

DESCRIPTION

METHOD FOR PRODUCING *cis*-5-HYDROXY-2-PIPERIDINECARBOXYLIC
ACID DERIVATIVE, AND METHOD FOR PURIFYING *cis*-5-HYDROXY-2-
5 PIPERIDINECARBOXYLIC ACID

TECHNICAL FIELD

[0001]

The present invention relates to an industrial method for producing a
10 derivative of *cis*-5-hydroxy-2-piperidinecarboxylic acid (which is also called *cis*-5-hydroxypipicolic acid). The present invention also relates to a method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid.

BACKGROUND ART

15 [0002]

cis-5-Hydroxy-2-piperidinecarboxylic acid is a useful intermediate for
pharmaceuticals. However, since this compound has 2 asymmetric carbons, 4 kinds
of isomers are present, and selective synthesis of a single kind of isomer, or single
kind of diastereomer, is very difficult. Thus, improvement of the purity by
20 separation of unnecessary isomers, or purification, by chemical conversion is
necessary.

[0003]

For example, methods for obtaining *cis*-5-hydroxy-2-piperidinecarboxylic
acid by introduction of a hydroxyl group to 2-piperidinecarboxylic acid by
25 hydroxylation reaction using a microorganism or enzyme have been reported.
However, it has been reported that a compound having a substituted hydroxyl group
at another position, such as a 3-position-hydroxylated compound, may be produced

as a by-product in addition to the compound of interest, *cis*-5-hydroxy-2-piperidinecarboxylic acid (Non-patent Document 1). Separation of the by-product is not described in the document. A method in which 5-hydroxy-2-piperidinecarboxylic acid is synthesized from 5-hydroxylysine by enzymatic reaction has also been reported (Patent Document 1), but the document does not describe separation of the generated *cis/trans* isomers. In a report describing synthesis of 5-hydroxy-2-piperidinecarboxylic acid from 5-hydroxylysine by a method similar to the method of Patent Document 1 (Patent Document 2), isomers are separated using an ion-exchange column. Since this method requires use of excessive amounts of a filler and eluent with respect to the substrate, the method is not realistic from the viewpoint of industrial production.

Thus, there is no known method by which a necessary stereoisomer can be selectively obtained with high purity from *cis*-5-hydroxy-2-piperidinecarboxylic acid synthesized using a microorganism or enzyme.

[0004]

There are known chemical synthesis methods using, as a material, L-amino acid, in which the stereochemistry of one of the two asymmetric carbons, the 2-position carbon, can be fixed. For example, a method using L-pyrroglutamic acid as a material (Patent Document 3) has been reported. This method requires use of an iridium catalyst, which is expensive, for formation of a piperidine ring, which is problematic. Methods using L-glutamic acid as a material (Non-patent Documents 2 and 3) and a method using a proline derivative as a material (Non-patent Document 4) have also been reported, but all of these methods require use of a diazo compound, which is highly risky, and also require a multistep complex process. Moreover, for separation of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid, the reaction is carried out via 5-oxo-2-piperidinecarboxylic acid to preferentially obtain the (2*S*,5*S*) compound by its reduction (Non-patent Document 4). This requires separation

using a silica gel column for removal of impurities such as isomers. Because of the load of this process, this method is not industrially satisfactory.

[0005]

It is also known that (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-

5 piperidinecarboxylic acid and its esters are lactonized to give benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (Non-patent Documents 4 and 5).

These documents on lactonization do not mention about impurities such as isomers, and the effects of purification by these methods have been unclear. Non-patent

Document 4 describes that benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-

10 carboxylate was obtained as an oily substance after purification with a silica gel column.

Non-patent Document 5 describes that crystallization of benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate occurs, but, since the

lactonization is carried out using *cis*-5-hydroxy-2-piperidinecarboxylic acid as a material, behavior of impurities such as isomers and the effect of purification are not

15 clear. There is a report describing separation of a *cis*/*trans* mixture of methyl *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate by acid treatment (Non-patent Document 3), but, since the compound was a carboxylic acid ester, it was impossible to separate lactone obtained from the *cis* isomer from the ester of the *trans* isomer by a simple method such as solvent extraction. Moreover, Non-patent Document 3

20 does not describe the yield in this process. When the present inventors studied this method, the yield of the lactone was low because of occurrence of a remarkable side reaction between lactone obtained from the *cis* isomer (benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate)) and the residual *trans* isomer ((2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid ester).

25 As described above, there are problems to be solved for industrial production of highly pure *cis*-5-hydroxy-2-piperidinecarboxylic acid by an inexpensive method.

PRIOR ART DOCUMENTS

[Patent Documents]

[0006]

Patent Document 1: JP 4590981 B

5 Patent Document 2: JP 2010-88395 A

Patent Document 3: WO 2010/126820

[Non-patent Documents]

[0007]

Non-patent Document 1: Adv. Synth. Catal., 2011, 353, 1375.

10 Non-patent Document 2: Chem. Commun., 1996, 349.

Non-patent Document 3: Tetrahedron Lett., 1988, 29, 2231.

Non-patent Document 4: Tetrahedron: Asym., 2006, 17, 2479.

Non-patent Document 5: Rec. Trav. Chim. Pays-Bas, 1959, 78, 648.

15 SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0008]

The present invention aims to provide a method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid, which is a useful intermediate for pharmaceuticals, and a

20 method for producing its derivative.

MEANS FOR SOLVING THE PROBLEMS

[0009]

The present inventors intensively studied to solve the problems described

25 above. As a result, the present inventors solved the problems by a process of reacting low-purity *cis*-5-hydroxy-2-piperidinecarboxylic acid containing impurities with an acid halide and/or acid anhydride to induce a *cis*-5-hydroxy-2-

piperidinecarboxylic acid derivative, thereby solving the problems.

In a more specific mode, the present inventors discovered that impurities such as isomers can be separated by reacting *cis*-5-hydroxy-2-piperidinecarboxylic acid with an acid halide and/or acid anhydride to convert the *cis*-5-hydroxy-2-
5 piperidinecarboxylic acid into a lactone whose amino group is protected, and then performing crystallization and/or solvent extraction. That is, studies by the present inventors revealed that the *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative in the present invention can be selectively converted to lactone by reaction with an acid halide and/or acid anhydride, and that this lactone can be crystallized under specific
10 conditions. The present inventors then developed a method in which the lactone is crystallized to achieve removal of impurities such as isomers having low crystallizability.

