

Description

BACKGROUND OF THE INVENTION

5 This invention relates to a novel process for preparing (2S, 3R)-3-phenylglycidic acid esters.
 (2S, 3R)-3-phenylglycidic acid esters are important compounds as synthetic intermediates of 1,5-benzothiazepine derivatives (U.S. Patent No. 4,590,188) having coronary blood vessel vasodilating activity or platelet aggregation-inhibiting activity, and other various pharmaceutical compounds. In the prior art, as the method for preparing such a (2S, 3R) type compound, there has been known the method in which a
 10 racemic mixture of methyl trans-3-(4-methoxyphenyl)glycidate is hydrolyzed to form a corresponding carboxylic acid, which carboxylic acid is optically resolved with an optically active amine and then esterified to give methyl (2S, 3R)-3-(4-methoxyphenyl)glycidate (Chemical Abstract, 103, 71179w and 71180q (1985)).

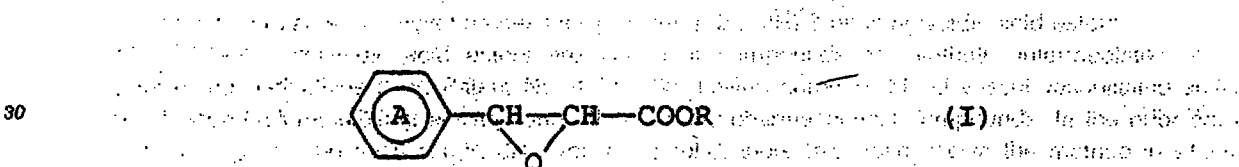
However, the above-mentioned method has many steps, and also there was involved the drawback that the product of the optically active methyl 3-(4-methoxyphenyl)glycidate could be obtained only as an oily
 15 product with low purity.

SUMMARY OF THE INVENTION

The present inventors have studied intensively in order to solve such drawbacks, and consequently
 20 found that microorganisms of the genus *Micrococcus* have an ability of stereoselectively hydrolyzing specifically (2R, 3S)-3-phenylglycidic acid ester compounds, to accomplish the present invention.

More specifically, according to the present invention, there is provided a process for preparing an optically active (2S, 3R)-3-phenylglycidic acid ester compound which comprises

(a) permitting a culture broth, cells or treated cells of a microorganism having the ability of stereoselec-
 25 tively hydrolyzing a (2R, 3S)-3-phenylglycidic acid ester compound to act on a racemic 3-phenylglycidic acid ester compound represented by the formula:



wherein Ring A is a substituted or unsubstituted phenyl group, and R is an ester residue,
 35 thereby stereoselectively hydrolyzing (2R, 3S) optical active isomer, and then
 (b) separating and collecting a (2S, 3R)-3-phenylglycidic acid ester compound from a reaction mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

40 Fig. 1 is an IR absorption spectrum of methyl (2S, 3R)-3-(4-methylphenyl)glycidate, Fig. 2 is an NMR spectrum of the same compound; and Fig. 3 is a mass analysis spectrum of the same compound.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

45 The process of the present invention can be practiced similarly in either the case when Ring A has a substituent selected from a lower alkyl group, a lower alkoxy group and a halogen atom or not. As such a substituent, for example, there is methyl group, methoxy group or chlorine atom at the 4-position. As the ester residue R, lower alkyl groups are generally preferred, as exemplified by methyl group, ethyl group, isopropyl group or t-butyl group.

50 In the present invention, as the racemic 3-phenylglycidic acid ester compound (I) which is the starting material, not only one containing equal amounts of (2S, 3R) isomer and (2R, 3S) isomer, but any one containing both of these optically active isomers can be employed.

As the microorganism which can be used in the present invention, a microorganism having an ability of hydrolyzing stereoselectively a (2R, 3S)-3-phenylglycidic acid ester compound (I) may be employed. For
 55 example, microorganisms such as bacteria, yeasts, molds and actinomycetes having such ability can be suitably employed. More specifically, there may be included bacteria belonging to the genus *Micrococcus*, the genus *Achromobacter*, the genus *Agrobacterium*, the genus *Corynebacterium*, the genus *Enterobacter*, the genus *Flavobacterium*, the genus *Microbacterium*, the genus *Pseudomonas*, the genus

Rhizobium, the genus *Sarcina*, the genus *Bacillus*, the genus *Brevibacterium*, the genus *Citrobacter* and the genus *Xanthomonas*, yeasts belonging to the genus *Candida*, the genus *Debaryomyces*, the genus *Hanseniaspora*, the genus *Hansenula*, the genus *Pichia*, the genus *Rhodospiridium*, the genus *Schizosaccharomyces*, the genus *Sporobolomyces*, the genus *Torula*, the genus *Kloeckera*, the genus *Rhodotorula*, the genus *Saccharomycopsis*, the genus *Torulapsora* and the genus *Trigonopsis*, molds belonging to the genus *Absidia* and the genus *Aspergillus*, and actinomycetes belonging to the genus *Streptomyces*. Specific examples of such microorganisms may include, for example, *Micrococcus ureae* IAM 1010 (FERM BP-2996, deposited under the Budapest Treaty on July 9, 1990 at the Fermentation Research Institute, Agency of Industrial Science and Technology),

