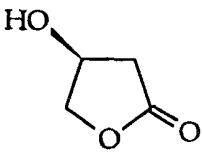




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| <p>(21) International Application Number: PCT/KR99/00398 (22) International Filing Date: 23 July 1999 (23.07.99) (30) Priority Data: 1998/29912 24 July 1998 (24.07.98) KR (71) Applicant (for all designated States except US): SAM-SUNG FINE CHEMICALS CO., LTD. [KR/KR]; 190, Yecheon-dong, Nam-ku, Ulsan 680-090 (KR). (72) Inventors; and (75) Inventors/Applicants (for US only): CHUN, JongPil [KR/KR]; 514-1405 Chungsol Apt., Songgang-dong, Yusung-ku, Daejeon 305-503 (KR). CHO, Yik-Haeng [KR/KR]; 103-205 Parangsae Apt., Dunsan-dong, Seo-ku, Daejeon 302-120 (KR). ROH, KyoungRok [KR/KR]; 105-1007 Sejong Apt., Jeonmin-dong, Yusung-ku, Daejeon 305-390 (KR). PARK, YoungMi [KR/KR]; 513-1401 Chungsol Apt., Songgang-dong, Yusung-ku, Daejeon 305-503 (KR). YU, HoSung [KR/KR]; 109-406 Sejong Apt., Jeonmin-dong, Yusung-ku, Daejeon 305-390 (KR). HWANG, DaeIl [KR/KR]; 513-1410 Chungsol Apt., Songgang-dong, Yusung-ku, Daejeon 305-503 (KR).</p> | <p>(74) Agent: HUH, SangHoon; Hyecheon Building, 13th floor, 831 Yeoksam-dong, Kangnam-ku, Seoul 135-792 (KR). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> | |
| <p>(54) Title: CONTINUOUS PROCESS FOR PREPARING OPTICALLY PURE (S)-3-HYDROXY-GAMA-BUTYROLACTONE</p> | | |
| <div style="text-align: center;">  <p>(1)</p> </div> | | |
| <p>(57) Abstract</p> <p>The present invention relates to a continuous process for preparing optically pure (S)-3-hydroxy-γ-butyrolactone expressed by formula (1) and more particularly, to a process which enables preparing optically pure (S)-3-hydroxy-γ-butyrolactone economically in large quantities, by: (a) preparing α-(1,4) linked oligosaccharide with adequate sugar distribution by reacting amylopectin which is easily available from natural product with enzyme under a specific condition; and (b) performing oxidation, esterification and cyclization sequentially under a specific condition.</p> | | |

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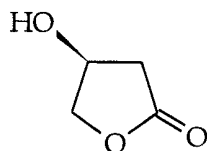
CONTINUOUS PROCESS FOR PREPARING OPTICALLY PURE (S)-3-HYDROXY-GAMA-BUTYROLACTONE

BACKGROUND OF THE INVENTION

5 Field of the Invention

The present invention relates to a continuous process for preparing optically pure (S)-3-hydroxy- γ -butyrolactone expressed by the following Formula 1 and more particularly, to the continuous process which enables preparing optically pure (S)-3-hydroxy- γ -butyrolactone economically in large
10 quantities, by:

- (a) Preparing α -(1,4) linked oligosaccharide having adequate sugar distribution by reacting amylopectin which is easily available from natural product with enzyme under a specific condition; and
- (b) Performing oxidation by running basic anion exchange resin with an
15 oxidant to give (S)-3,4-dihydroxybutyric acid-anion exchange resin complex, dissociating the (S)-3,4-dihydroxy-butyrac acid from anion exchange resin complex, esterification and cyclization sequentially under a specific condition.



(1)

20 Description of the Related Arts

(S)-3,4-Dihydroxybutyric acid derivatives and (S)-3-hydroxy- γ -butyrolactone are used as synthetic intermediates for preparing various chiral compounds. For example, it is well known that they act as key intermediates for preparing neuromediator (R)-GABOB [*Tetrahedron*, **46**,

4277(1990)], treatment for hyperlipemia (Atorvastatin; HMG-CoA reductase inhibitor) [*Tetrahedron Lett.*, **33**, 2279(1992)], (S)-oxiracetam which is improvement agent in brain metabolism [International patent publication WO93/06,826], L-carnitine which is health supplement agent [International patent publication WO99/05,092], (S)-3-hydroxytetrahydrofuran [*J. Am. Chem. Soc.*, **117**, 1181(1995); International patent publication WO94/05,639] which is an essential intermediate of AIDS drug (Agenerase; HIV protease inhibitor), (S)-mono-betalactam [Japanese patent publication 64-13,069(1989)], ester of (S)-3-hydroxy-4-bromobutyric acid [Japanese patent publication 4-149,151(1992); Japanese patent publication 6-172,256(1994)], potentiating intermediate of satiety agent [*Bull. Chem. Soc. Jpn.*, **61**, 2025(1988)] and neuroleptic drug [USP 4,138,484] and useful intermediates in synthetic efforts towards natural products [*J. Org. Chem.*, **50**, 1144 (1985), *Can. J. Chem.*, **65**, 195 (1987), *Tetrahedron Lett.*, 507 (1992)]. Optical purity is the most important factor in preparing these chiral compounds.

The conventional technologies for preparing (S)-3,4-dihydroxybutyric acid derivatives and (S)-3-hydroxy- γ -butyrolactone, which are useful for preparing the said chiral compounds, are explained in detail hereunder.

Methods of preparing (S)-3-hydroxybutyric acid derivatives from the enzymatic or catalytic reduction of β -ketoester were known [*J. Am. Chem. Soc.*, **105**, 5925~5926(1983); *Tetrahedron Lett.*, **31**, 267~270(1990); European patent publication 452,143A2]. These methods have difficulty in that the prochiral center should be reduced to one-side to generate chiral center and expensive metal catalyst should be used.

A technology preparing ester of (S)-3,4-dihydroxybutyric acid and (S)-3-hydroxy- γ -butyrolactone by selective reduction of (L)-malic acid ester was known [*Chem. Lett.*, 1389~1392(1984); USP 5,808,107]. This technology has disadvantage in that reduction should be performed selectively to only one of

isomers due to tautomerization and a mixture of cyclic compounds and hydrates derived from dicarbonyl compound (A). So, the dicarbonyl compound (A) cannot be separated in good yields from the reaction mixture. Another problem is that the prepared (S)-3,4-dihydroxybutyric acid is degraded
5 to formic acid and glycolic acid due to the overoxidation.

A similar technology for preparing (S)-3,4-dihydroxybutyric acid from carbohydrate either using base only or using oxygen in base was known. It proposed that the dicarbonyl compound (A) was a synthetic intermediate for (S)-3,4-dihydroxybutyric acid as shown in the Scheme 1. But the yield was
10 reported to be as low as about 30% [*J. Res. Natl. Bur. Stand.*, **32**, 45(1944); *J. Am. Chem. Soc.*, 2245~2247(1953); *J. Am. Chem. Soc.*, 1431~1435(1955); *Carbohyd. Res.*, **11**, 17~25(1969); *J. Chromatography*, **549**, 113~125(1991)]. In these methods, (S)-3,4-dihydroxybutyric acid is produced with various kind of mixtures including glycolic acid (D), isosaccharinic acid (B), formic acid, ketone, diketone
15 and glyceric acid. Since the yield of (S)-3,4-dihydroxybutyric acid is very low, these methods are also considered as not suitable for industrial use.

A method for preparing (S)-3,4-dihydroxybutyric acid from disaccharide (lactose) using base and oxidant has been reported [International patent publication WO98/04543]. In this work, (S)-3,4-dihydroxybutyric
20 acid was cyclized to (S)-3-hydroxy- γ -butyrolactone under the reaction condition and purified by protection of the two hydroxy groups to acetonide ester compound, methyl (S)-3,4-O-isopropylidene-3,4-dihydroxybutanoate, which was recycled to (S)-3-hydroxy- γ -butyrolactone under acidic media.