In another specific mode, the present inventors discovered that impurities such as isomers having a carboxyl group can be removed by solvent extraction after
15 reacting *cis*-5-hydroxy-2-piperidinecarboxylic acid with an acid halide and/or acid anhydride, and then with an alcohol in the presence of an acid catalyst to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into an ester whose amino group is protected. That is, the present inventors studied to develop a method in which, in the presence of an acid catalyst, a *cis*-5-hydroxy-2-piperidinecarboxylic acid
20 derivative is selectively converted into lactone, and the lactone is reacted with an alcohol present in the reaction system to efficiently and selectively convert the *cis* isomer into an ester while suppressing a side reaction, followed by removing residual impurities such as isomers having a carboxyl group by solvent extraction.

The present inventors then discovered that highly pure *cis*-5-hydroxy-2-
25 piperidinecarboxylic acid can be obtained by hydrolyzing the resulting lactone and/or ester to give *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid, and then removing the protective group for the amino group, thereby completing the present

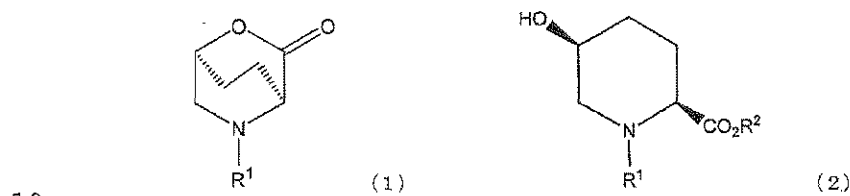
invention.

[0010]

That is, the present invention can be summarized as follows.

[1] A method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid
 5 derivative, the method comprising a step of converting *cis*-5-hydroxy-2-
 piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2)
 (wherein R¹ represents a protective group for an amino group, and R² represents a
 C₁-C₆ alkyl group).

[0011]



[0012]

[2] The method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid
 derivative according to [1], comprising a step of reacting *cis*-5-hydroxy-2-
 piperidinecarboxylic acid with an acid halide and/or acid anhydride to convert the
 15 *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound of Formula (1).

[3] The method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid
 derivative according to [1], comprising a step of reacting *cis*-5-hydroxy-2-
 piperidinecarboxylic acid with an acid halide and/or acid anhydride, and then with an
 alcohol in the presence of an acid catalyst, to convert the *cis*-5-hydroxy-2-
 20 piperidinecarboxylic acid into a compound of Formula (2).

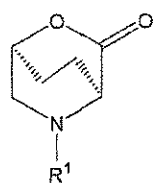
[4] A method for regenerating *cis*-5-hydroxy-2-piperidinecarboxylic acid,
 the method comprising the steps of:

converting *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of
 Formula (1) and/or Formula (2) (wherein R¹ represents a protective group for an

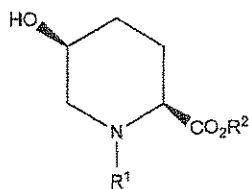
amino group, and R^2 represents a C_1 - C_6 alkyl group); and

converting the compound(s) of Formula (1) and/or Formula (2) into *cis*-5-hydroxy-2-piperidinecarboxylic acid.

[0013]



(1)



(2)

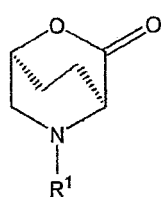
[0014]

[5] The method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative according to any one of [1] to [3], wherein the *cis*-5-hydroxy-2-piperidinecarboxylic acid is *cis*-5-hydroxy-2-piperidinecarboxylic acid synthesized by bacterial reaction and/or enzymatic reaction.

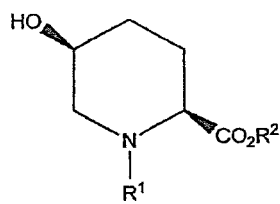
[6] The method for regenerating *cis*-5-hydroxy-2-piperidinecarboxylic acid according to [4], wherein the *cis*-5-hydroxy-2-piperidinecarboxylic acid is *cis*-5-hydroxy-2-piperidinecarboxylic acid synthesized by bacterial reaction and/or enzymatic reaction.

[7] A method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid, the method comprising a step of reacting a mixture containing *cis*-5-hydroxy-2-piperidinecarboxylic acid and an impurity with an acid halide and/or acid anhydride, or with an acid halide and/or acid anhydride and an alcohol, to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2), separating the compound(s) and then converting the separated compound(s) into *cis*-5-hydroxy-2-piperidinecarboxylic acid.

[0015]



(1)



(2)

[0016]

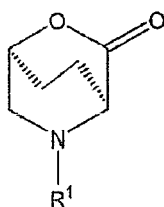
[8] The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to [7], wherein the impurity is 2-piperidinecarboxylic acid or an analogue thereof.

[9] The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to [8], wherein the 2-piperidinecarboxylic acid or an analogue thereof is *trans*-5-hydroxy-2-piperidinecarboxylic acid.

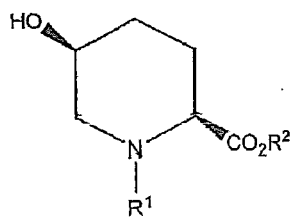
[10] The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to any one of [7] to [9], wherein the step of separating the compound(s) of Formula (1) and/or Formula (2) is carried out by crystallization or solvent extraction.

[11] The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to any one of [7] to [10], wherein the mixture containing *cis*-5-hydroxy-2-piperidinecarboxylic acid and an impurity is a mixture synthesized by bacterial reaction and/or enzymatic reaction.

[0016a] In one aspect, there is provided a method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative, (wherein R¹ represents a protective group for an amino group, and R² represents a C₁-C₆ alkyl group).



(1)



(2)

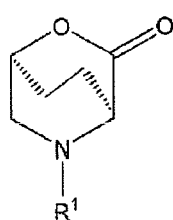
comprising a step of reacting *cis*-5-hydroxy-2-piperidinecarboxylic acid with an acid halide and/or acid anhydride to convert said *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound of Formula (1); or

comprising a step of reacting *cis*-5-hydroxy-2-piperidinecarboxylic acid with an acid halide and/or acid anhydride, and then with an alcohol in the presence of an acid catalyst, to convert said *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound of Formula (2).

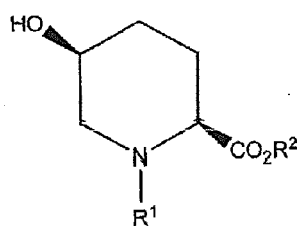
[0016b] In another aspect, there is provided a method for regenerating *cis*-5-hydroxy-2-piperidinecarboxylic acid, said method comprising the steps of:

converting *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2) according to the method described herein (wherein R^1 represents a protective group for an amino group, and R^2 represents a C_1 - C_6 alkyl group); and

converting the compound(s) of Formula (1) and/or Formula (2) into *cis*-5-hydroxy-2-piperidinecarboxylic acid.