Achromobacter butyri OUT 8004, *Achromobacter dendriticum* OUT 8009, *Achromobacter liquidum* OUT 8012, *Agrobacterium radiobacter* IAM 1526, Ditto IFO 13259, *Corynebacterium murisepticum* ATCC 21374, *Enterobacter cloacae* NCTC 9394, *Flavobacterium arborescens* IAM 1100, *Flavobacterium* sp. FERM P-6901, *Microbacterium* sp. ATCC 21376, *Pseudomonas dacunhae* IAM 1199, *Pseudomonas gelidicola* OUT 8116, *Pseudomonas fluorescens* IAM 1219, Ditto IAM 1236, *Pseudomonas taetrolens* IFO 3460, *Pseudomonas ovalis* IAM 1002, Ditto IAM 1177, *Pseudomonas putida* IAM 1512, Ditto FERM P-3505, *Rhizobium meliloti* IFO 13336, *Sarcina subflava* AHU 1485, *Bacillus subtilis* OUT 8234, *Brevibacterium helvolum* IAM 1637, *Brevibacterium imperiale* IAM 1654, *Citrobacter freundii* ATCC 8090, *Xanthomonas campestris* IAM 1671, *Candida boidinii* IFO 10240, *Candida guilliermondii* IFO 0566, *Candida melinii* IFO 0747, *Candida rugosa* ATCC 10571, Ditto IFO 0591, *Candida succiphila* IFO 1911, *Candida tropicalis* IFO 1400, *Candida utilis* IFO 0626, Ditto IFO 1086, *Debaryomyces hansenii* var. *fabryi* IFO 0015, *Debaryomyces nepalensis* IFO 0039, *Hanseniaspora valbyensis* IFO 0115, *Hansenula polymorpha* IFO 1024, *Hansenula saturnus* HUT 7087, *Pichia farinosa* IFO 0607, *Pichia pastoris* IFO 0948, Ditto IFO 1013, Ditto IAM 12267, *Pichia wickernhamii* IFO 1278, *Rhodospiridium toruloides* IFO 0559, *Schizosaccharomyces pombe* IFO 0358, *Sporobolomyces gracilis* IFO 1033, *Torula fermentati* HUT 7524, *Torula utilis* HUT 7526, *Kloeckera corticis* IFO 0633, *Rhodotorula minuta* OUT 6153, *Rhodotorula rubra* OUT 6158, *Torulapsora delbrueckii* IFO 0422, *Saccharomycopsis capsularis* IFO 0672, *Candida saitoana* IFO 0768, *Candida glabrata* IFO 0005, *Trigonopsis variabilis* IFO 0671, *Absidia glauca* var. *paradoxa* IFO 4007, *Aspergillus flavus* IFO 5839, *Aspergillus oryzae* IFO 5710, *Streptomyces griseus* subsp. *griseus* IFO 3430, Ditto IFO 3355 and *Streptomyces lavendulae* subsp. *lavendulae* IFO 3361, Ditto IFO 3145 and Ditto IFO 3146. These may be either wild strains or mutant strains, and further may be those derived from these microorganisms according to the bioengineering methods such as gene recombination and cell fusion.

As the medium for culturing the microorganism, any medium in which the above-mentioned microorganism can be grown. For example, there can be preferably used a medium containing, as carbon sources, 0.4 to 15 % of saccharides such as glucose, sucrose and molasses, organic acids such as fumaric acid or citric acid or alcohols such as glycerol, and as nitrogen sources, 0.3 to 2.0 % of inorganic ammonium salts such as ammonium sulfate and ammonium chloride or urea, or peptone, meat extract, corn-steep liquor, yeast extract and casein hydrolyzate. Further, if necessary, inorganic salts such as phosphate, magnesium salt, potassium salt and calcium salt, and metal ions of manganese and zinc, may be also present in appropriate amounts in the medium. When a synthetic medium is employed, if necessary, it is effective to add, for example, an amino acid such as proline and histidine, or biotin or thiamine. Further, if necessary, 0.1 to 1.0 % of a vegetable oil, a racemic 3-phenylglycidic acid ester compound (I) and a surfactant can be also added as an enzyme deriving substance or a defoaming agent to enhance the enzyme activity. These media is preferably employed with adjustment of pH to 5 to 7.

Cultivation can be performed in conventional manner after inoculation of the microorganism onto the above-mentioned medium, for example, according to any of shaking culture, aeration stirring culture, stationary culture and continuous culture.

The cultural conditions may be chosen suitably depending on the kind of the medium, the cultural method, and not particularly limited, provided that the above-mentioned microorganism can grow to produce an esterase. Generally, it is desired to adjust the pH at initiation of culturing to 5 to 7, and carry out cultivation at room temperature or under heating, for example, at 20 to 40 °C.

As the treated product of such microbial cells, there may be included lyophilized cells, acetone dried cells, self-digested product of cells, extract of cells, ground cells, sonicated cells of the above-mentioned microbial cells and the culture supernatant. Further, the microbial cells or treated cells of the present invention can be also immobilized by known methods such as the polyacrylamide method, the sulfur-containing polysaccharide gel method (e.g. carrageenan gel method), the alginic acid gel method or the agar gel method, before use. Further, an enzyme obtained by purification from the extract of microbial cells by combination of known methods can be also employed.

The stereoselective hydrolysis reaction according to the present invention can be practiced by permitting the culture broth of the microorganism, the cells collected from said culture broth or the treated cells to contact with a racemic 3-phenylglycidic acid ester compound (I).

The substrate concentration may be generally 0.1 to 80 % by weight, particularly preferably 20 to 80 % by weight, and the reaction can proceed at normal temperature or under heating, preferably at 10 to 50 °C, particularly preferably at 20 to 40 °C. During the reaction, it is preferred to adjust the pH of the reaction mixture to 4 to 9, above all 5 to 7. As the reaction mixture, an aqueous solvent such as water or water-methanol mixture can be employed, but from the standpoint of stabilization of the substrate, the reaction can be also practiced in a two-phase solvent system of water or an aqueous solvent and an organic solvent. As such organic solvent, there may be included, for example, toluene, xylene, carbon tetrachloride, dichloromethane, trichloroethylene, chlorobenzene, ethyl acetate, butyl acetate, methyl isobutyl ketone, isooctane, acetone, isopropyl ether, tert-butyl methyl ether, methyl alcohol, ethyl alcohol, n-propyl alcohol, isopropyl alcohol, n-butyl alcohol and t-butyl alcohol, particularly preferably toluene, methyl isobutyl ketone, ethyl alcohol and carbon tetrachloride. Also, by practicing the above reaction in the presence of a surfactant, shortening of the reaction time and increase of yield of the (2S, 3R)-3-phenylglycidic acid ester compound (I) can be effected. As such a surfactant, there can be employed cetylpyridinium bromide, cetyltrimethylammonium bromide, polyethylene glycol and polyoxyethylene octylphenyl ether, and its amount added may be preferably about 0.0001 to 0.1 % by weight based on the reaction mixture.

Isolation of the (2S, 3R)-3-phenylglycidic acid ester compound (I) thus obtained from the hydrolysis reaction mixture can be easily practiced according to the conventional method. For example, when the hydrolysis reaction is carried out in a water-organic solvent two-phase system, the (2R, 3S)-3-phenylglycidic acid ester type compound (I) is hydrolyzed to be migrated into the aqueous layer, while the (2S, 3R) optically active isomer remains in the organic solvent, and therefore by separating the organic solvent layer and concentrating it under reduced pressure, the (2S, 3R)-3-phenylglycidic acid ester compound can be collected as crystals. On the other hand, when the hydrolysis reaction is practiced in an aqueous solvent, the desired product can be obtained by extracting with an organic solvent such as toluene after the reaction, and concentrating the extract under reduced pressure.