Preparing methods of (S)-3,4-dihydroxybutyric acid including the
25 process of alkaline oxidation of carbohydrate containing glucose substituent in the 4-position have been known [USP 5,292,939, 5,319,110 & 5,374,773(1994)]. In these methods, dicarbonyl compound (A) intermediate is formed at first, oxidized to (S)-3,4-dihydroxybutyric acid (C) and glycolic acid (D).

However, optical purity, the most important physical property of chiral compounds, is not mentioned at all. Also, purification of target compound is very difficult, considering the reaction mechanism. In the case of disaccharides such as maltose or lactose, only one sugar unit in the disaccharide forms (S)-3,4-dihydroxybutyric acid and the other sugar unit functions as leaving group, so that the target product and leaving group coexist as 1:1 mixture. Accordingly, it is very difficult to separate and purify (S)-3,4-dihydroxybutyric acid or (S)-3-hydroxy- γ -butyrolactone from the reaction mixture. The maximum mass conversion obtainable is 28.3 wt%. In other words, 28.3g of (S)-3-hydroxy- γ -butyrolactone can be obtained from 100g of disaccharide. For polysaccharides, such as maltodextrin, starch and cellulose, mentioned in the above patents, the (1,4) and/or (1,6) glucose units are linked complexly like nets. The problem is that the step-by-step oxidation proceeding from the reducing end units comprising (1,4) linkage terminates at (1,6) linkage unit. Therefore no more target product is formed. Also, the polysaccharides are degraded by overoxidation of reducing end units to complex acid mixtures containing formic acid, oxalic acid, glycolic acid and erythronic acid [*J. Am. Chem. Soc.*, **81**, 3136(1959); *Starch* **41** Nr. 8, S. 303~309(1989); *Synthesis*, 597~613(1997)].

There was an attempt to improve the yield of (S)-3,4-dihydroxybutyric acid or (S)-3-hydroxy- γ -butyrolactone for polysaccharide by degradation of higher-molecular sugars to relatively lower-molecular sugars through acid or base hydrolysis. Though the reactivity by this method is increased to a degree, (1,4) linkage and (1,6) linkage are not hydrolyzed selectively to afford random distribution. Accordingly, there is a fundamental problem in preparing (S)-3,4-dihydroxybutyric acid and its derivatives in high yield [*Encyclopedia of Chemical Technology*, 3th ed. 492~507].

Regarding the preparation of (S)-3-hydroxy- γ -butyrolactone using (1,4)

linked polysaccharide, the step-by-step oxidation proceeds continuously from the reducing-end units to non-reducing end units to afford (S)-3,4-dihydroxybutyric acid until the last chain unit (a leaving group) is remained. Namely, if (1,4) linked polysaccharide is used as a source material for preparing (S)-3-hydroxy- γ -butyrolactone, the maximum mass conversion obtainable is 63 wt%, about two times more compared with the method using disaccharide. In other words, 63g of (S)-3-hydroxy- γ -butyrolactone can be obtained from 100g of (1,4)-linked polysaccharide. Also, since the small amount of leaving group is produced in the reaction mixture compared with disaccharide, the target product is easily purified. Therefore, the use of (1,4) linked polysaccharide promises the enhanced productivity.

However, regarding conventional polysaccharides, the target product and by-products (acids such as formic acid, oxalic acid, glycolic acid and erythronic acid) is formed competitively in the step-by-step oxidation due to the compact structure having random (1,4) linkage and (1,6) linkage. Thus, selective degradation technique of polysaccharide to a suitable sugar distribution range having (1,4) linkage is required.

On the other hand, there have been many reports of transforming higher-molecular sugars to lower-molecular sugars using biological enzymatic treatment process for industrial use.

The reported technologies include preparing glucose, maltose and ethanol through enzymatic treatment of starch [USP 3,791,865(1974); USP 3,922,200(1975); USP 4,855,232(1989): Japanese patent publication 4-158,795(1992); *Methods Carbohydr. Chem.*, 10, 231~239(1994); *Methods Carbohydr. Chem.*, 10, 245~248(1994)], and preparing maltodextrin with adequate dextrose equivalent (DE) [USP 3,986,890(1976); USP 4,447,532(1984); USP 4,612,284(1986); USP 5,506,353(1996)]. In these references, through the degradation or transformation of high molecular polysaccharides, it is converted to adequate

materials for medicines, food additives and diagnostic reagents.

But, method for preparing (1,4) linked oligosaccharides suitable for the mass production of (S)-3-hydroxy- γ -butyrolactone by biological treatment of higher molecular polysaccharides with enzymes is not known at present.

5

SUMMARY OF THE INVENTION

The inventors of the present invention made intensive efforts to develop a continuous method for preparing optically pure (S)-3-hydroxy- γ -butyrolactone from commercially available amylopectin with ease. As a result, a process which enables preparing optically pure (S)-3-hydroxy- γ -butyrolactone economically in large quantities is found by preparing oligosaccharide with structural specificity which can minimize formation of by-products from amylopectin by enzymatic reaction, performing oxidation by running basic anion exchange resin with an oxidant to give (S)-3,4-dihydroxybutyric acid-anion exchange resin complex, dissociating the (S)-3,4-dihydroxybutyric acid from anion exchange resin complex, esterification and cyclization sequentially under a specific condition. Because glucose generated from the above oxidation as a leaving group does not adsorbed on anion exchange resin, it can be easily removed by washing anion exchange resin with water compared with conventional oxidation methods using inorganic base. Also, anion exchange resin used above oxidation can be simultaneously regenerated by dissociation process. Therefore, it can be re-used for oxidation of oligosaccharide. This is one of superior advantages of the present invention.

25 Accordingly, an object of this invention is to provide a continuous method for preparing optically pure (S)-3-hydroxy- γ -butyrolactone in high yield by preparing oligosaccharide with structural specificity which can minimize formation of by-products from amylopectin by enzymatic reaction,

performing oxidation by running basic anion exchange resin with an oxidant to give (S)-3,4-dihydroxybutyric acid-anion exchange resin complex, dissociating the (S)-3,4-dihydroxybutyric acid from anion exchange resin complex, esterification and cyclization sequentially without additional purification of intermediates.

Brief Description of the Drawings

Fig. 1a represents the optical purity analysis results by gas chromatography (GC) of racemic 3-hydroxy- γ -butyrolactone.

Fig. 1b represents the optical purity analysis results by gas chromatography (GC) of 3-hydroxy- γ -butyrolactone prepared from disaccharide of the conventional method.

Fig. 1c represents the optical purity analysis results by gas chromatography (GC) of 3-hydroxy- γ -butyrolactone prepared from oligosaccharide of the present invention.

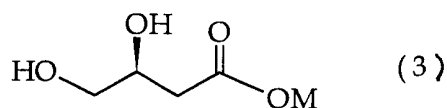
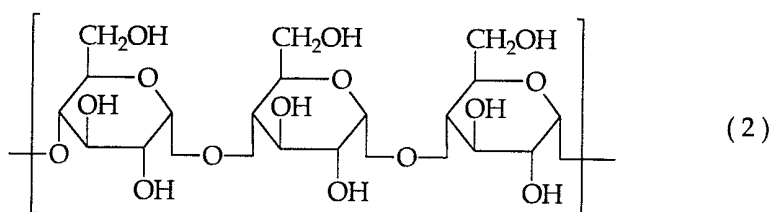
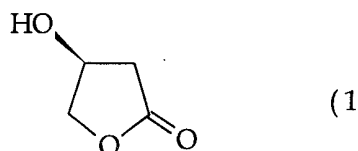
Detailed Description of the Invention

The present invention is characterized by comprising the following steps:

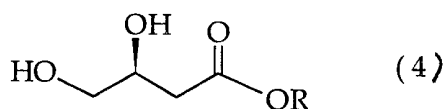
- (a) Enzymatic reaction of amylopectin to α -(1,4) linked oligosaccharide expressed by the Formula 2;
- (b) Oxidation of the oligosaccharide by running basic anion exchange resin with an oxidant to afford (S)-3,4-dihydroxybutyric acid expressed by the Formula 3; and
- (c) Subsequent esterification with alcohol in the presence of an acid catalyst to afford ester of (S)-3,4-dihydroxybutyric acid expressed by the Formula 4; and
- (d) Cyclization of the prepared ester of (S)-3,4-dihydroxybutyric acid in

the presence of an acid catalyst without additional separation and purification process to afford (S)-3-hydroxy- γ -butyrolactone expressed by the Formula 1.