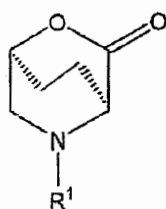
(1)



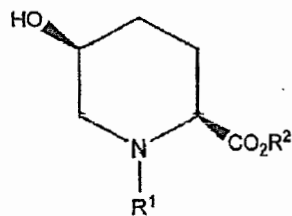
(2)

[0016c] In another aspect, there is provided a method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid, said method comprising a step of reacting a mixture containing *cis*-5-hydroxy-2-piperidinecarboxylic acid and an impurity with an acid halide and/or acid anhydride, or with an acid halide and/or acid anhydride and an alcohol, to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s)

of Formula (1) and/or Formula (2), separating said compound(s) and then converting the separated compound(s) into *cis*-5-hydroxy-2-piperidinecarboxylic acid.



(1)



(2)

5 EFFECT OF THE INVENTION

[0017]

By the method of the present invention, highly pure *cis*-5-hydroxy-2-piperidinecarboxylic acid and its derivatives can be provided.

10 BRIEF DESCRIPTION OF THE DRAWINGS

[0018]

Fig. 1 is a schematic diagram illustrating routes for purifying *cis*-5-hydroxy-2-

piperidinecarboxylic acid from *cis*-5-hydroxy-2-piperidinecarboxylic acid that may contain an impurity(s). The route 1 is a route in which *cis*-5-hydroxy-2-piperidinecarboxylic acid is reacted with an acid halide and/or acid anhydride to give a compound of Formula (1) directly or via *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid, and the compound is then converted into *cis*-5-hydroxy-2-piperidinecarboxylic acid. The route 2 is a route in which *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid is reacted with an acid catalyst to give a compound of Formula (1), and the compound is then converted into *cis*-5-hydroxy-2-piperidinecarboxylic acid by hydrolysis. The route 3 is a route in which *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid is reacted with an alcohol in the presence of an acid catalyst to give a compound of Formula (2), and the compound is then converted to *cis*-5-hydroxy-2-piperidinecarboxylic acid by hydrolysis.

Fig. 2. is a diagram showing the result of HPLC analysis of hydroxylysines obtained with recombinant lysine hydroxylases.

Fig. 3 is a diagram showing the result of HPLC analysis of hydroxylysines obtained with recombinant lysine hydroxylases.

DESCRIPTION OF THE EMBODIMENTS

[0019]

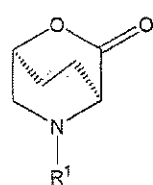
The present invention is described below in detail.

In the present invention, examples of the *cis*-5-hydroxy-2-piperidinecarboxylic acid include (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid, (2*R*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid, and mixtures thereof. The *cis*-5-hydroxy-2-piperidinecarboxylic acid may be a racemic compound. The *cis*-5-hydroxy-2-piperidinecarboxylic acid may be forming a salt with an acid or base.

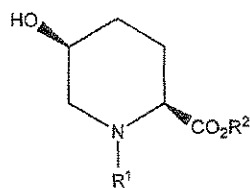
[0020]

The method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid

derivative of the present invention comprises a step of converting *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2).



(1)



(2)

[0021]

5 In Formula (1) and Formula (2), R¹ represents a protective group for an amino group, and specific examples of the protective group include the following groups. However, the protective group is not limited to these examples.

10 Examples of the protective group for the amino group include acyl groups such as formyl, acetyl, chloroacetyl, dichloroacetyl, trichloroacetyl, trifluoroacetyl, propionyl, benzoyl, and 4-chlorobenzoyl; alkoxycarbonyl groups such as methoxycarbonyl, ethoxycarbonyl, *tert*-butoxycarbonyl, benzyloxycarbonyl, and allyloxycarbonyl; arylalkyl groups such as benzyl, 4-methoxybenzyl, 4-bromobenzyl, and 1-phenethyl; and sulfonyl groups such as methanesulfonyl, *p*-toluenesulfonyl, and 2-nitrobenzenesulfonyl.

15 [0022]

Among these, acyl groups and alkoxycarbonyl groups are preferred since these groups can be easily removed; acetyl, chloroacetyl, trifluoroacetyl, benzoyl, *tert*-butoxycarbonyl, and benzyloxycarbonyl are more preferred; and acetyl, *tert*-butoxycarbonyl, and benzyloxycarbonyl are still more preferred since these are 20 industrially inexpensive. The protective group is especially preferably benzyloxycarbonyl since benzyloxycarbonyl can be removed by hydrogenation without leaving a non-volatile component, and the load of purification of *cis*-5-hydroxy-2-piperidinecarboxylic acid can therefore be reduced.

[0023]

In Formula (2), R² represents a C₁-C₆ alkyl, and specific examples of R² include primary alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, and hexyl; secondary alkyl groups such as isopropyl, isobutyl, isopentyl, cyclopentyl, and cyclohexyl; and tertiary alkyl groups such as *tert*-butyl. Among these, methyl, ethyl, isopropyl, butyl, and *tert*-butyl are preferred, and ethyl and isopropyl are more preferred.

[0024]

In the present invention, the *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative may be the compound(s) of Formula (1) and/or Formula (2) itself/themselves.

Specific examples of the compound of Formula (1) include benzyl 5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate, *tert*-butyl 5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate, and 5-acetyl-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane.

Specific examples of the compound of Formula (2) include methyl *cis-N*-(benzyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate, ethyl *cis-N*-(benzyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate, isopropyl *cis-N*-(benzyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate, methyl *cis-N*-(*tert*-butyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate, ethyl *cis-N*-(*tert*-butyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate, and isopropyl *cis-N*-(*tert*-butyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate.

[0025]

The *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative may be a compound obtained by chemical conversion of a compound(s) of Formula (1) and/or Formula (2).

Specific examples of such a *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative include carboxylic acids such as *cis-N*-(benzyloxycarbonyl)-5-hydroxy-2-

piperidinecarboxylic acid and *cis-N*-(*tert*-butyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylic acid; esters such as benzyl *cis-N*-(benzyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate and benzyl *cis-N*-(*tert*-butyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylate; carboxylic acid amides such as *cis-N*-(benzyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylic acid amide, *cis-N*-(*tert*-butyloxycarbonyl)-5-hydroxy-2-piperidinecarboxylic acid amide, benzyl *cis*-5-hydroxy-2-(methylcarbamoyl)piperidine-1-carboxylate, *tert*-butyl *cis*-5-hydroxy-2-(methylcarbamoyl)piperidine-1-carboxylate, benzyl *cis*-2-(1-*tert*-butyloxycarbamoylpyrrolidine-3-ylcarbamoyl)-5-hydroxypiperidine-1-carboxylate, *tert*-butyl *cis*-2-(1-benzyloxycarbamoylpyrrolidine-3-ylcarbamoyl)-5-hydroxypiperidine-1-carboxylate, benzyl *cis*-2-(1-*tert*-butyloxycarbamoylpiperidine-4-ylcarbamoyl)-5-hydroxypiperidine-1-carboxylate, and *tert*-butyl *cis*-2-(1-benzyloxycarbamoylpiperidine-4-ylcarbamoyl)-5-hydroxypiperidine-1-carboxylate.