The (2S, 3R)-3-phenylglycidic acid ester compound thus obtained can be converted into (-)-cis-2-(4'-methylphenyl)-3-acetyloxy-5-[2-(dimethylamino)ethyl]-8-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one according to the known method as described in U.S. Patents No. 4,567,175 and No. 4,590,188.

As described above, the process of the present invention can obtain a desired (2S, 3R)-3-phenylglycidic acid ester compound in a single step and yet as crystals of high purity from a racemic 3-phenylglycidic acid ester compound, and therefore can be an industrially advantageous preparation process. Further, the microorganism to be used in the present invention can be easily found depending on the kind thereof by effecting screening using a technique known in this field of the art. For example, screening can be easily carried out by cultivating various microorganisms in a medium containing carbon and nitrogen sources, and if necessary, adding various minerals, amino acids and surfactants, at room temperature or under heating at a pH of 5 to 7 as described at page 5 of the present specification, and then effecting a reaction by adding a substrate (racemic 3-phenylglycidic acid ester compound) or if necessary, adding a solvent and surfactant as described above at room temperature or under heating at a pH of 4 to 9, and then analyzing the reaction completed solution by HPLC, etc.

Example 1

An amount 100 ml of a medium (pH 7) containing 0.5 % of glucose, 1 % of peptone, 1 % of meat extract, 1.25 % of yeast extract, 0.5 % of sodium chloride, 0.3 % of a polyalkylene glycol derivative series surfactant (trade name: Karalin, available from Sanyo Kasei Kogyo K.K.) and 0.5 % of a polyoxyethylene derivative series surfactant (trade name: Tween 80®, available from Atlas Powder Co., USA) was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into the medium was inoculated a platinum loop of *Micrococcus ureae* IAM 1010 (FERM BP-2996), and shaking culture was carried out at 30 °C for 24 hours. The above culture broth (250 ml) was concentrated by an ultra-filtration membrane (molecular weight fraction 10,000) to 1/10, to obtain concentrated microbial cells. To the concentrated microbial cells were added 25 ml of a 1 M phosphate buffer (pH 7) and 50 ml of methanol containing 10 g of a racemic methyl 3-(4-methylphenyl)glycidate, and by carrying out the stereoselective hydrolysis reaction at 30 °C for 48 hours, methyl (2R, 3S)-3-(4-methylphenyl)glycidate was completely decomposed. After the reaction, toluene was added for extraction, and the toluene layer was separated and concentrated under reduced pressure to obtain 3.2 g of methyl (2S, 3R)-3-(4-methylphenyl)glycidate as crude crystals. To 3.2 g of the crude crystals was added n-hexane, and the crystals were dissolved by

heating and then recrystallized by cooling to obtain 2.5 g of crystals of methyl (2S, 3R)-3-(4-methylphenyl)glycidate.

M. P.: 50 °C

$[\alpha]_D^{20}$: +209 ° (C=0.2, methanol)

Optical purity: 100 %

IR-absorption spectrum: Fig. 1

NMR spectrum: Fig. 2

Mass analysis spectrum: Fig. 3

Quantitation of the above optically active isomer was performed by high performance liquid chromatography by use of a chiral cell OB Φ 4.6 x 250 mm manufactured by Daicel Kagaku Kogyo K.K.

Example 2

To the microbial cells collected by centrifugation from 250 ml of the culture broth obtained in the same manner as in Example 1 was added 25 ml of a 0.05 M phosphate buffer (pH 7), and the mixture was subjected to sonication treatment and centrifugation. To the supernatant was added ammonium sulfate, and the precipitates obtained were dialyzed against a 0.05 M phosphate buffer to obtain 25 ml of a dialyzed inner solution. To the dialyzed inner solution were added 25 ml of a 0.5 M phosphate buffer (pH 7) and 25 ml of a toluene solution containing 5 g of a racemic methyl 3-(4-methylphenyl)glycidate, and by carrying out the stereoselective hydrolysis reaction at 30 °C for 48 hours, methyl (2R, 3S)-3-(4-methylphenyl)glycidate was completely decomposed. The toluene layer was separated and concentrated under reduced pressure to obtain 1.5 g of methyl (2S, 3R)-3-(4-methylphenyl)glycidate. The physical constants of this product were identical with the product obtained in Example 1.

Example 3

An amount 50 ml of a medium (pH 6.0) containing 1 % of glucose, 0.5 % of peptone, 0.3 % of yeast extract, 0.3 % of malt extract, 1 % of olive oil, 0.1 % of monopotassium phosphate and 1.0 % of calcium carbonate was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into the medium was inoculated a platinum loop of a yeast or a mold shown below in Table 1, and the yeast was subjected to shaking culture at 27 °C for 20 hours, and the mold for 68 hours. To 45 ml of the above culture broth was added 1.5 ml of ethanol containing 60 mg of a racemic methyl 3-(4-methylphenyl)glycidate, and further cetyltrimethylammonium bromide in an amount to the final concentration in the reaction mixture of 0.01 % and calcium chloride in an amount to the final concentration of 1 mM were added, and the stereoselective hydrolysis reaction was carried out at 30 °C for 20 to 68 hours. After the reaction, the substrate and the (2S, 3R) type optically active isomer which is the reaction product were extracted with 15 ml of ethyl acetate. The content of the (2S, 3R) isomer in the reaction mixture is as shown below in Table 1, and substantially no (2R, 3S) isomer which is the antipode thereof was detected in the reaction mixture.

Quantitation of the above optically active isomer was performed by high performance liquid chromatography by use of a chiral cell OJ Φ 4.6 x 250 mm manufactured by Daicel Kagaku Kogyo K.K. (hereinafter the same).