5



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Wherein, M represents hydrogen, alkali metal or alkali earth metal atom; R represents linear or branched alkyl group with 1~5 carbons.

15

The detailed description of the present invention is given hereunder.

20

The fundamental inventive concept of the present invention is selective degradation of α -(1,4) linkage and α -(1,6) linkage within amylopectin using specific enzymes, i.e., transforming amylopectin to α -(1,4) linked oligosaccharide with the optimal sugar distribution for preparing the target compound. And subsequent oxidation by running basic anion exchange resin with an oxidant to give (S)-3,4-dihydroxybutyric acid removing simultaneously a by-product, glucose, easily and effectively, esterification and

cyclization is performed for preparing optically pure (S)-3-hydroxy- γ -butyrolactone.

Namely, focusing on the specificity of enzymes, amylopectin is degraded sequentially with specific enzymes to α -(1,4) linked oligosaccharide, and optically pure (S)-3-hydroxy- γ -butyrolactone is prepared from the transformed oligosaccharide by oxidation running basic anion exchange resin to give (S)-3,4-dihydroxybutyric acid removing simultaneously a by-product, glucose, easily, esterification and cyclization sequentially in high yield. Optical purity of the desired product prepared by a sequential method is over 99.9 %ee.

Amylopectin, the source material of the present invention, is commercially easily available. Especially, since amylopectin is highly soluble in water or buffer solution of pH 4.0~8.0 used as reaction solvent for enzymatic reaction of the present invention compared with other polysaccharides such as starch and cellulose, the relative reactivity to enzyme is greatly increased. Thus, the same is very effective material for preparing of oligosaccharide having suitable sugar distribution for the preparation of (S)-3-hydroxy- γ -butyrolactone.

When using pullulanase as an enzyme for selective degradation of α -(1,6) linkage in amylopectin, it causes the solubility problem of amylopectin and reduced enzyme activity. So, rather than using pullulanase only, α -amylase was used to improve reactivity in degrading amylopectin to a suitable sugar distribution, and then pullulanase was used. However, in this case, activity of the remaining α -amylase persists and thus amylopectin is degraded excessively, so that the desired oligosaccharide is not formed. Accordingly, a technology of inactivating the remaining α -amylase before the pullulanase reaction was introduced.

The detailed explanation of the preparation process of this invention is

as follow. It comprises; 1) a step preparing oligosaccharide with characteristic α -(1,4) linkage expressed in Formula 2 by selective degradation of amylopectin using biological treatment of specific enzymes, 2) oxidation of the oligosaccharide by running basic ion exchange resin with an oxidant to afford
5 (S)-3,4-dihydroxybutyric acid, 3) subsequent esterification with alcohol in the presence of an acid catalyst to afford ester of (S)-3,4-dihydroxybutyric acid, and 4) a step preparing optically pure (S)-3-hydroxy- γ -butyrolactone in high yield through cyclization of the prepared ester compound. Especially, the preparation process of this invention is characterized by preparing (S)-3-
10 hydroxy- γ -butyrolactone in the same reactor without additional purification of the intermediates (oligosaccharide and ester of (S)-3,4-dihydroxybutyric acid).

The enzymatic reaction of the present invention uses α -amylase and pullulanase sequentially. α -Amylase degrades α -(1,4) linkage and pullulanase degrades α -(1,6) linkage selectively.

15 The superiority of the present invention lies in that optically pure (S)-3-hydroxy- γ -butyrolactone is prepared in high yield under a mild condition by using enzymes selectively degrading α -(1,4) linkage or α -(1,6) linkage, while the chemical hydrolysis method has no selectivity.

The enzymatic reaction of the present invention is performed in water
20 or buffer solution of pH 4.0~8.0 at 40~120°C. α -Amylase is used in the range of 0.001~10 wt% of amylopectin, and enzymatic reaction of α -amylase is performed for 30 min ~ 4 hr, and then remaining α -amylase is inactivated. Inactivation is performed under acidic (pH 2.0~4.5) and high temperature (60~150°C) conditions and maintained for 10 min ~ 4 hr. In the enzymatic
25 reaction of pullulanase, pullulanase is used in the range of 0.001~10 wt% of amylopectin, and most of the oligosaccharides distribute within 3~50 glucose units by 10~40 hr of the pullulanase enzymatic treatment. Reducing end units and molecular weight distribution of the prepared oligosaccharide is

analyzed from reducing end units and dextrose equivalent analysis by an optical analyzer, HPLC analysis, and gel permeation chromatography (GPC) analysis.

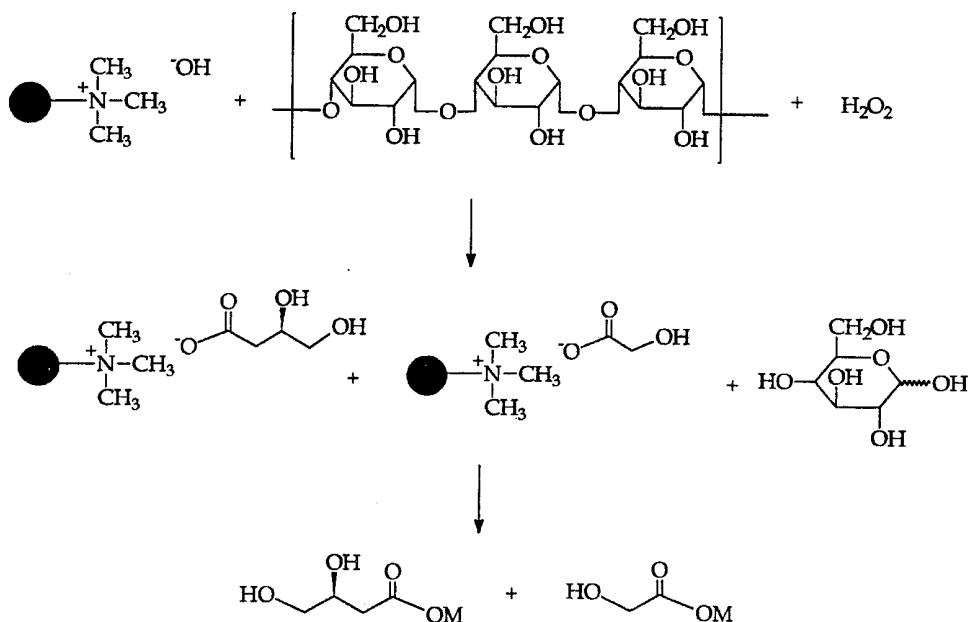
The oligosaccharide is obtained from the selective enzymatic reaction
5 and has distribution mostly between 3~50 glucose units, and preferably 5~50
glucose units. Since most of the glucose units are linked with α -(1,4)
linkage, (S)-3-hydroxy- γ -butyrolactone can be obtained in high yield through
continuous sequential reactions with minimizing the by-products (e.g., acid
mixtures of formic acid, oxalic acid, glycolic acid and erythronic acid).
10 Furthermore, the obtained (S)-3-hydroxy- γ -butyrolactone was identified to be
optically very pure (>99.9%ee).