[0026]

In one mode of the method of the present invention, *cis*-5-hydroxy-2-piperidinecarboxylic acid is reacted with an acid halide and/or acid anhydride to protect the amino group of the *cis*-5-hydroxy-2-piperidinecarboxylic acid, and subsequent lactonization of the resultant causes its conversion into a compound of Formula (1).

[0027]

The acid halide and/or acid anhydride is/are not limited as long as the acid halide and/or acid anhydride can protect the amino group and allow(s) the lactonization. Examples of the acid halide include acid chlorides and acid bromides. In view of ease of handling, acid chlorides are preferred. Examples of the acid anhydride include carboxylic acid anhydrides, and sulfonic acid anhydrides. In view of ease of handling and the cost, carboxylic acid anhydrides are preferred.

Specific examples of the acid halide and/or acid anhydride include acylating

agents such as formic acid-acetic anhydride, acetic anhydride, acetyl chloride, chloroacetyl chloride, dichloroacetyl chloride, trichloroacetyl chloride, trifluoroacetic anhydride, propionyl chloride, benzoyl chloride, 4-chlorobenzoyl chloride, acetyl bromide, propionyl bromide, and benzoyl bromide; alkoxycarbonylating agents such as di-*tert*-butyldicarbonate, benzyloxycarbonyl chloride, allyloxycarbonyl chloride, benzyloxycarbonyl bromide, and allyloxycarbonyl bromide; and sulfonylating agents such as methanesulfonyl chloride, *p*-toluenesulfonyl chloride, 2-nitrobenzenesulfonyl chloride, methanesulfonyl bromide, *p*-toluenesulfonyl bromide, and 2-nitrobenzenesulfonyl bromide.

10 Among these, acylating agents and alkoxycarbonylating agents are preferred. Acetic anhydride, chloroacetyl chloride, trichloroacetyl chloride, trifluoroacetic anhydride, di-*tert*-butyldicarbonate, benzyloxycarbonyl chloride, and allyloxycarbonyl chloride are more preferred since these can be easily removed after the protection of the amino group. Acetic anhydride, di-*tert*-butyldicarbonate, and benzyloxycarbonyl chloride are still more preferred since these are industrially inexpensive. Benzyloxycarbonyl chloride is especially preferred since it can be removed by hydrogenation without leaving a non-volatile component, and the load of purification of *cis*-5-hydroxy-2-piperidinecarboxylic acid can therefore be reduced. [0028]

20 In the present invention, two or more kinds of acid halide and/or acid anhydride may be used. In cases where two or more kinds of acid halide and/or acid anhydride are used, these may be added to the reaction system at once, but it is preferred to use different kinds of acid halide and/or acid anhydride for in each the process of amino group protection and the process of lactonization. For example, it is preferred to carry out a method in which benzyloxycarbonyl chloride is used for the protection of the amino group, and acetic anhydride is used for the lactonization. In cases where the protection of the amino group and the lactonization are carried out

in a single reactor, a single kind of acid halide and/or acid anhydride is preferably used for suppression of by-products having different protective groups. In cases where the protection of the amino group and the lactonization are carried out in separate reactors, two or more kinds of acid halide and/or acid anhydride are preferably used in view of optimization of the reactions and cost reduction. In particular, the protection of the amino group and the lactonization are preferably carried out in a single reactor since the number of reactors required for the production can be decreased, and the cost can therefore be reduced.

[0029]

The amount of the acid halide and/or acid anhydride to be used is usually 1 to 10 molar equivalents, preferably 1.2 to 5 molar equivalents, more preferably 1.5 to 3 molar equivalents relative to the total amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid and the amine compound to be protected. The acid halide and/or acid anhydride may be added dividedly in a plurality of times.

[0030]

In the reaction with the acid halide and/or acid anhydride, a base may be used, if necessary. Specific examples of the base include, but are not limited to, tertiary amines such as triethylamine, diisopropylethylamine, *N*-methylmorpholine, quinuclidine, and 1,4-diazabicyclo[2.2.2]octane; pyridines such as pyridine, 4-dimethylaminopyridine, and 2,6-lutidine; organic strong bases such as 1,8-diazabicyclo[5.4.0]undeca-7-ene and tetramethylguanidine; metal amides such as lithium diisopropylamide and sodium hexamethyldisilazide; alkyl metals such as *n*-butyllithium, *sec*-butyllithium, *tert*-butyllithium, and isopropylmagnesium bromide; metal hydrides such as sodium hydride and calcium hydride; metal alkoxides such as sodium methoxide, sodium ethoxide, sodium *tert*-butoxide, and potassium *tert*-butoxide; carbonates such as sodium hydrogen carbonate and potassium carbonate; phosphates such as potassium phosphate and sodium hydrogen phosphate;

hydroxides such as sodium hydroxide, potassium hydroxide, lithium hydroxide, and calcium hydroxide; and cyanides such as sodium cyanide and potassium cyanide.

Preferred bases vary depending on the protective reagent to be used. In cases where a preferred protective reagent, that is, acetic anhydride, di-*tert*-butyldicarbonate, or benzyloxycarbonyl chloride, is used, the base is preferably tertiary amines, pyridines, carbonates, or hydroxides. In cases where the especially preferred protective reagent, that is, benzyloxycarbonyl chloride, is used, the base is preferably a hydroxide, which is inexpensive.

In cases where a base is used, the amount of base is preferably added such that the pH of the reaction mixture becomes 7 to 12. In cases where water is used as the solvent, the pH of the reaction mixture herein means the pH of the layer containing water. In cases where a solvent other than water is used, the pH of the reaction mixture herein means the pH of the layer which contains water when an equal volume of water is added to the reaction mixture. In cases where water is used as the solvent, hydrolysis of the compound of Formula (1) may occur when the basicity of the reaction mixture is too strong. The pH is therefore more preferably 7 to 12, especially preferably 7 to 11.

[0031]

Examples of the reaction solvent include water; esters such as ethyl acetate and butyl acetate; alcohols such as methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-ethyl-1-hexanol, and 2-butanol; ethers such as diethyl ether, di-*n*-butyl ether, diisopropyl ether, di-*n*-butyl ether, *tert*-butyl methyl ether, tetrahydrofuran, and dioxane; ketones such as acetone, methyl ethyl ketone, and methyl isobutyl ketone; nitriles such as acetonitrile; amides such as dimethylformamide, dimethylacetamide, and *N*-methylpyrrolidinone; sulfones such as dimethylsulfoxide and sulfolane; hydrocarbons such as hexane, heptane, and toluene; and mixed solvents of two or more of these. Among these, water, and mixed solvents containing water and one

or more of the above solvents, are preferred. In particular, in cases where the *cis*-5-hydroxy-2-piperidinecarboxylic acid is synthesized by bacterial reaction and/or enzymatic reaction, the *cis*-5-hydroxy-2-piperidinecarboxylic acid is obtained as a solution whose solvent is mainly water. Therefore, in view of simplicity of the production process, it is preferred to use the solution as it is, or to concentrate part of the solution, for carrying out the reaction using water as a main solvent.

