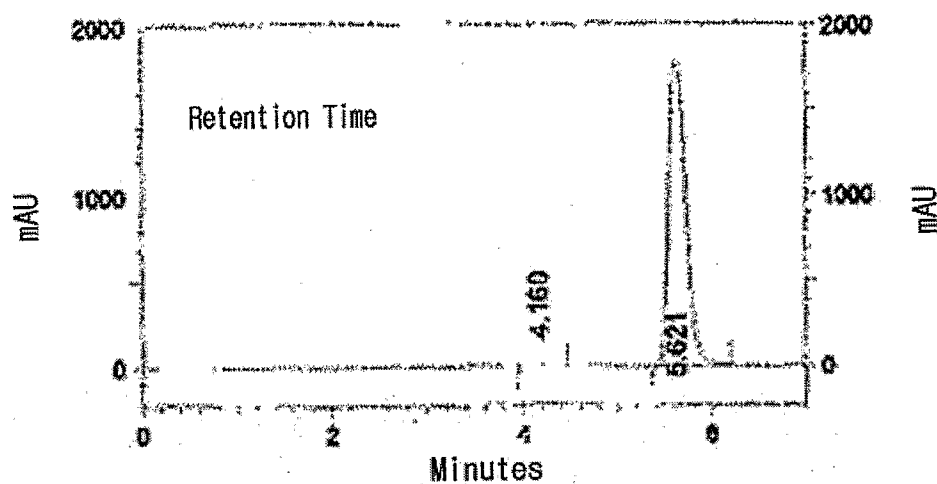
R1, R2, R3, R4 and R5 are each independently one selected from the group consisting of hydrogen, (C1-C10)alkyl, (C5-C6)cycloalkyl, fluoro(C1-C10)alkyl, OR', aryl, aryl(C1-C10)alkyl, NO₂, NR'R'', C(O)R', CO₂R', C(O)NR'R'', N(R'')C(O)R', N(R'')CO₂R', N(R'')C(O)NR'R'', S(O)_mNR'R'', S(O)_mR', CN and N(R'')S(O)_mR', provided that two adjacent Rs among the groups R1, R2, R3, R4 and R5 may share carbon atoms to form a fused aromatic or cycloalkene ring, the R' and R'' are each independently one selected from the group consisting of hydrogen, (C1-C10)alkyl, aryl and aryl(C1-C10)alkyl, provided that, if the R' and R'' are attached to the same nitrogen atom, they may bind to each other to form a 5-, 6- or 7-membered ring containing 1 to 3 heteroatoms selected from the group consisting of N, O and S, and m is an integer from 0 to 2;

(b) removing enzymes from the hydrolysate of the step (a) and removing benzoic acid in the resultant hydrolysate by extracting the benzoic acid with a hydrophobic organic solvent; and

(c) extracting the benzoic acid-free hydrolysate of the step (b) with a hydrophilic organic solvent at 0 °C or below to obtain S-HGB.

- [9] The method according to claim 8, wherein the hydrolysis of S-BBL is carried out in a hydrophilic solvent or a two-phase system of a hydrophilic solvent-hydrophobic organic solvent.
- [10] The method according to claim 9, wherein the hydrophilic solvent is at least one selected from the group consisting of water, lower alcohol, acetic acid, acetone, ethylacetate, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, tetrahydrofuran, dioxane, dimethylformamide and dimethylsulfoxide.
- [11] The method according to claim 9, wherein the hydrophobic organic solvent is at least one selected from the group consisting of isopropyl ether, tert-butyl methyl ether (TBME), chloroform, dichloromethane, carbon tetrachloride, hexane, toluene and cyclohexane.
- [12] The method according to claim 8, wherein the hydrolase is at least one selected from the group consisting of lipase, proteinase and esterase.
- [13] The method according to claim 12, wherein the hydrolase is *Candida rugosa*-derived lipase, *Alcaligenes sp.*-derived lipase or their mixture.
- [14] The method according to claim 8, wherein the hydrolase is an immobilized enzyme.
- [15] The method according to claim 8, wherein the enzyme in the step (b) is removed using a filtration method.
- [16] The method according to claim 8, wherein the hydrophobic organic solvent of the step (b) is at least one selected from the group consisting of isopropyl ether, tert-butyl methyl ether (TBME), chloroform, dichloromethane, carbon tetrachloride, hexane, toluene and cyclohexane.
- [17] The method according to claim 8, wherein the hydrophilic organic solvent of the step (c) is at least one selected from the group consisting of methanol, lower alcohol, acetic acid, acetone, ethylacetate, acetonitrile, N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, tetrahydrofuran, dioxane, dimethylformamide and dimethylsulfoxide.