Table 1

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Candida boidinii</i> IFO 10240	1.0
<i>Candida guilliermondii</i> IFO 0566	1.1
<i>Candida melinii</i> IFO 0747	0.7
<i>Candida tropicalis</i> IFO 1400	1.1
<i>Candida utilis</i> IFO 0626	0.8
<i>Candida rugosa</i> ATCC 10571	0.9
<i>Candida rugosa</i> IFO 0591	0.9
<i>Candida succiphila</i> IFO 1911	0.9
<i>Debaryomyces hansenii</i> var. <i>fabryi</i> IFO 0015	1.1
<i>Debaryomyces nepalensis</i> IFO 0039	1.6
<i>Hanseniaspora valbyensis</i> IFO 0115	1.7
<i>Hansenula polymorpha</i> IFO 1024	1.0
<i>Pichia farinosa</i> IFO 0607	1.0
<i>Pichia pastoris</i> IFO 0948	1.1
<i>Pichia pastoris</i> IFO 1013	0.8
<i>Pichia pastoris</i> IAM 12267	1.1
<i>Pichia wickerhamii</i> IFO 1278	1.5
<i>Rhodospiridium toruloides</i> IFO 0559	0.9
<i>Schizosaccharomyces</i> <i>pombe</i> IFO 0358	0.8
<i>Sporobolomyces gracilis</i> IFO 4033	1.6
<i>Torula fermentati</i> HUT 7524	0.9

Table 1 (Contd)

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Torula utilis</i> HUT 7526	1.1
<i>Absidia glauca</i> var. <i>paradoxa</i> IFO 4007	0.9

Example 4

An amount 50 ml of a medium (pH 7.0) containing 0.4 % of glucose, 0.4 % of yeast extract, 1 % of malt extract, 1 % of olive oil, 0.1 % of monopotassium phosphate and 1.0 % of calcium carbonate was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. To the medium was inoculated a platinum loop of an actinomycete shown below in Table 2, and the actinomycete was subjected to shaking culture at 30 °C for 68 hours. To 45 ml of the above culture broth was added 1.5 ml of ethanol containing 60 mg of racemic methyl 3-(4-methylphenyl)glycidate, and further cetyltrimethylammonium in an amount to the final concentration of 0.01 % in the reaction mixture and calcium chloride in an amount to the final concentration of 1 mM were added to carry out the stereoselective hydrolysis reaction at 30 °C for 20 to 68 hours. After the reaction, the substrate and the (2S, 3R) optically active isomer were extracted with 15 ml of ethyl acetate. The content of the (2S, 3R) isomer in the reaction mixture is as shown in Table 2, and substantially no (2R, 3S) isomer which is the antipode thereof was detected in the reaction mixture.

Table 2

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Streptomyces griseus</i> subsp. <i>griseus</i> IFO 3355	1.8
<i>Streptomyces griseus</i> subsp. <i>griseus</i> IFO 3430	1.6
<i>Streptomyces lavendulae</i> subsp. <i>lavendulae</i> IFO 3145	1.4
<i>Streptomyces lavendulae</i> subsp. <i>lavendulae</i> IFO 3146	1.4
<i>Streptomyces lavendulae</i> subsp. <i>lavendulae</i> IFO 3361	1.3

Example 5

An amount 50 ml of a medium A (pH 7.0) containing 1 % of a soluble starch, 0.5 % of peptone, 0.5 % of yeast extract, 1 % of olive oil and 0.1 % of monopotassium phosphate was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into the medium A or into 50 ml of a medium B in which 1.5 ml of ethanol containing 60 mg of a racemic methyl 3-(4-methylphenyl)glycidate added to the medium A was inoculated a platinum loop of a bacterium shown below in Table 3, and subjected to shaking culture at 30 °C for 20 hours. Into 45 ml of the above culture broth A was added 1.5 ml of ethanol containing 60 mg of a racemic methyl 3-(4-methylphenyl)glycidate. To the media A and B were added cetyltrimethylammonium bromide in an amount to the final concentration of 0.01 % in the reaction mixture and calcium chloride in an amount to the final concentration of 1 mM, and the stereoselective hydrolysis reaction was carried out at 30 °C for 20 to 68 hours. After the reaction, the substrate and the optically active (2S, 3R) isomer which is the reaction product were extracted with 15 ml of ethyl acetate. The content of the (2S, 3R) isomer in the reaction mixture is as shown below in Table 3, and substantially no (2R, 3S) isomer which is the antipode thereof was detected.

Table 3

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Achromobacter butyl</i> OUT 8004	1.3
<i>Achromobacter dendriticum</i> OUT 8009	1.0
<i>Agrobacterium radiobacter</i> IAM 1526	1.2
<i>Agrobacterium radiobacter</i> IFO 13259	0.8
<i>Corynebacterium murisepticum</i> ATCC 21374	1.3 (Medium B)
<i>Enterobacter cloacae</i> NCTC 9394	1.5
<i>Flavobacterium arborescens</i> IAM 1100	1.0 (Medium B)
<i>Microbacterium</i> sp. ATCC 21376	1.0
<i>Micrococcus ureae</i> IAM 1010 (FERM BP-2996)	1.0
<i>Pseudomonas gelidicola</i> OUT 8116	1.1
<i>Rhizobium melloti</i> IFO 13336	0.8
<i>Sarcina subflava</i> AHU 1485	0.9

Example 6

An amount 50 ml of medium (pH 7.0) containing 0.5 % of glucose, 1 % of peptone, 1 % of meat extract, 1.25 % of yeast extract, 0.1 % of monopotassium phosphate, 0.5 % of sodium chloride and 1 % of olive oil was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into the medium, one platinum loop of *Micrococcus ureae* IAM 1010 (FERM BP-2996) was inoculated, and cultivation was performed under shaking at 30 °C for 20 hours. 3.75 ml of McIlvaine buffer (pH 5.0) and 7.5 ml of organic solvent shown in Table 4 containing 30 mg of racemic methyl 3-(4-methylphenyl) glycidate were added into 3.75 ml of the above microbial cells solution concentrated ten-fold by treating with an ultrafiltration membrane (cut-off molecular weight : 10,000). Then, the stereoselective hydrolysis reaction was carried out at 30 °C for 20 hours in emulsion state. After the reaction, the organic solvent layer was separated to obtain a reaction mixture containing the (2S, 3R) isomer. The content of the (2S, 3R) isomer in the reaction mixture was shown in Table 4, and substantially no (2R, 3S) isomer which is its antipode was detected in the reaction mixture.