[0032]

The reaction temperature is usually -20°C to 100°C, preferably -10°C to 50°C. Since reaction at high temperature may cause decomposition of reagents and products, the reaction temperature is more preferably 0°C to 30°C.

[0033]

The protection of the amino group and the lactonization are preferably carried out by adding an acid halide and/or acid anhydride (the route 1 in Fig. 1). It is especially preferred to carry out the protection of the amino group and the lactonization using a single acid halide and/or acid anhydride in a single reactor in view of simplicity of the operation (the upper formula in the route 1 in Fig. 1).

[0034]

The protection of the amino group and the lactonization may also be carried out under different conditions (the route 2 in Fig. 1). That is, the amino group may be protected by addition of the acid halide and/or acid anhydride, and then an acid catalyst may be used to perform the lactonization. In cases where the protection of the amino group and the lactonization are carried out under different reaction conditions, it is preferred to suppress production of the compound of Formula (1) at the stage of protection of the amino group. The protection of the amino group can be selectively and effectively carried out by using the acid halide and/or acid anhydride in the same molar amount as that of the amino group, or by adding the acid halide and/or acid anhydride dividedly such that excessive presence of the acid halide

and/or acid anhydride relative to the amino group is avoided, and then stopping the reaction when the material *cis*-5-hydroxy-2-piperidinecarboxylic acid has almost disappeared. In cases where the protection of the amino group is carried out in a solvent containing water, the compound of Formula (1) generated by lactonization, if any, can be hydrolyzed by a method such as making the pH of the reaction mixture strongly basic, increasing the temperature of the reaction mixture to a temperature of not less than room temperature, performing the reaction for long time, or a combination of one or more of these methods, and, as a result, the amino-protected compound can be obtained. By subsequently reacting the amino-protected compound with an acid catalyst, the compound of Formula (1) can be obtained.

[0035]

The compound of Formula (1) produced can be isolated by a method such as the crystallization described below.

[0036]

In another mode of the method of the present invention, *cis*-5-hydroxy-2-piperidinecarboxylic acid is reacted with an acid halide and/or acid anhydride, and then with an alcohol in the presence of an acid catalyst, to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound of Formula (2) (the route 3 in Fig. 1). By the reaction with an alcohol in the presence of an acid catalyst, the compound of Formula (1) produced by the reaction with an acid halide and/or acid anhydride is converted to the compound of Formula (2). In this process, in cases where compounds having a carboxyl group other than the compound of Formula (1), especially the 2-piperidinecarboxylic acid or an analogue thereof described later, are contained as impurities, these compounds are not converted to lactone like the compound of Formula (1). Therefore, the compound derived from *cis*-5-hydroxy-2-piperidinecarboxylic acid can be selectively converted to the compound of Formula (2), which is an ester, while the other compounds are left in the reaction system as

carboxylic acids. Thereafter, by extraction with a basic aqueous solution, the compound of Formula (2) can be separated into the organic layer, while the impurities other than the compound of Formula (2) can be separated into the aqueous layer.

5 [0037]

The alcohol used herein is not limited as long as it is a C₁-C₆ alcohol. Examples of the alcohol include primary alcohols such as methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, and 1-hexanol; secondary alcohols such as 2-propanol, 2-butanol, cyclopentanol, and cyclohexanol; and tertiary alcohols such as
10 *tert*-butanol. Among these, secondary alcohols are preferred, and 2-propanol is more preferred since it is inexpensive, has a low boiling point, and can be easily removed. In cases where a primary alcohol is used, a side reaction may occur to cause direct conversion of a carboxylic acid into its ester in the presence of an acid catalyst, resulting in low effect of removal of impurities. In cases where a tertiary
15 alcohol is used, reactivity with the compound of Formula (1) is low. Therefore, the compound of Formula (2), which is a secondary alcohol, may react with the compound of Formula (1) to partially form a dimer, resulting in low yield of the compound of Formula (2). Thus, in the present invention, a secondary alcohol is preferably used.

20 An ester of the above-described alcohol may be added in the presence of an acid catalyst and water so that hydrolysis reaction of the ester allows the alcohol to be present in the reaction system, and the alcohol may be reacted with the *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid in the presence of an acid catalyst.

The alcohol, or the ester which generates the alcohol, may be used in an
25 excess amount, and the molar amount of the alcohol or ester to be used is usually 1 to 500 times the molar amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid. In cases where the amount of the alcohol or ester is too small, a side reaction in which

the compound of Formula (2) reacts with the compound of Formula (1) may occur to form a dimer, while in cases where the amount of the alcohol or ester is too large, the effect of the acid catalyst decreases. Thus, the molar amount of the alcohol or ester is preferably 2 to 100 times, more preferably 3 to 50 times, especially preferably 5 to 20 times the molar amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid

[0038]

Examples of the acid catalyst include inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, and polyphosphoric acid; sulfonic acids such as *p*-toluenesulfonic acid, methanesulfonic acid, and trifluoromethanesulfonic acid; and carboxylic acids such as acetic acid, formic acid, trifluoroacetic acid, trichloroacetic acid, benzoic acid, and oxalic acid. Among these, sulfonic acids are preferred since they have high solubility in organic solvents and sufficiently strong acidity for allowing the reaction to proceed. *p*-Toluenesulfonic acid and methanesulfonic acid are more preferred since they are industrially inexpensive.

The molar amount of the acid catalyst to be added is usually 0.001 to 10 times the molar amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid. In cases where the molar amount of the acid catalyst is too small, the reaction proceeds slowly, while the molar amount of the acid catalyst is too large, the load of the post treatment is large. The molar amount of the acid catalyst is therefore preferably 0.01 to 5 times, more preferably 0.02 to 1 times, especially preferably 0.05 to 0.5 times the molar amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid.

[0039]

Examples of the reaction solvent include organic acids such as formic acid and acetic acid; esters such as ethyl acetate and butyl acetate; alcohols such as methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-ethyl-1-hexanol, and 2-butanol; ethers such as diethyl ether, di-*n*-butyl ether, diisopropyl ether, di-*n*-butyl

ether, *tert*-butyl methyl ether, tetrahydrofuran, and dioxane; ketones such as acetone, methyl ethyl ketone, and methyl isobutyl ketone; nitriles such as acetonitrile; amides such as dimethylformamide, dimethylacetamide, and *N*-methylpyrrolidinone; sulfones such as dimethylsulfoxide and sulfolane; hydrocarbons such as hexane, heptane, and toluene; and mixed solvents of two or more of these. Among these, solvents which do not show coordination of a proton are preferred since they do not weaken the activity of the acid catalyst. More specifically, hydrocarbons are preferred. In addition, organic acids, which themselves are acids, and esters, which produce acids by hydrolysis, are preferred. Toluene is more preferred because of its low cost, high reaction rate, and high solubility of acid catalysts therein.