Oxidation of oligosaccharide is performed by running basic anion
exchange resin with an oxidant to afford (S)-3,4-dihydroxybutyric acid
absorbed on the resin in the column. Aqueous alkali metal or alkali earth
15 metal solution is used for dissociating the absorbed (S)-3,4-dihydroxybutyric
acid.

Oxidation of oligosaccharide explained above is in the following scheme

2.

Scheme 2



wherein,  represents supporter; M represents hydrogen, alkali metal or alkali earth metal atom.

5 In oxidation of oligosaccharide of scheme 2, basic anion exchange resin acts as a base and oligosaccharide is oxidized by an oxidant to produce (S)-3,4-dihydroxybutyric acid and glycolic acid which are absorbed on the anion exchange resin. The last glucose (a leaving group) of oligosaccharide stays in the reaction solution.

10 After oxidation, anion exchange resin is washed with water to remove a leaving group, glucose, and other by-products and then aqueous alkali metal or alkali earth metal solution is eluted to dissociate (S)-3,4-dihydroxybutyric acid with a rate of 2 BV/hr. One of the advantages of this invention is regeneration of the basic anion exchange resin in the dissociation step, which
15 can be re-used for oxidation step semi-permanently.

Oxidation of oligosaccharide is performed at 30~65°C for 6~36 hr. Hydrogen peroxide, alkali metal peroxides, alkaline earth metal peroxides and alkyl hydroperoxides are used for the oxidants, and hydrogen peroxides is the

most preferable. The oxidant is used in the range of 1~3 equivalents per molar glucose unit of amylopectin. The basic anion exchange resin is used for a base and strong basic anion exchange resin with quaternary ammonium hydroxide is preferable. The basic anion exchange resin is used in the
5 range of 2~4 equivalents per molar glucose unit of amylopectin.

The preparation yields of (*S*)-3-hydroxy- γ -butyrolactone depending on the source material are compared as follows [Refer to Experimental example 1]. If maltose (disaccharide) or lactose (disaccharide) obtained from cheese by-product is used as source material, the theoretical mass conversion yield of (*S*)-
10 3-hydroxy- γ -butyrolactone is not more than 28.3 wt% of the source material weight used. On the other hand, if amylose (α -(1,4) linked polysaccharide) with more than 50 glucose units is used, the theoretical mass conversion yield of (*S*)-3-hydroxy- γ -butyrolactone is equal to that of amylopectin. But the
15 double helix structure due to very strong intramolecular hydrogen bond limits the step-by-step oxidation, so the yield becomes very low. However, by using oligosaccharide of the present invention as source material, the yield of (*S*)-3-hydroxy- γ -butyrolactone is very high as 56.6 wt% of the source material weight used.

In order to synthesize (*S*)-3-hydroxy- γ -butyrolactone from the
20 dissociated solution containing (*S*)-3,4-dihydroxybutyric acid, esterification and cyclization are performed sequentially.

Esterification of the present invention is performed in the presence of acid catalyst using alcohol as both solvent and reagent in the range of 30~80°C. Inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid and
25 nitric acid, and organic acids such as fluoroalkylsulfonic acid, aralkylsulfonic acid, hydrate of aralkylsulfonic acid and trifluoroacetic acid are used as acid catalyst. Linear or branched alcohol with 1~5 carbon atoms is used for the alcohol.

Cyclization of the present invention is performed at the temperature range of 30~80°C for 2~5 hr in the presence of acid catalyst to afford the target compound, (S)-3-hydroxy- γ -butyrolactone.

Inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid and nitric acid, and organic acids such as fluoroalkylsulfonic acid, aralkylsulfonic acid, hydrate of aralkylsulfonic acid and trifluoroacetic acid are used as acid catalyst.

According to the conventional method of preparing (S)-3-hydroxy- γ -butyrolactone from (S)-3,4-dihydroxybutyric acid and its derivatives disclosed in USP 5,319,110, various side products including glucose leaving groups and glycolic acid are mixed substantially with the target product. Also, since (S)-3-hydroxy- γ -butyrolactone is highly soluble in water, it is very difficult to separate and purify from the aqueous reaction solution. In WO98/04543, (S)-3,4-dihydroxybutyric acid was cyclized to (S)-3-hydroxy- γ -butyrolactone under the reaction condition and purified by protection of the two hydroxy groups to acetonide ester compound, methyl (S)-3,4-O-isopropylidene-3,4-dihydroxybutanoate, which was recycled to (S)-3-hydroxy- γ -butyrolactone under acidic media. In this method, additional procedures are required after obtaining the target product because methyl (S)-3,4-O-isopropylidene-3,4-dihydroxybutanoate with the dihydroxy group protected should be separated and cyclized by adding acid to obtain (S)-3-hydroxy- γ -butyrolactone.

On the other hand, in the preparing method of the present invention, after oxidation, a leaving group (glucose) and other by-products are easily removed by washing the resin with water, and the sequential dissociation, esterification and cyclization afford optically pure (S)-3-hydroxy- γ -butyrolactone in high yield. Therefore, the present invention provides advantages of simple preparing routes and enables the reaction to proceed continuously and thereby produces (S)-3-hydroxy- γ -butyrolactone economically in large quantities.

As explained above, the present invention is excellent in that the low reactivity of amylopectin to oxidation is overcome by transforming amylopectin to oligosaccharide with the application of specific enzymes. Furthermore, by-product formation is minimized and by performing oxidation with basic anion exchange resin, it can be easily removed by simple process (washed with water). Thus, (S)-3-hydroxy- γ -butyrolactone can be prepared in high yield with very simple purification process.

The following examples are intended to be illustrative of the present invention and should not be construed as limiting the scope of this invention defined by the appended claims.

Example 1: Preparation of methyl (S)-3,4-dihydroxybutanoate

10 L of water and 5 kg of dried amylopectin were put into a 50 L reactor. After heating the reactor to 55°C, 12 g of α -amylase (BAN; EC 3.2.1.1 from *Bacillus licheniformis*, Novo Nordisk) was added. After heating this reaction solution to 75°C, the same was stirred for 2 hours at the same temperature. 5 mL of 0.1N HCl solution was added to adjust the pH of the reaction solution to 3.0~3.5, and then the same was stirred for 1 hr at 90°C to inactivate the remaining α -amylase. After slowly cooling the reaction mixture to 30°C, 3.7 L of 4M acetic acid buffer solution (pH 5) and 1.3 L of water were added to adjust the pH to 5. The reaction solution was heated to 60°C, and then 62.5 g of pullulanase (Promozyme; EC 3.2.1.4 from *Bacillus acidopullulyticus*, Novo Nordisk) was added and the solution was stirred for 22 hr at the same temperature.

Ion exchange resin (Amberlite IRA 402 OH; 0.95 eq/L, Rohm and Hass, 75 L) containing quaternary ammonium hydroxide solution was placed in other 100 L reactor and heated to 50°C. To the reaction solution were added dropwise a solution of oligosaccharide prepared by the above enzyme reaction

and 30% of hydrogen peroxide for 24 hr and the mixture was stirred for 1 hr at the same temperature. The reaction solution was cooled to room temperature and poured into the column. 100 kg of water was eluted to remove a leaving group, glucose, and other side-products, and 110 kg of 3 wt% of NaOH aqueous solution was eluted to obtain (S)-3,4-dihydroxybutyric acid sodium salt. The prepared (S)-3,4-dihydroxybutyric acid sodium salt was identified using NMR analysis.