Table 4

Solvent	(2S, 3R) isomer content (mg/ml)
Carbon tetrachloride	1.8
Toluene	1.8
n-Butyl acetate	1.1
p-Xylene	1.1
Methyl isobutyl ketone	1.6
Isooctane	0.7
Acetone	0.8
Ethyl alcohol	1.5
Tert-Butyl methyl ether	0.2

Example 7

An amount 50 ml of a medium (pH 7.0) containing 0.5 % of glucose, 1 % of peptone, 1 % of meat extract, 1.25 % of yeast extract, 0.5 % of sodium chloride, 0.03 % of a polyalkylene glycol derivative series surfactant (trade name: Karalin, available from Sanyo Kasei Kogyo K.K.), 0.1 % of a polyoxyethylene derivative series surfactant (trade name: Tween® 80, available from Atlas Powder Co., USA) was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into the medium was inoculated a bacterium shown below in Table 5, and subjected to shaking culture at 30 °C for 24 hours.

To 45 ml of the above culture broth was added 4.5 ml of methanol containing 450 mg of a racemic methyl 3-(4-methylphenyl)glycidate, and further calcium chloride in an amount to the concentration of 1 mM in the reaction mixture and cetyltrimethylammonium bromide in an amount to 0.01 % were added to carry out stereoselective hydrolysis at 30 °C for 72 hours. After the reaction, the mixture was extracted with addition of 45 ml of toluene, and the content of the content of methyl (2S, 3R)-3-(4-methylphenyl)glycidate was measured. As the result, the content of the (2S, 3R) isomer in the reaction mixture was as shown below in Table 5, and substantially no (2R, 3S) isomer which is the antipode thereof was detected in the reaction mixture.

Table 5

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Achromobacter dentriticum</i> OUT 8009	0.8
<i>Achromobacter liquidum</i> OUT 8012	0.8
<i>Agrobacterium, radiobacter</i> IAM 1526	3.0
<i>Bacillus subtilis</i> OUT 8234	0.8
<i>Brevibacterium helvolum</i> IAM 1637	2.4
<i>Brevibacterium imperiale</i> IAM 1654	1.6
<i>Citrobacter freundii</i> ATCC 8090	2.4
<i>Flavobacterium</i> sp. FERP-P No.6901	1.1
<i>Micrococcus ureae</i> IAM 1010	3.4
<i>Pseudomonas dacunhae</i> IAM 1199	2.0
<i>Pseudomonas fluorescent</i> IAM 1219	2.2
<i>Pseudomonas fluorescent</i> IAM 1236	2.4
<i>Pseudomonas taetrolens</i> IFO 3460	2.5
<i>Pseudomonas ovalis</i> IAM 1002	1.5
<i>Pseudomonas ovalis</i> IAM 1177	1.9
<i>Pseudomonas putida</i> IAM 1512	2.6
<i>Pseudomonas putida</i> FERP-P No. 3505	2.2
<i>Xanthomonas campestris</i> IAM 1671	3.2

Example 8

An amount 50 ml of a medium (pH 6.2) containing 1 % of glucose, 0.5 % of peptone, 0.3 % of yeast extract, 0.3 % of malt extract, 0.03 % of a polyalkylene glycol derivative series surfactant (trade name: Karalin, available from Sanyo Kasei Kogyo K.K.), 0.1 % of a polyoxyethylene derivative series surfactant (trade name: Tween 80, available from Atlas Powder Co., USA) was charged into a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into the medium was inoculated a mold or yeast shown below

in Table 6, and subjected to shaking culture at 27 °C for 72 hours for the mold, and for 48 hours for the yeast. By use of 45 ml of the above culture broth, the reaction was carried out in the same manner as in Example 7 and the content of methyl (2S, 3R)-3-(4-methylphenyl)glycidate was measured. As the result, the content of the (2S, 3R) isomer in the reaction was as shown below in Table 6, and substantially no (2R, 3S) isomer which is the antipode thereof was detected in the reaction mixture.

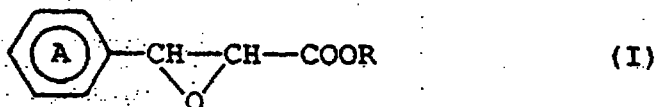
Table 6

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Absidia glauca</i> var. <i>paradoxa</i> IFO 4007	1.0
<i>Aspergillus flavus</i> IFO 5839	1.6
<i>Aspergillus oryzae</i> IFO 5710	2.3
<i>Candida guilliermondii</i> IFO 0566	1.4
<i>Candida utilis</i> IFO 0626	1.0
<i>Candida utilis</i> IFO 1086	0.8
<i>Hansenula saturnus</i> HUT 7087	0.8
<i>Kloeckera corticis</i> IFO 0633	1.0
<i>Rhodotorula minuta</i> OUT 6153	1.6
<i>Rhodotorula rubra</i> OUT 6158	2.4
<i>Torulaspora delbrueckii</i> IFO 0422	1.5
<i>Saccharomycopsis capsularis</i> IFO 0672	1.4
<i>Torula utilis</i> HUT 7526	1.9
<i>Candida saitoana</i> IFO 0768	1.0
<i>Candida glabrata</i> IFO 0005	1.6
<i>Torigonopsis variabilis</i> IFO 0671	0.9

Claims

1. A process for preparing an optically active (2S, 3R)-3-phenylglycidic acid ester compound which comprises

(a) permitting a culture broth, cells or treated cells of a microorganism having the ability of stereoselectively hydrolyzing a (2R, 3S)-3-phenylglycidic acid ester compound to act on a racemic 3-phenylglycidic acid ester compound represented by the formula:



wherein Ring A is a substituted or unsubstituted phenyl group, and R is an ester residue, thereby stereoselectively hydrolyzing (2R, 3S) optical active isomer; and then

(b) separating and collecting a (2S, 3R)-3-phenylglycidic acid ester compound from a reaction mixture.

2. The process according to Claim 1, wherein the microorganism is a microorganism belonging to the genus *Micrococcus*, the genus *Achromobacter*, the genus *Agrobacterium*, the genus *Corynebacterium*

in Table 6, and subjected to shaking culture at 27 °C for 72 hours for the mold, and for 48 hours for the yeast. By use of 45 ml of the above culture broth, the reaction was carried out in the same manner as in Example 7 and the content of methyl (2S, 3R)-3-(4-methylphenyl)glycidate was measured. As the result, the content of the (2S, 3R) isomer in the reaction was as shown below in Table 6, and substantially no (2R, 3S) isomer which is the antipode thereof was detected in the reaction mixture.