[0040]

The reaction temperature is usually 0°C to 150°C, preferably 20°C to 120°C. Reaction at higher temperature may cause a side reaction, but reaction at lower temperature is slow and takes a long time. The reaction temperature is therefore more preferably 40°C to 80°C.

[0041]

The compound of Formula (2) generated can be isolated by a method such as the extraction method using an organic solvent described later.

[0042]

The compounds of Formulae (1) and (2) can be converted to *cis-N*-protected-5-hydroxy-2-piperidinecarboxylic acid by treatment with a base.

[0043]

Examples of the base to be used herein include hydroxides such as sodium hydroxide, potassium hydroxide, lithium hydroxide, and calcium hydroxide; phosphates such as sodium phosphate, potassium phosphate, and calcium phosphate; carbonates such as lithium carbonate, sodium carbonate, potassium carbonate, magnesium carbonate, and calcium carbonate; and hydrogen carbonates such as

sodium hydrogen carbonate and potassium hydrogen carbonate. The base is preferably a hydroxide.

The molar amount of the base to be used is usually 0.1 to 10 times, preferably 0.5 to 5 times, more preferably 0.9 to 3 times the total molar amount of the compounds of Formulae (1) and (2).

[0044]

By carrying out deprotection of the amino group of the *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid, *cis*-5-hydroxy-2-piperidinecarboxylic acid (*cis*-5-hydroxypipericolic acid) can be produced. In particular, in cases where R¹ in the Formulae (1) and (2) is a benzyloxycarbonyl group, deprotection of the amino group can be carried out by hydrogenation reaction. The hydrogenation reaction can be carried out by, for example, using a palladium carbon catalyst. By such a reaction, the *cis*-5-hydroxy-2-piperidinecarboxylic acid can be regenerated.

[0045]

The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid of the present invention is a method in which highly pure *cis*-5-hydroxy-2-piperidinecarboxylic acid is obtained by purification from *cis*-5-hydroxy-2-piperidinecarboxylic acid containing an impurity(s).

[0046]

Examples of the impurity include 2-piperidinecarboxylic acid or an analogue thereof, amino acids, peptides, sugars, and fatty acids. Since, among these, 2-piperidinecarboxylic acid or an analogue thereof, amino acids, and peptides have an amino group(s) and carboxyl group(s), these can be hardly separated from the desired *cis*-5-hydroxy-2-piperidinecarboxylic acid of interest by an ordinary purification method such as ion-exchange resin purification. In particular, since 2-piperidinecarboxylic acid or an analogue thereof has the same skeleton as *cis*-5-hydroxy-2-piperidinecarboxylic acid, its separation is very difficult, and

establishment of removal process is very important.

Examples of the 2-piperidinecarboxylic acid or an analogue thereof include isomers of *cis*-5-hydroxy-2-piperidinecarboxylic acid; and 2-piperidinecarboxylic acid and analogues thereof. Examples of the isomers of *cis*-5-hydroxy-2-

5 piperidinecarboxylic acid include stereoisomers such as *trans* isomers, and structural isomers having hydroxyl groups at different positions. It should be noted that optical isomers of *cis*-5-hydroxy-2-piperidinecarboxylic acid are not included in the impurity of the present invention.

Examples of the *trans* isomers include *trans*-5-hydroxy-2-
 10 piperidinecarboxylic acid, that is, (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid, (2*R*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid, and mixtures of these. Examples of the structural isomers include *cis*-2-hydroxy-2-piperidinecarboxylic acid, *trans*-2-hydroxy-2-piperidinecarboxylic acid, *cis*-3-hydroxy-2-piperidinecarboxylic acid, *trans*-3-hydroxy-2-piperidinecarboxylic acid, and *trans*-4-hydroxy-2-
 15 piperidinecarboxylic acid. It should be noted that *cis*-4-hydroxy-2-piperidinecarboxylic acid is not included in the impurity of the present invention.

Examples of the 2-piperidinecarboxylic acid and analogues thereof include 2-piperidinecarboxylic acid and 1,2,3,4-tetrahydro-2-pyridinecarboxylic acid.

Examples of the amino acids include essential amino acids such as proline,
 20 lysine, and isoleucine, and unnatural amino acids such as 3-hydroxyproline, 4-hydroxyproline, and 5-hydroxylysine.

Examples of the peptides include dipeptides, oligopeptides, and proteins.

Examples of the sugars include glucose, gluconic acid, sodium gluconate, potassium gluconate, and calcium gluconate.

25 Examples of the fatty acids include C₂-C₄ short-chain fatty acids, C₅-C₁₂ medium-chain fatty acids, long-chain fatty acids having not less than 13 carbon atoms, and glycerol esters.

[0047]

A mixture containing *cis*-5-hydroxy-2-piperidinecarboxylic acid and an impurity(s) is reacted with an acid halide and/or acid anhydride, or with an acid halide and/or acid anhydride and an alcohol, to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2).
5 Subsequently, the compound(s) is/are separated by crystallization, solvent extraction, and/or the like and then treated with a base to convert the compound(s) into *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid. By subsequently carrying out deprotection of the amino group, *cis*-5-hydroxy-2-piperidinecarboxylic acid which
10 the impurity has been removed can be obtained.

That is, *cis*-5-hydroxy-2-piperidinecarboxylic acid can be separated from the impurity utilizing specific lactonization of the *cis*-5-hydroxy-2-piperidinecarboxylic acid. Even in cases where the impurity other than the above-described impurities is contained, the *cis*-5-hydroxy-2-piperidinecarboxylic acid can be separated also from
15 the impurity.

In the present description, the crystallization includes not only ordinary crystallization by adding a poor solvent, acid, base, or the like to a solution, or azeotropically removing a good solvent such as water, to decrease solubility of the desired product and recover the product as crystals; but also recrystallization in
20 which crude crystals that have once been obtained are dissolved in an appropriate solvent and then recrystallized. The crystallization may be promoted by addition of seed crystals to the solution.

In the crystallization of the compound of Formula (1), crystallization is preferably carried out for a crude product of the compound of Formula (1) obtained
25 by the reaction with the acid halide and/or acid anhydride. This is because a small amount of acidic component(s) generated from the acid halide and/or acid anhydride, and/or other components derived from reagents, may remain to induce crystallization.