$^1\text{H-NMR}$ (D_2O , ppm) δ 2.27 (dd, 1H), 2.39 (dd, 1H), 3.41 (dd, 1H), 3.51 (dd, 1H), 3.8~3.9 (m, 1H)

The reaction solution was concentrated, and 10 L of methanol was added. Sulfuric acid was added to adjust the pH to 4~5, and then the same was stirred for 3 hr at 50°C. Sodium carbonate was added to neutralize the solution, and the same was filtered to remove the by-product, and then methanol was concentrated to obtain methyl (S)-3,4-dihydroxybutanoate.

The formation of methyl (S)-3,4-dihydroxybutanoate (conversion ratio: 91%) was identified through NMR analysis by comparison with internal standard.

$^1\text{H-NMR}$ (CDCl_3 , ppm) δ 2.5 (dd, 2H), 3.5 (dd, 1H), 3.6 (dd, 1H), 3.7 (s, 3H), 4.1 (m, 1H)

20 Example 2: Preparation of (S)-3-hydroxy- γ -butyrolactone

10 L of water and 5 kg of dried amylopectin were put into a 50 L reactor. After heating the reactor to 55°C, 12 g of α -amylase (Teramyl; EC 3.2.1.1 from *Bacillus amyloliquefaciens*, Novo Nordisk) was added. After heating this reaction solution to 85°C, the same was stirred for 2 hr at the same temperature. 5 mL of 0.1N HCl solution was added to adjust the pH 3.0~3.5, and then the same was stirred for 1 hr at 90°C to inactivate the remaining α -amylase. After slowly cooling the reaction to 30°C, 3.7 L of 4M acetic acid buffer solution (pH 5) and 1.3 L of water were added to adjust the pH to 5. The reaction

solution was heated to 60°C, and then 62.5 g of pullulanase (Promozyme; EC 3.2.1.4 from *Bacillus acidopullulyticus*, Novo Nordisk) was added and the solution was stirred for 22 hr at the same temperature. A column with 10 cm in diameter and 100 cm in height was packed with basic anion exchange resin (Amberlite IRA 402 OH; 0.95 eq/L, Rohm and Hass, 75 L) and heated to 50°C. Oligosaccharide prepared by the above enzyme reaction and 30 % of hydrogen peroxide solution (5.25 kg) were added dropwise for 24 hr. The reaction mixture in the column was cooled down to room temperature. 100 kg of water was eluted to remove a leaving group, glucose, and other side products, and 110 kg of 3 wt% of NaOH aqueous solution was eluted to dissociate the absorbed (S)-3,4-dihydroxybutyric acid. The prepared (S)-3,4-dihydroxybutyric acid sodium salt was identified using NMR analysis.

¹H-NMR (D₂O, ppm) δ 2.27 (dd, 1H), 2.39 (dd, 1H), 3.41 (dd, 1H), 3.51 (dd, 1H), 3.8~3.9 (m, 1H)

15

The reaction solution was concentrated, and 10 L of methanol was added. In this solution, methanesulfonic acid was added to adjust the pH to 4~5, and then the same was stirred for 3 hr at 50°C. After cooling, sodium carbonate was added to neutralize the solution, and the same was filtered to remove the by-product, and then methanol was concentrated to obtain methyl (S)-3,4-dihydroxybutanoate. Formation of methyl (S)-3,4-dihydroxybutanoate (conversion ratio: 92%) was identified using NMR analysis comparing with the internal standard.

¹H-NMR (CDCl₃, ppm) δ 2.5 (dd, 2H), 3.5 (dd, 1H), 3.6 (dd, 1H), 3.7 (s, 3H), 4.1 (m, 1H)

25

The prepared methyl (S)-3,4-dihydroxybutanoate was cyclized at 65°C under reduced pressure by adding 0.5 wt% of concentrated HCl without any separation. The resultant solution was dissolved in ethyl acetate and the

same was neutralized with sodium carbonate. After filtrating and concentrating the same, (*S*)-3-hydroxy- γ -butyrolactone (2.83 kg, 56.6 wt% of the amylopectin weight used) was obtained.

¹H-NMR (CDCl₃, ppm) δ 2.28 (dd, 1H), 2.74 (dd, 1H), 4.13 (dd, 1H), 4.32 (dd,
5 1H), 4.4~4.5 (m, 1H)

Example 3: Preparation of (*S*)-3-hydroxy- γ -butyrolactone

As in the Example 2, however using 1 wt% of methanesulfonic acid rather than concentrated HCl in the cyclization of the prepared methyl (*S*)-3,4-dihydroxybutanoate, the same was cyclized at 65°C under reduced pressure.
10 The resultant solution was dissolved in ethyl acetate and the same was neutralized with sodium carbonate. After filtrating and concentrating the same, (*S*)-3-hydroxy- γ -butyrolactone (2.80 kg, 56 wt% of the amylopectin weight used) was obtained.

15 ¹H-NMR (CDCl₃, ppm) δ 2.28 (dd, 1H), 2.74 (dd, 1H), 4.13 (dd, 1H), 4.32 (dd, 1H), 4.4~4.5 (m, 1H)

Example 4: Preparation of methyl (*S*)-3,4-dihydroxybutanoate

As in the Example 1, however, using *t*-butylhydroperoxide (4.16 kg)
20 rather than H₂O₂ for the oxidant, methyl (*S*)-3,4-dihydroxybutanoate was obtained. The formation of methyl (*S*)-3,4-dihydroxybutanoate (conversion ratio: 91%) was identified using NMR analysis by comparison with internal standard.

¹H-NMR (CDCl₃, ppm) δ 2.5 (dd, 2H), 3.5 (dd, 1H), 3.6 (dd, 1H), 3.7 (s, 3H), 4.1
25 (m, 1H)

Comparative example 1: Preparation of (*S*)-3-hydroxy- γ -butyrolactone from starch

20 L of water and 5 kg of dried starch were put into a 50 L reactor, and the temperature was raised to 70°C. 40% NaOH (8.64 kg) solution and 30% H₂O₂ (5.25 kg) solution were added dropwise for 48 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The same was esterified and cyclized as in Example 2 to obtain (S)-3-hydroxy- γ -butyrolactone (1.1 kg, 22.0 wt% of starch weight used).

Comparative example 2: Preparation of (S)-3-hydroxy- γ -butyrolactone from starch

10 10 L of 0.5N HCl solution and 5 kg of dried starch were put into a 50 L reactor, and the starch was hydrolyzed for 20 min at 100°C. After cooling the solution to 20°C, the same was neutralized with 100 mL of 40% NaOH solution and the temperature was raised to 70°C. 40% NaOH (8.64 kg) solution and 30% H₂O₂ (5.25 kg) solution were added dropwise for 48 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The same was esterified and cyclized as in Example 2 to obtain (S)-3-hydroxy- γ -butyrolactone (1.22 kg, 24.4 wt% of starch weight used).

Comparative example 3: Preparation of (S)-3-hydroxy- γ -butyrolactone from amylose

20 20 L of water and 5 kg of dried amylose were put into a 50 L reactor, and the temperature was raised to 70°C. 40% NaOH (8.64 kg) solution and 30% H₂O₂ (5.25 kg) solution were added dropwise for 48 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The same was esterified and cyclized as in the Example 2 to obtain (S)-3-hydroxy- γ -butyrolactone (1.35 kg, 27.0 wt% of amylose weight used).

Experimental example 1: Comparison of (S)-3-hydroxy- γ -butyrolactone yield

depending on the source material

For the reaction solutions containing each of the carbohydrates shown in Table 1, oxidation, esterification and cyclization were performed as in the Example 2 to obtain (S)-3-hydroxy- γ -butyrolactone. The yields of (S)-3-
5 hydroxy- γ -butyrolactone are shown in Table 1.