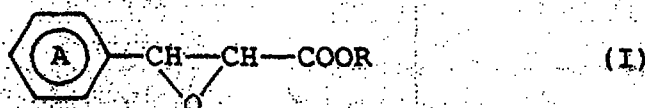
Table 6

Microorganism employed	(2S, 3R) isomer content (mg/ml)
<i>Absidia glauca</i> var. <i>paradoxa</i> IFO 4007	1.0
<i>Aspergillus flavus</i> IFO 5839	1.6
<i>Aspergillus oryzae</i> IFO 5710	2.3
<i>Candida guilliermondii</i> IFO 0566	1.4
<i>Candida utilis</i> IFO 0626	1.0
<i>Candida utilis</i> IFO 1086	0.8
<i>Hansenula saturnus</i> HUT 7087	0.8
<i>Kloeckera corticis</i> IFO 0633	1.0
<i>Rhodotorula minuta</i> OUT 6153	1.6
<i>Rhodotorula rubra</i> OUT 6158	2.4
<i>Torulasporea delbrueckii</i> IFO 0422	1.5
<i>Saccharomycopsis capsularis</i> IFO 0672	1.4
<i>Torula utilis</i> HUT 7526	1.9
<i>Candida saltoana</i> IFO 0768	1.0
<i>Candida glabrata</i> IFO 0005	1.6
<i>Torigonopsis variabilis</i> IFO 0671	0.9

Claims

1. A process for preparing an optically active (2S, 3R)-3-phenylglycidic acid ester compound which comprises

(a) permitting a culture broth, cells or treated cells of a microorganism having the ability of stereoselectively hydrolyzing a (2R, 3S)-3-phenylglycidic acid ester compound to act on a racemic 3-phenylglycidic acid ester compound represented by the formula:



wherein Ring A is a substituted or unsubstituted phenyl group, and R is an ester residue, thereby stereoselectively hydrolyzing (2R, 3S) optical active isomer, and then

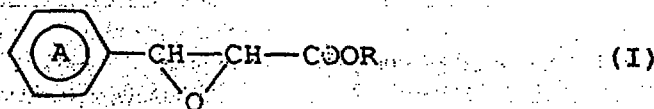
(b) separating and collecting a (2S, 3R)-3-phenylglycidic acid ester compound from a reaction mixture.

2. The process according to Claim 1, wherein the microorganism is a microorganism belonging to the genus *Micrococcus*, the genus *Achromobacter*, the genus *Agrobacterium*, the genus *Corynebacterium*



terium, the genus *Enterobacter*, the genus *Flavobacterium*, the genus *Microbacterium*, the genus *Pseudomonas*, the genus *Rhizobium*, the genus *Sarcina*, the genus *Bacillus*, the genus *Brevibacterium*, the genus *Citrobacter*, the genus *Xanthomonas*, the genus *Candida*, the genus *Debaryomyces*, the genus *Hanseniaspora*, the genus *Hansenula*, the genus *Pichia*, the genus *Rhodospiridium*, the genus *Schizosaccharomyces*, the genus *Sporobolomyces*, the genus *Torula*, the genus *Kloeckera*, the genus *Rhodotorula*, the genus *Saccharomycopsis*, the genus *Torulapsora*, the genus *Trigonopsis*, the genus *Absidia*, the genus *Aspergillus* or the genus *Streptomyces*.

3. The process according to Claim 1, wherein the configuration of a compound to be separated and collected is (2S, 3R).
4. The process according to Claim 1, wherein the compound to be separated and collected is a lower alkyl (2S, 3R)-3-(4-methylphenyl)glycidate.
5. The process according to Claim 1, wherein the compound to be separated and collected is methyl (2S, 3R)-3-(4-methylphenyl)glycidate.
6. The process according to Claim 1, wherein the stereoselective hydrolysis reaction is practiced by contacting the microorganism with a racemic 3-phenylglycidic acid ester compound in a solvent.
7. The process according to Claim 6, wherein the reaction is practiced with a substrate concentration of 0.1 to 80 % by weight at a temperature of 10 to 50 °C and a pH of 4 to 9.
8. Methyl (2S, 3R)-3-(4-methylphenyl)glycidate.
9. A process for preparing (-)-cis-2-(4'-methylphenyl)-3-acetyloxy-5-(2-(dimethylamino)ethyl)-8-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one, which comprises
 - (a) permitting a culture broth, cells or treated cells of a microorganism having an ability of stereoselectively hydrolyzing a (2R, 3S)-type 3-phenylglycidic acid ester compound to act on a racemic 3-phenylglycidic acid ester compound represented by the formula:



wherein Ring A is a substituted or unsubstituted phenyl group, and R is an ester residue, thereby hydrolyzing the (2R, 3S) optically active isomer and

- (b) separating and collecting the (2S, 3R) antipode from the reaction mixture, and then
- (c) converting said compound to (-)-cis-2-(4'-methylphenyl)-3-acetyloxy-5-(2-(dimethylamino)ethyl)-8-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-one according to a known method.
10. The process according to Claim 9, wherein the microorganism is a microorganism belonging to the genus *Micrococcus*, the genus *Achromobacter*, the genus *Agrobacterium*, the genus *Corynebacterium*, the genus *Enterobacter*, the genus *Flavobacterium*, the genus *Microbacterium*, the genus *Pseudomonas*, the genus *Rhizobium*, the genus *Sarcina*, the genus *Bacillus*, the genus *Brevibacterium*, the genus *Citrobacter*, the genus *Xanthomonas*, the genus *Candida*, the genus *Debaryomyces*, the genus *Hanseniaspora*, the genus *Hansenula*, the genus *Pichia*, the genus *Rhodospiridium*, the genus *Schizosaccharomyces*, the genus *Sporobolomyces*, the genus *Torula*, the genus *Kloeckera*, the genus *Rhodotorula*, the genus *Saccharomycopsis*, the genus *Torulapsora*, the genus *Trigonopsis*, the genus *Absidia*, the genus *Aspergillus* or the genus *Streptomyces*.
11. Lower alkyl (2S,3R)-3-(4-methylphenyl)glycidate.
12. Methyl (2S,3R)-3-(4-methylphenyl)glycidate of high purity.
13. Lower alkyl (2S,3R)-3-(4-methylphenyl)glycidate of high purity.