[0048]

Examples of the crystallization solvent for the crystallization treatment include water; organic acids such as acetic acid and propionic acid; esters such as ethyl acetate, isopropyl acetate, and butyl acetate; alcohols such as methanol, ethanol, 5 1-propanol, 2-propanol, 1-butanol, 2-ethyl-1-hexanol, and 2-butanol; ethers such as diethyl ether, di-*n*-butyl ether, diisopropyl ether, methyl-*tert*-butyl ether, di-*n*-butyl ether, tetrahydrofuran, and dioxane; ketones such as acetone, methyl ethyl ketone, and methyl isobutyl ketone; nitriles such as acetonitrile; aliphatic hydrocarbons such as hexane and heptane; aromatic hydrocarbons such as toluene and xylene; and 10 mixed solvents of two or more of these. Among these, alcohols, aliphatic hydrocarbons, aromatic hydrocarbons, and mixed solvents of two or more of these are preferred since solubility of the compound(s) of Formula (1) and/or Formula (2) in these solvents is sufficiently low. Aliphatic hydrocarbons, aromatic hydrocarbons, and mixed solvents of two or more of these are more preferred, and 15 hexane, heptane, toluene, and mixed solvents of two or more of these are especially preferred.

[0049]

Examples of the organic solvent to be used for the extraction include water-insoluble solvents, for example, esters such as ethyl acetate, isopropyl acetate, and 20 butyl acetate; ethers such as diethyl ether, methyl-*tert*-butyl ether, di-*n*-butyl ether, diisopropyl ether, di-*n*-butyl ether, *tert*-butyl methyl ether, and tetrahydrofuran; aliphatic hydrocarbons such as hexane and heptane; and aromatic hydrocarbons such as toluene and xylene.

[0050]

25 The above-described method is especially useful in cases where *cis*-5-hydroxy-2-piperidinecarboxylic acid is to be purified from a mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic

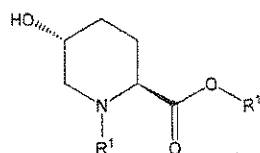
acid.

[0051]

That is, in a mode of the purification method of the present invention (the route 1 or route 2 in Fig. 1), a mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic acid is reacted with an acid halide and/or
5 acid anhydride to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into the compound of Formula (1). The resulting compound is then separated by crystallization and/or extraction with an organic solvent, and then treated with a base to convert the compound into *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid.
10 By subsequently carrying out deprotection of the amino group, *cis*-5-hydroxy-2-piperidinecarboxylic acid separated from the *trans* isomer can be obtained.

In such cases, the acid halide and/or acid anhydride is/are preferably used in an excessive amount over 1 molar equivalent relative to the total amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid and the *trans*-5-hydroxy-2-piperidinecarboxylic
15 acid. The amount of the acid halide and/or acid anhydride is usually 1 to 10 molar equivalents, preferably 1.2 to 5 molar equivalents, more preferably 1.5 to 3 molar equivalents. In cases where both an acid halide and an acid anhydride are used, these are added such that their total amount exceeds 1 molar equivalent relative to the total amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid and the *trans*-5-
20 hydroxy-2-piperidinecarboxylic acid.

By the reaction with the acid halide and/or acid anhydride, the *cis*-5-hydroxy-2-piperidinecarboxylic acid is converted to the compound of Formula (1). On the other hand, the *trans*-5-hydroxy-2-piperidinecarboxylic acid remains in the reaction system without being converted, or is converted to the mixed acid anhydride of *trans*-
25 5-hydroxy-2-piperidinecarboxylic acid described below. This mixed acid anhydride can be converted into the *trans*-5-hydroxy-2-piperidinecarboxylic acid again by post treatment. Thus, its separation by crystallization reaction is possible.

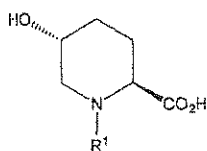


[0052]

In another mode of the purification method of the present invention (the route 3 in Fig. 1), a mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic acid is reacted with an acid halide and/or acid anhydride to protect the amino group. Subsequently, the *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid generated is reacted with an alcohol (or an ester that generates an alcohol) in the presence of an acid catalyst to allow conversion into the compound of Formula (2) via the compound of Formula (1). The resulting compound is separated by extraction with an organic solvent, and then treated with a base to allow conversion into *cis*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid. By subsequently carrying out deprotection of the amino group, *cis*-5-hydroxy-2-piperidinecarboxylic acid separated from the *trans* isomer can be obtained.

In such cases, the acid halide and/or acid anhydride is/are preferably used in an amount of about 1 molar equivalent relative to the total amount of the *cis*-5-hydroxy-2-piperidinecarboxylic acid and the *trans*-5-hydroxy-2-piperidinecarboxylic acid.

By performing the reaction with the acid halide and/or acid anhydride to protect the amino group, and then with the alcohol (or ester that generates an alcohol) in the presence of the acid catalyst, the *cis*-5-hydroxy-2-piperidinecarboxylic acid is converted into the compound of Formula (2), while the *trans*-5-hydroxy-2-piperidinecarboxylic acid is converted to the *trans*-*N*-protected-5-hydroxy-2-piperidinecarboxylic acid described below. Thus, their separation is possible by extraction with an organic solvent.



[0053]

The “*cis*-5-hydroxy-2-piperidinecarboxylic acid containing an impurity(s)” and “mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic acid” may be those synthesized by bacterial reaction and/or enzymatic reaction. For example, by the method described in Example 29 of JP 4590981 B, a “mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic acid” can be obtained. The “*cis*-5-hydroxy-2-piperidinecarboxylic acid containing an impurity(s)” and “mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic acid” may also be products recovered after separation of a *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative by the method of the present invention.

[0054]

In cases where *cis*-5-hydroxy-2-piperidinecarboxylic acid is purified by the method of the present invention from the “*cis*-5-hydroxy-2-piperidinecarboxylic acid containing an impurity(s)” or “mixture of *cis*-5-hydroxy-2-piperidinecarboxylic acid and *trans*-5-hydroxy-2-piperidinecarboxylic acid” synthesized by bacterial reaction and/or enzymatic reaction, the obtained *cis*-5-hydroxy-2-piperidinecarboxylic acid is preferably further subjected to adsorption purification using activated carbon and/or crystallization using a solvent containing water to remove impurities such as a colored substance, to further increase purity of the *cis*-5-hydroxy-2-piperidinecarboxylic acid.

[0055]

As the activated carbon, an arbitrary known activated carbon may be used, and examples of the known activated carbon include coal-based, wood-based,

coconut shell-based, and resin-based activated carbons. The activated carbon may also be prepared by activating a material activated carbon such as a coal-based, wood-based, coconut shell-based, or resin-based material activated carbon by a method such as gas activation, steam activation, or chemical activation using zinc chloride, phosphoric acid, or the like.