Table 1

| Source material (5kg) | | Product (wt% compared with source material weight) |
|--|-------------|--|
| Oligosaccharide of the present invention (Example 2) | | 2.83 kg (56.6 wt%) |
| Polysaccharide | Amylopectin | 1.01 kg (20.2 wt%) |
| | Amylose | 1.35 kg (27.0 wt%) |
| Disaccharide (maltose) ^{a)} | | 1.19 kg (23.7 wt%) |
| ^{a)} Examples 1 & 2 of USP 5,292,939, 5,319,110 & 5,374,773 | | |

Table 1 shows that for disaccharide the relative mass conversion yield is
10 low as 23.7 wt%. On the other hand, if amylopectin is converted to oligosaccharide by specific enzyme treatment, the relative mass conversion yield is enhanced to 56.6 wt%, almost two times compared with disaccharide. If amylopectin is not treated with enzymes, the relative mass conversion yield is relatively low as 20.2 wt%.

15

Experimental example 2: Optical purity analysis of (S)-3-hydroxy- γ -butyrolactone

(S)-3-Acetoxy- γ -butyrolactone was synthesized by the following method in order to analyze optical purity of (S)-3-hydroxy- γ -butyrolactone prepared

from the present invention and the conventional preparing method.

102 mg (1 mmol) of (*S*)-3-hydroxy- γ -butyrolactone prepared from each method was dissolved in 3 mL of methylene chloride, and 0.4 mL (5 mmol) of pyridine and 0.47 mL (5 mmol) of acetic anhydride were added to the same.

5 After 3 hr, the reaction was quenched with 1N HCl. (*S*)-3-Acetoxy- γ -butyrolactone was extracted with the methylene chloride. After work up, the same was purified with silica gel column chromatography. The obtained (*S*)-3-acetoxy- γ -butyrolactone was dissolved in methylene chloride, and 0.5 μ l was taken with syringe for GC analysis. The result is shown in

10 the following Table 2 and Figs 1a~1c.

Table 2

| Source Material | Optical Purity |
|--|----------------|
| Disaccharide (maltose) ^{a)} | 94%ee |
| Oligosaccharide of the present invention (Example 2) | 99.9%ee |
| ^{a)} Examples 1 & 2 of USP 5,292,939, 5,319,110 & 5,374,773 | |

To improve the medicinal efficiency and minimize the side effect, more than 99.5%ee of high optical purity is required for chiral compounds. Table

15 2 and Figs. 1a~1c show that the optical purity of (*S*)-3-hydroxy- γ -butyrolactone prepared from the present invention is very high as 99.9%ee. So, the same is very useful for the intermediates of other chiral compounds. The results are illustrated in Figure 1a, 1b, and 1c respectively

As described above, the preparing method of the present invention

20 provides the continuous method for production of (*S*)-3-hydroxy- γ -butyrolactone in a large quantities by preparing oligosaccharide with structural specificity which minimizes formation of by-products from amylopectin, and performing oxidation running basic anion exchange resin with an oxidant to make easy removal of by-products without additional separation and

purification.

Additionally, amylopectin as the source material is easily available form natural product with a low price compared with lactose and maltose and provides optically pure form. Use of oligosaccharide prepared from amylopectin provides that productivity is two times more compared with use of disaccharide.

Therefore, the present invention has overcome the disadvantage of using expensive metal catalyst for selective asymmetric reduction, and enables easy preparation from inexpensive natural product having optically pure chiral center, thereby the industrial utility as chiral intermediates of various medicine can be maximized. Furthermore, the relative mass conversion yield is almost double compared with disaccharides.

CLAIMS

What is claimed is :

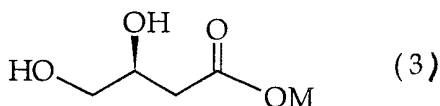
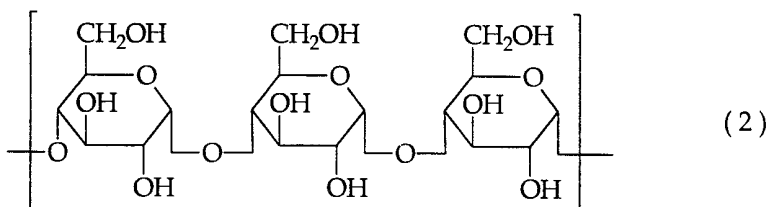
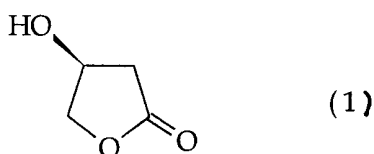
1. A continuous process for producing optically pure (*S*)-3-hydroxy- γ -butyrolactone expressed by the following Formula 1 from polysaccharide source, which comprises the following steps:

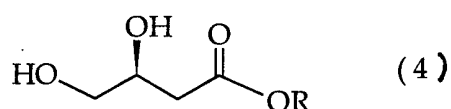
(a) enzymatic reaction of amylopectin to α -(1,4) linked oligosaccharide expressed by the Formula 2;

(b) oxidation of the oligosaccharide by running basic anion exchange resin with an oxidant to afford (*S*)-3,4-dihydroxybutyric acid expressed by the Formula 3;

(c) subsequent esterification with alcohol in the presence of an acid catalyst to afford ester of (*S*)-3,4-dihydroxybutyric acid expressed by the Formula 4; and

(d) cyclization of the prepared ester of (*S*)-3,4-dihydroxybutyric acid in the presence of an acid catalyst without additional separation and purification process to form (*S*)-3-hydroxy- γ -butyrolactone expressed by the Formula 1.





wherein, M represents hydrogen, alkali metal or alkali earth metal
5 atom; R represents linear or branched alkyl group with 1~5 carbon
atoms.

2. The continuous process according to claim 1, wherein the said
oligosaccharide prepared by enzymatic reaction has the 3~50 glucose
10 units.
3. The continuous process according to claim 1, wherein the said
enzymatic reaction is performed in water or in pH 4~8 buffer solution.
4. The continuous process according to claim 1, wherein the said
15 enzymatic reaction is performed by α -amylase enzymatic reaction
followed by pullulanase enzymatic reaction.
5. The continuous process according to claim 4, wherein the said α -
amylase enzymatic reaction is performed under the condition of pH
20 4.0~8.0 and temperature of 40~120°C.
6. The continuous process according to claim 4, wherein the said α -
amylase is used in the range of 0.001~10 wt% of amylopectin .
- 25 7. The continuous process according to claim 4, wherein the said
pullulanase enzymatic reaction is performed under the condition of pH
4.0~8.0 and temperature of 40~120°C.

8. The continuous process according to claim 4, wherein the said pullulanase is used in the range of 0.001~10 wt% of amylopectin .
9. The continuous process according to claim 4, wherein the remaining α -amylase after the α -amylase enzymatic reaction is inactivated under the condition of pH 2.0~4.5 and temperature of 60~150°C, before the pullulanase enzymatic reaction is performed.
10. The continuous process according to claim 1, wherein the said oxidation is performed in the range of 30~65°C.
11. The continuous process according to claim 1, wherein the oxidant used in the said oxidation is selected from hydrogen peroxide, alkali metal peroxide, alkaline earth metal peroxide and alkylhydroperoxide.
12. The continuous process according to claim 11, wherein the said oxidant is hydrogen peroxide.
13. The continuous process according to claim 11, wherein the said oxidant is *t*-butylhydroperoxide.
14. The continuous process according to claim 1 or claim 11, wherein the said oxidant is used in the range of 1~3 equivalents per molar glucose unit of amylopectin.
15. The continuous process according to claim 1, wherein the basic anion exchange resin used in the said oxidation is a strong basic ion exchange containing quaternary ammonium functional group.