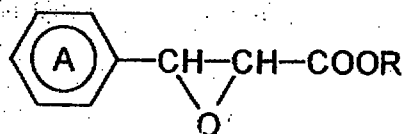
14. Methyl (2S,3R)-3-(4-methylphenyl)glycidate in crystal form.

15. Lower alkyl (2S,3R)-3-(4-methylphenyl)glycidate in crystal form.

5 Patentansprüche

1. Verfahren zur Herstellung einer optisch aktiven (2S,3R)-3-Phenylglycidssäureester-Verbindung, umfassend:

(a) Einwirkenlassen von einer Kulturbrühe, Zellen oder behandelten Zellen eines Mikroorganismus, der die Fähigkeit zur stereoselektiven Hydrolyse von (2R,3S)-3-Phenylglycidssäureester-Verbindungen besitzt, auf ein Racemat einer 3-Phenylglycidssäureester-Verbindung der Formel



(I)

worin der Ring A eine substituierte oder unsubstituierte Phenylgruppe und R ein Esterrest ist, wodurch das (2R,3S)-optisch aktive Isomer stereoselektiv hydrolysiert wird, und dann

(b) Abtrennen und Auffangen der (2S,3R)-3-Phenylglycidssäureester-Verbindung aus der Reaktionsmischung.

2. Verfahren gemäss Anspruch 1, dadurch gekennzeichnet, dass der Mikroorganismus ein Mikroorganismus ist, gehörend zur Gattung Micrococcus, zur Gattung Achromobacter, zur Gattung Agrobacterium, zur Gattung Corynebacterium, zur Gattung Enterobacter, zur Gattung Flavobacterium, zur Gattung Microbacterium, zur Gattung Pseudomonas, zur Gattung Rhizobium, zur Gattung Sarcina, zur Gattung Bacillus, zur Gattung Brevibacterium, zur Gattung Citrobacter, zur Gattung Xanthomonas, zur Gattung Candida, zur Gattung Debaryomyces, zur Gattung Hanseniaspora, zur Gattung Hansenula, zur Gattung Pichia, zur Gattung Rhodosporidium, zur Gattung Schizosaccharomyces, zur Gattung Sporobolomyces, zur Gattung Torula, zur Gattung Kloeckera, zur Gattung Rhodotorula, zur Gattung Saccharomycopsis, zur Gattung Torulapsora und zur Gattung Trigonopsis, zur Gattung Absidia, zur Gattung Aspergillus oder zur Gattung Streptomyces.

3. Verfahren gemäss Anspruch 1, dadurch gekennzeichnet, dass die Konfiguration der abzutrennenden und aufzusammelnden Verbindung (2S,3R) ist.

4. Verfahren gemäss Anspruch 1, dadurch gekennzeichnet, dass die abzutrennende und aufzusammelnde Verbindung ein Niederalkyl-(2S,3R)-3-(4-methylphenyl)glycidat ist.

5. Verfahren gemäss Anspruch 1, dadurch gekennzeichnet, dass die abzutrennende und aufzusammelnde Verbindung Methyl-(2S,3R)-3-(4-methylphenyl)glycidat ist.

6. Verfahren gemäss Anspruch 1, dadurch gekennzeichnet, dass die stereoselektive Hydrolysereaktion durchgeführt wird durch Kontaktierung des Mikroorganismus mit einer racemischen 3-Phenylglycidssäureester-Verbindung in einem Lösungsmittel.

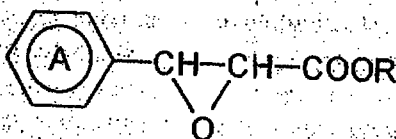
7. Verfahren gemäss Anspruch 6, dadurch gekennzeichnet, dass die Reaktion durchgeführt wird bei einer Substratkonzentration von 0,1 bis 80 Gew.% bei einer Temperatur von 10 bis 50 °C und einem pH von 4 bis 9.

8. Methyl-(2S,3R)-3-(4-methylphenyl)glycidat.

9. Verfahren zur Herstellung von (-)-cis-2-(4-Methylphenyl)-3-acetyloxy-5-[2-(dimethylamino)ethyl]-8-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-on, umfassend



(a) Einwirkenlassen von einer Kulturbrühe, Zellen oder behandelten Zellen eines Mikroorganismus, der die Fähigkeit zur stereoselektiven Hydrolyse von (2R,3S)-3-Phenylglycidssäureester-Verbindungen besitzt, auf ein Racemat einer 3-Phenylglycidssäureester-Verbindung der Formel



(I)

worin der Ring A eine substituierte oder unsubstituierte Phenylgruppe und R ein Esterrest ist, wodurch das (2R,3S)-optisch aktive Isomer stereoselektiv hydrolysiert wird, und

(b) Abtrennen und Auffangen der (2S,3R)-Antipode aus der Reaktionsmischung, und dann
(c) Konvertierung besagter Verbindung zu (-)-cis-2-(4-Methylphenyl)-3-acetoxy-5-[2-(dimethylamino)ethyl]-8-methyl-2,3-dihydro-1,5-benzothiazepin-4(5H)-on nach einem bekannten Verfahren.

10. Verfahren gemäss Anspruch 9, dadurch gekennzeichnet, dass der Mikroorganismus ein Mikroorganismus ist, gehörend zur Gattung Micrococcus, zur Gattung Achromobacter, zur Gattung Agrobacterium, zur Gattung Corynebacterium, zur Gattung Enterobacter, zur Gattung Flavobacterium, zur Gattung Microbacterium, zur Gattung Pseudomonas, zur Gattung Rhizobium, zur Gattung Sarcina, zur Gattung Bacillus, zur Gattung Brevibacterium, zur Gattung Citrobacter, zur Gattung Xanthomonas, zur Gattung Candida, zur Gattung Debaryomyces, zur Gattung Hanseniaspora, zur Gattung Hansenula, zur Gattung Pichia, zur Gattung Rhodosporidium, zur Gattung Schizosaccharomyces, zur Gattung Sporobolomyces, zur Gattung Torula, zur Gattung Kloeckera, zur Gattung Rhodotorula, zur Gattung Saccharomycopsis, zur Gattung Torulapsora und zur Gattung Trigonopsis, zur Gattung Absidia, zur Gattung Aspergillus oder zur Gattung Streptomyces.