FIG. 1

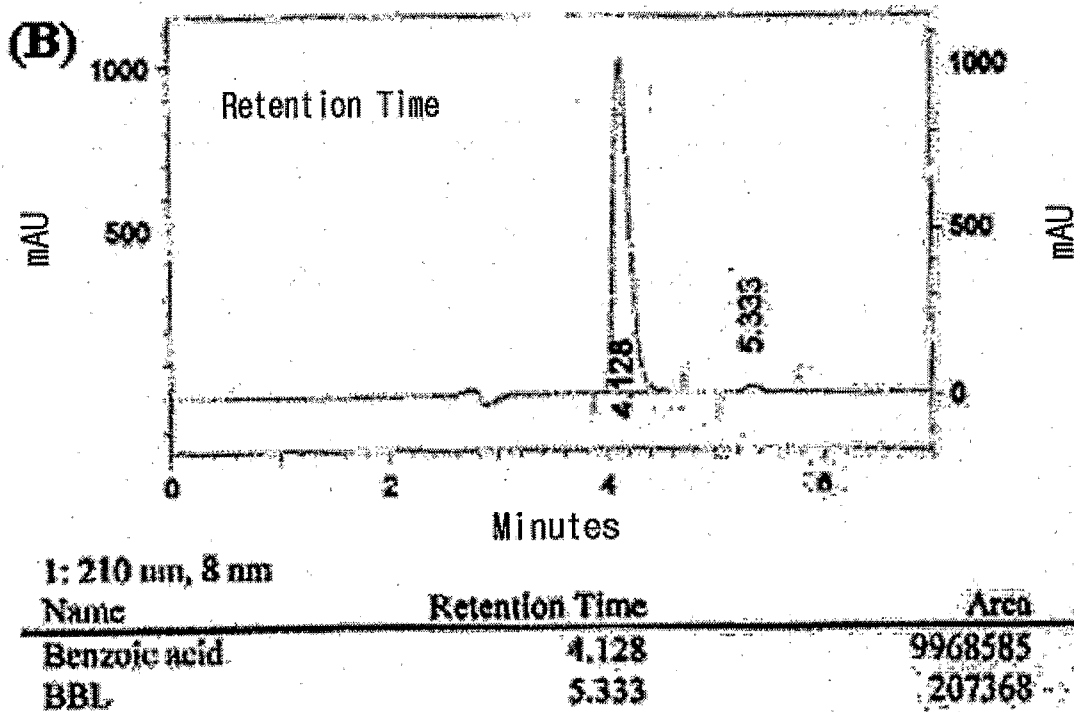
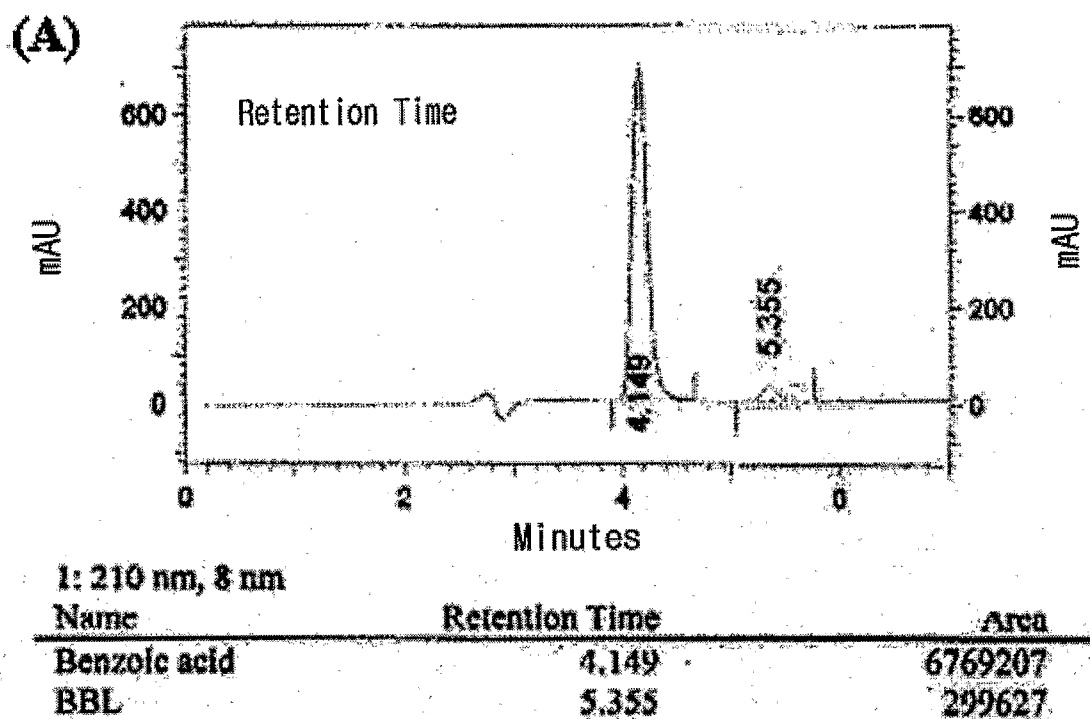