16. The continuous process according to claim 1 or claim 15, wherein the said basic anion exchange resin is used in the range of 2~4 equivalents per molar glucose unit of amylopectin.
- 5 17. The continuous process according to claim 1, wherein the said anion exchange resin after oxidation is eluted with 2~50 wt% of aqueous alkali metal hydroxide or alkaline earth metal hydroxide solution to obtain (S)-3,4-dihydroxybutyric acid.
- 10 18. The continuous process according to claim 17, wherein the said aqueous solution is sodium hydroxide solution.
19. The continuous process according to claim 1, wherein the acid catalyst used in the said esterification is an inorganic acid selected from
15 hydrochloric acid, sulfuric acid, phosphoric acid and nitric acid.
20. The continuous process according to claim 1, wherein the acid catalyst used in the said esterification is an organic acid selected from
20 fluoroalkylsulfonic acid, aralkylsulfonic acid, hydrate of aralkylsulfonic acid and trifluoroacetic acid.
21. The continuous process according to claim 1, wherein the said esterification is performed in the range of 30~80°C.
- 25 22. The continuous process according to claim 1, wherein the acid catalyst used in the said cyclization is an inorganic acid selected from hydrochloric acid, sulfuric acid, phosphoric acid and nitric acid.

23. The continuous process according to claim 1, wherein the acid catalyst used in the said cyclization is an organic acid selected from fluoroalkylsulfonic acid, aralkylsulfonic acid, hydrate of aralkylsulfonic acid and trifluoroacetic acid.