11. Niederalkyl-(2S,3R)-3-(4-methylphenyl)glycidat.

12. Methyl-(2S,3R)-3-(4-methylphenyl)glycidat hoher Reinheit.

13. Niederalkyl-(2S,3R)-3-(4-methylphenyl)-glycidat hoher Reinheit.

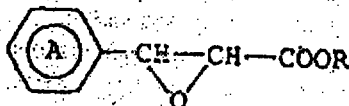
14. Methyl-(2S,3R)-3-(4-methylphenyl)glycidat in kristalliner Form.

15. Niederalkyl-(2S,3R)-3-(4-methylphenyl)glycidat in kristalliner Form.

Revendications

1. Procédé pour la préparation d'un ester d'acide (2S, 3R)-3-phénylglycidique optiquement actif, qui comprend :

(a) la réaction d'un bouillon de culture, de cellules ou de cellules traitées d'un microorganisme ayant la capacité d'hydrolyser de façon stéréosélective un ester d'acide (2R, 3S)-3-phénylglycidique, sur un ester d'acide 3-phénylglycidique racémique représenté par la formule :



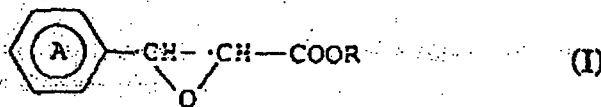
(I)

dans laquelle le cycle A est un groupe phényl substitué ou non substitué, et R est un résidu ester, de façon à hydrolyser de façon stéréosélective l'isomère (2R, 3S) optiquement actif, et ensuite,

(b) la séparation et la récupération d'un ester d'acide (2S, 3R)-3-phénylglycidique à partir du mélange réactionnel.



2. Procédé suivant la revendication 1, dans lequel le microorganisme est un microorganisme appartenant au genre *Micrococcus*, au genre *Achromobacter*, au genre *Agrobacterium*, au genre *Corynebacterium*, au genre *Enterobacter*, au genre *Flavobacterium*, au genre *Microbacterium*, au genre *Pseudomonas*, au genre *Rhizobium*, au genre *Sarcina*, au genre *Bacillus*, au genre *Brevibacterium*, au genre *Citrobacter*, au genre *Xanthomonas*, au genre *Candida*, au genre *Debaryomyces*, au genre *Hanseniaspora*, au genre *Hansenula*, au genre *Pichia*, au genre *Rhodospiridium*, au genre *Schizosaccharomyces*, au genre *Sporobolomyces*, au genre *Torula*, au genre *Kloeckera*, au genre *Rhodotorula*, au genre *Saccharomycopsis*, au genre *Torulapsora*, au genre *Trigonopsis*, au genre *Absidia*, au genre *Aspergillus* ou au genre *Streptomyces*.
3. Procédé suivant la revendication 1, dans lequel la configuration d'un composé à séparer et à récupérer (2S, 3R).
4. Procédé suivant la revendication 1, dans lequel le composé à séparer et à récupérer est un (2S, 3R)-3-(4-méthylphényl)-glycidate d'alkyle inférieur.
5. Procédé suivant la revendication 1, dans lequel le composé à séparer et à récupérer est le (2S, 3R)-3-(4-méthylphényl)-glycidate de méthyle.
6. Procédé suivant la revendication 1, dans lequel la réaction d'hydrolyse stéréosélective est réalisée par mise en contact du microorganisme avec un ester d'acide 3-phénylglycidique racémique dans un solvant.
7. Procédé suivant la revendication 6, dans lequel la réaction est réalisée avec une concentration du substrat comprise entre 0,1 et 80% en poids, à une température de 10 à 50°C et à un pH de 4 à 9.
8. (2S, 3R)-3-(4-Méthylphényl)-glycidate de méthyle.
9. Procédé pour la préparation de la (-)-cis-2-(4'-méthylphényl)-3-acétyloxy-5-(2-(diméthylamino)-éthyl)-8-méthyl-2,3-dihydro-1,5-benzothiazépin-4(5H)-one, qui comprend :
 - (a) la réaction d'un bouillon de culture, de cellules ou de cellules traitées d'un microorganisme ayant la capacité d'hydrolyser de façon stéréosélective un ester d'acide (2R, 3S)-3-phénylglycidique, sur un ester d'acide 3-phénylglycidique racémique représenté par la formule :



dans laquelle le cycle A est un groupe phényle substitué ou non substitué, et R est un résidu ester, de façon à hydrolyser de façon stéréosélective l'isomère (2R, 3S) optiquement actif, et ensuite.

(b) la séparation est la récupération l'antipode (2S, 3R) à partir du mélange réactionnel, et ensuite

(c) la conversion de ce composé en (-)-cis-2-(4'-méthylphényl)-3-acétyloxy-5-(2-(diméthylamino)-éthyl)-8-méthyl-2,3-dihydro-1,5-benzothiazépin-4(5H)-one par une procédure connue.

10. Procédé suivant la revendication 9, dans lequel le microorganisme est un microorganisme appartenant au genre *Micrococcus*, au genre *Achromobacter*, au genre *Agrobacterium*, au genre *Corynebacterium*, au genre *Enterobacter*, au genre *Flavobacterium*, au genre *Microbacterium*, au genre *Pseudomonas*, au genre *Rhizobium*, au genre *Sarcina*, au genre *Bacillus*, au genre *Brevibacterium*, au genre *Citrobacter*, au genre *Xanthomonas*, au genre *Candida*, au genre *Debaryomyces*, au genre *Hanseniaspora*, au genre *Hansenula*, au genre *Pichia*, au genre *Rhodospiridium*, au genre *Schizosaccharomyces*, au genre *Sporobolomyces*, au genre *Torula*, au genre *Kloeckera*, au genre *Rhodotorula*, au genre *Saccharomycopsis*, au genre *Torulapsora*, au genre *Trigonopsis*, au genre *Absidia*, au genre *Aspergillus* ou au genre *Streptomyces*.
11. (2S, 3R)-3-(4-Méthylphényl)-glycidate d'alkyle inférieur.
12. (2S, 3R)-3-(4-méthylphényl)-glycidate de méthyle de haute pureté.



13. (2S, 3R)-3-(4-Méthylphényl)-glycidate d'alkyle inférieur de haute pureté.

14. (2S, 3R)-3-(4-méthylphényl)-glycidate de méthyle sous forme cristalline.

15. (2S, 3R)-3-(4-Méthylphényl)-glycidate d'alkyle inférieur sous forme cristalline.