2:238nm, 8nm

Name	Retention Time	Area	Area Percent
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BBI	5.621	23157191	99.72

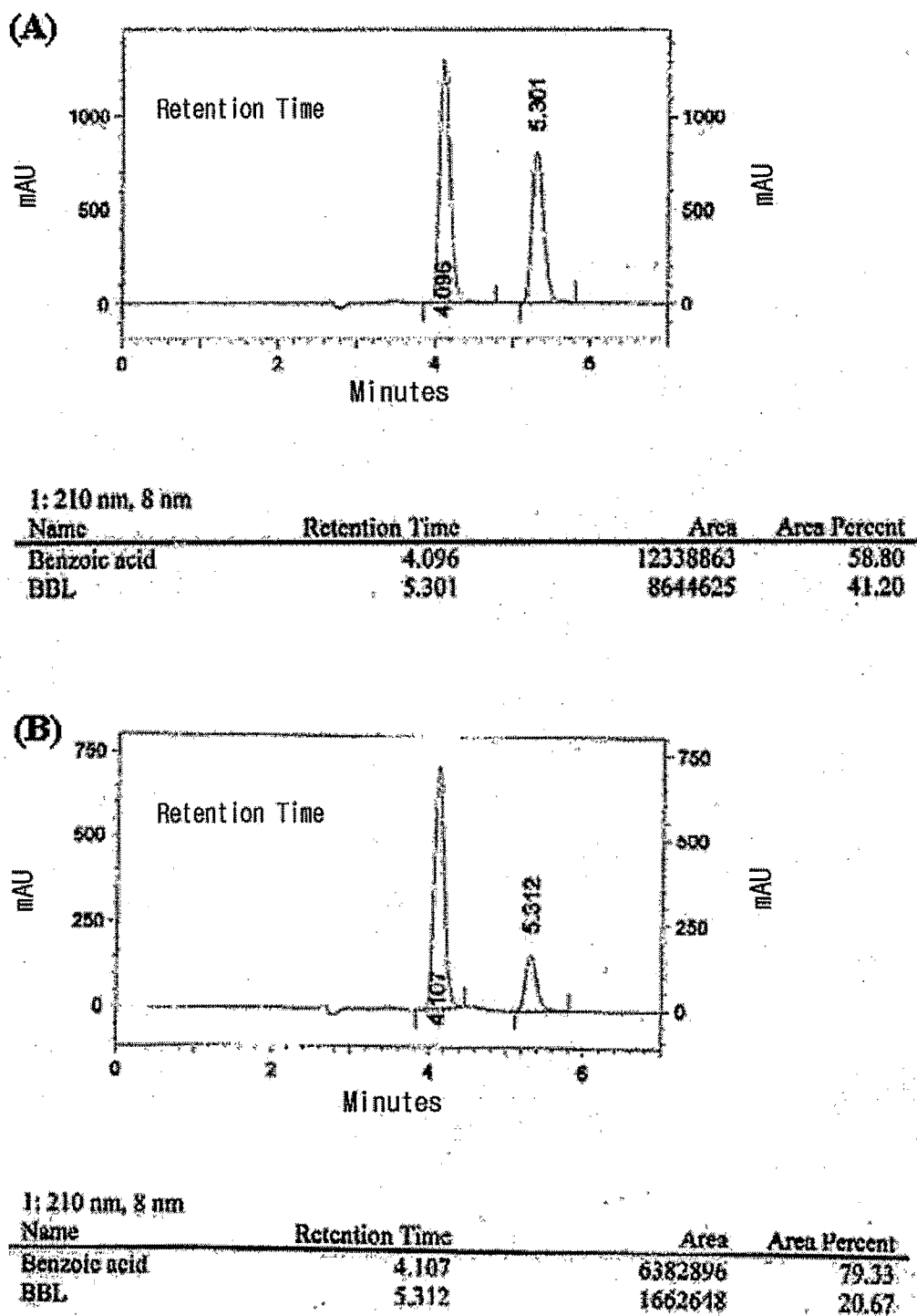
2/3

FIG. 2



3/3

FIG. 3



A. CLASSIFICATION OF SUBJECT MATTER*C07D 307/32(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 : as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PUB MED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2002/204699 A (Taisel kakaku kokyo Ltd. Co.) 23 July 2002 See abstract, claims 1-5.	1-17
A	US 5084392 A (Kazutoshi Miyazawa et al) 28 Jan. 1992 See abstract, claims 1-4.	1-17

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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"&" document member of the same patent family

Date of the actual completion of the international search

30 MAY 2007 (30.05.2007)

Date of mailing of the international search report

30 MAY 2007 (30.05.2007)

Name and mailing address of the ISA/KR

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Facsimile No. 82-42-472-7140

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Telephone No. 82-42-481-5608



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2007/001052

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2002/204699 A	23.07.2002	None	
US 5084392 A	28.01.1992	EP439779A2 JP254294B2	07.08.1991 09.10.1996