5

24. The continuous process according to claim 1, wherein the said cyclization is performed in the range of 30~80°C.

Fig. 1a

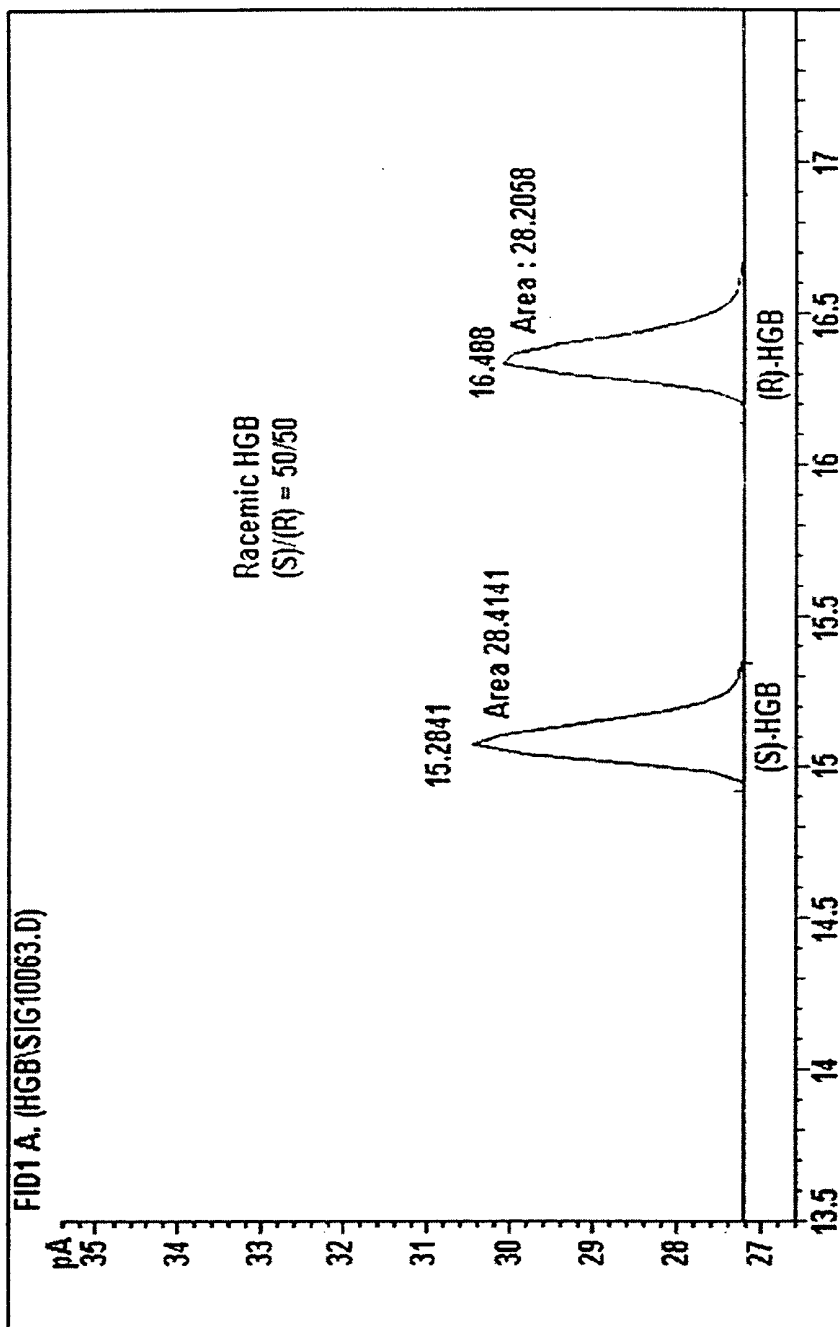


Fig. 1b

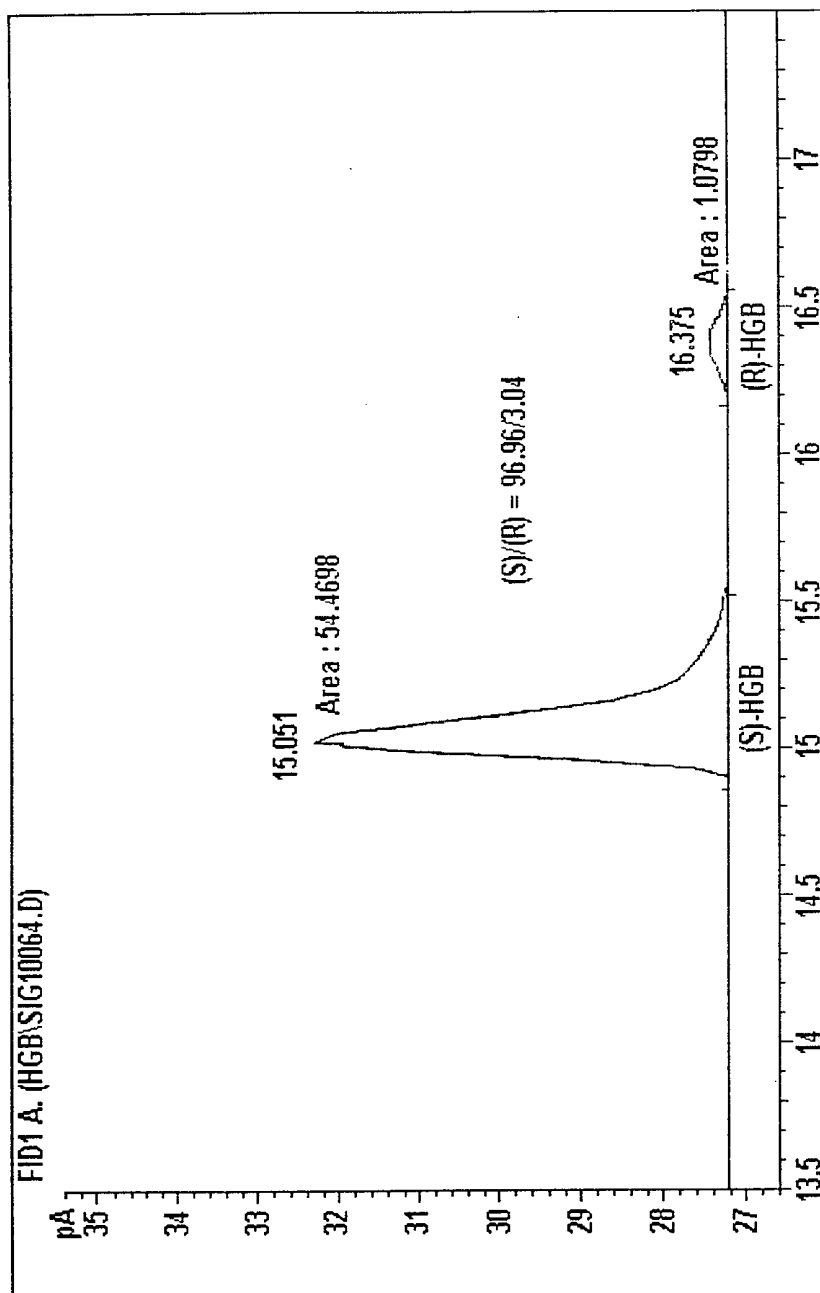
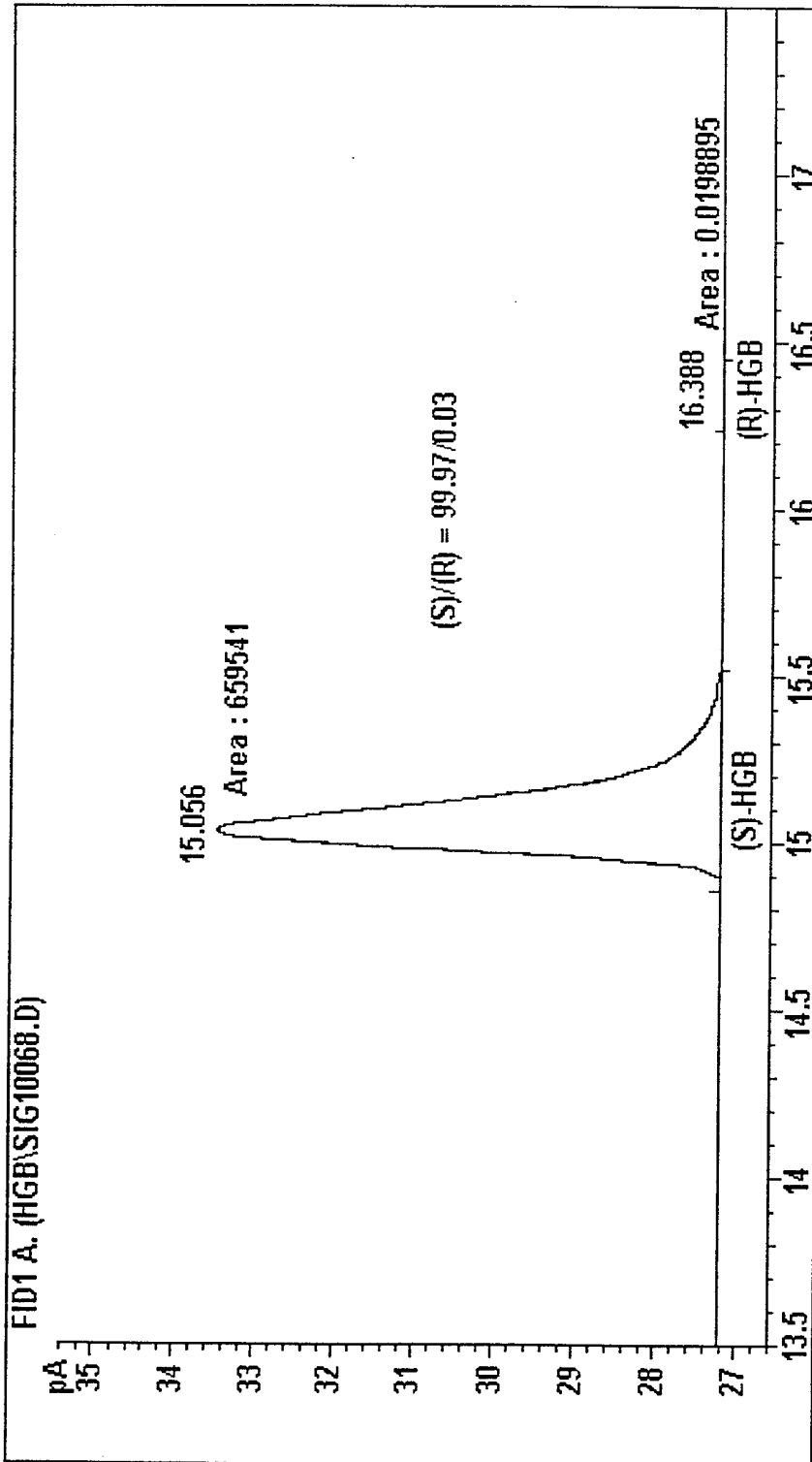


Fig. 1c



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00398

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|--|--|---|--|--|
| A. CLASSIFICATION OF SUBJECT MATTER IPC ⁷ : C 12 P 41/00; C 07 C 51/00 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 ⁷ : C 12 P 41/00; C 07 C 51/00 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) WPI, EPODOC, PAJ | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | |
| A | WO 98/04543 A1 (WARNER -LAMBERT COMPANY) 05 February 1998 (05.02.98) claim 1. | 1 | | |
| A | EP 0513430 A1 (THE BOARD OF TRUSTEES OF THE MICHIGAN STATE UNIVERSITY) 19 November 1992 (19.11.92) abstract. | 1 | | |
| A | US 5808107 A (HOLLINGSWORTH) 15 September 1998 (15.09.98) abstract. | 1 | | |
| A | JP 06-172256 A (KANEGAFUCHI CHEM IND CO LTD) Patent Abstracts of Japan, Vol. 18, No. 511, 27 September 1994 (27.09.94) (abstract). | 1 | | |
| ---- | | | | |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex. | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;"> * Special categories of cited documents: „A“ document defining the general state of the art which is not considered to be of particular relevance „E“ earlier application or patent but published on or after the international filing date „L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) „O“ document referring to an oral disclosure, use, exhibition or other means „P“ document published prior to the international filing date but later than the priority date claimed </td> <td style="width: 50%; border: none; vertical-align: top;"> „T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention „X“ document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone „Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art „&“ document member of the same patent family </td> </tr> </table> | | | * Special categories of cited documents: „A“ document defining the general state of the art which is not considered to be of particular relevance „E“ earlier application or patent but published on or after the international filing date „L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) „O“ document referring to an oral disclosure, use, exhibition or other means „P“ document published prior to the international filing date but later than the priority date claimed | „T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention „X“ document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone „Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art „&“ document member of the same patent family |
| * Special categories of cited documents: „A“ document defining the general state of the art which is not considered to be of particular relevance „E“ earlier application or patent but published on or after the international filing date „L“ document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) „O“ document referring to an oral disclosure, use, exhibition or other means „P“ document published prior to the international filing date but later than the priority date claimed | „T“ later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention „X“ document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone „Y“ document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art „&“ document member of the same patent family | | | |
| Date of the actual completion of the international search | | Date of mailing of the international search report | | |
| 08 November 1999 (08.11.99) | | 06 December 1999 (06.12.99) | | |
| Name and mailing address of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200 | | Authorized officer Wolf Telephone No. 1/53424/436 | | |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR 99/00398

| In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche | | Datum der Veröffentlichung Publication date Date de publication | Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets | Datum der Veröffentlichung Publication date Date de publication |
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| EP A1 | 513430 | 19-11-1992 | AT E 117668 CA AA 2049536 DE CO 69107062 DE J2 69107062 DK T3 513430 EP B1 513430 EP T3 2070384 GB T3 2014919 JP A2 4338359 JP B4 708440B US A 5292939 US A 5319110 US A 5674773 | 15-02-1995 14-11-1993 09-03-1995 18-05-1995 22-05-1995 25-01-1995 01-06-1995 31-05-1995 25-11-1995 13-09-1995 08-03-1994 07-04-1994 20-12-1994 |
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