Specific examples of the activated carbon include Calgon CPG, Calgon CAL, Calgon SGL, Diasorb W, Diahope MS10, Diahope M010, Diahope MS16, Diahope 6MD, Diahope 6MW, Diahope 8ED, Diahope ZGN4, and CENTUR, manufactured by Calgon Mitsubishi Chemical Corporation; GAC, GAC PLUS, GCN PLUS, C GRAN, RO, ROX, DARCO, CN, SX, SX PLUS, SA, SX, PK, and W, manufactured by Norit Japan Co., Ltd.; GW, GWH, GLC, 4GC, KW, PW, and PK, manufactured by Kuraray Chemical Co., Ltd.; HC-30S, GL-30S, 4G-3S, PS, and PC, manufactured by Tsurumicoal Co., Ltd.; P, W, CW, SG, SGP, S, GB, CA, and K, manufactured by Futamura Chemical Co., Ltd.; Shirasagi KL, Shirasagi W2C, Shirasagi WH2C, Shirasagi W5C, Shirasagi WH5C, Shirasagi WH5X, Shirasagi XS7100H-3, Carboraffin, Shirasagi A, Shirasagi C, and Shirasagi M, manufactured by Japan EnviroChemicals Ltd.; and Hokuetsu CL-K, Hokuetsu HS, and Hokuetsu KS, manufactured by Ajinomoto Fine-Techno Co., Inc.

By this operation, impurities showing absorption within the wavelength range of 400 nm to 800 nm can be removed.

[0056]

As the solvent containing water to be used for the crystallization, a water-soluble organic solvent can be used. Specific examples of the solvent include organic acids such as acetic acid and propionic acid; esters such as ethyl acetate; alcohols such as methanol, ethanol, 1-propanol, 2-propanol, and 1-butanol; ethers such as tetrahydrofuran and dioxane; ketones such as acetone, methyl ethyl ketone, and diethyl ketone; nitriles such as acetonitrile; amides such as dimethylformamide,

dimethylacetamide, and *N*-methylpyrrolidinone; sulfones such as dimethylsulfoxide and sulfolane; and mixed solvents of two or more of these. It is also preferred to further increase purity of the *cis*-5-hydroxy-2-piperidinecarboxylic acid by carrying out crystallization of the *cis*-5-hydroxy-2-piperidinecarboxylic acid using the above-described solvent. Among these, organic acids, alcohols, and ketones, whose solubility in water is higher, are preferred. Alcohols and ketones are more preferred, and ethanol and acetone are especially preferred.

EXAMPLES

10 [0057]

The present invention is described below in more detail by way of Examples. However, the present invention is not limited by these Examples.

[0058]

15 The quantitative analyses in the Examples were carried out by HPLC (High Performance Liquid Chromatography) under the following conditions.

<HPLC-1>

Column: Astec CLC-D (4.6 mm × 150 mm, 5 μm), manufactured by
SUPELCO

Mobile phase: 2 mmol/L aqueous copper sulfate solution

20 Flow rate: 1.0 mL/minute

Column temperature: 45°C

Detection wavelength: UV 254 nm

<HPLC-2>

25 Column: L-column (4.6 mm × 250 mm, 5 μm), manufactured by Chemicals Evaluation and Research Institute, Japan

Mobile phase:

A: 0.1 wt% aqueous trifluoroacetic acid solution

B: methanol

gradient (concentration of B): 0 minute, 20% → 2 minute, 20% → 10 minute, 80% → 20 minute, 80%

Flow rate: 1.0 mL/minute

5 Column temperature: 40°C

Detection wavelength: UV 200 nm

<HPLC-3>

Column: ZORBAX Eclipse Plus C18 (4.6 mm × 150 mm, 1.8 μm),
manufactured by Agilent Technologies

10 Mobile phase:

A: 0.1 wt% aqueous phosphoric acid solution

B: acetonitrile

gradient (concentration of B): 0 minute, 55% → 6 minute, 55% → 9 minute, 80% → 12 minute, 80%

15 Flow rate: 1.0 mL/minute

Column temperature: 40°C

Detection wavelength: UV 210 nm

<HPLC-4>

Column: COSMOSIL 5C₁₈-AR-II (4.6 mm × 150 mm), manufactured by
20 Nacalai Tesque, Inc.

Mobile phase: 50 mmol/L phosphate buffer (pH 2.7)

Flow rate: 1.0 mL/minute

Column temperature: 40°C

Detection wavelength: UV 340 nm

25 <HPLC-5>

Column: SUMICHIRAL OA-6100 (4.6 mm × 250 mm), manufactured by
Sumika Chemical Analysis Service, Ltd.

Mobile phase: 1 mmol/L copper sulfate

Flow rate: 1.0 mL/minute

Column temperature: 30°C

Detection wavelength: UV 254 nm

5 <HPLC-6>

Column: CLC-D (4.6 mm × 150 mm), manufactured by SUPELCO

Mobile phase: 2 mmol/L copper sulfate

Flow rate: 1.0 mL/minute

Column temperature: 30°C

10 Detection wavelength: UV 254 nm

[0059]

[Reference Example 1]

[Preparation Example of Recombinant *Escherichia coli* (*E. coli*) JM109/pKW32

15 (dpkA, aip, gdh, kr), in Which *N*-Methyl-L-amino Acid Dehydrogenase (Hereinafter Referred to as DpkA), L-Amino Acid Oxidase (Hereinafter Referred to as AIP), Glucose-1-dehydrogenase (Hereinafter Referred to as GDH), and Amino Acid Racemase (hereinafter referred to as KR) Are Co-expressed]

(1) Cloning of Genes

20 Based on a gene sequence of dpkA (SEQ ID NO:1) encoding DpkA (GenBank Accession No. BAD89743, SEQ ID NO:2) derived from *Pseudomonas putida* (*P. putida*), primers for amplifying the full-length sequence of the dpkA gene, dpkA_F (SEQ ID NO:9) and dpkA_R (SEQ ID NO:10), were designed and synthesized. PCR was carried out using chromosomal DNA of *P. putida* as a
25 template according to a conventional method, to obtain a DNA fragment of about 1.0 kbp.

A gene sequence of aip (SEQ ID NO:3) encoding a protein AIP (SEQ ID

NO:4), which has the amino acid sequence of L-amino acid oxidase derived from *Scomber japonicus* (GenBank Accession No.CAC00499) with the exception that the signal peptide is removed and methionine is added, was designed and artificially synthesized. Primers for amplifying the full-length sequence of the aip gene, aip_F (SEQ ID NO:11) and aip_R (SEQ ID NO:12), were designed and synthesized. PCR was carried out according to a conventional method, to obtain a DNA fragment of about 1.5 kbp.

Based on a gene sequence of gdh (SEQ ID NO:5) encoding a protein (SEQ ID NO:6) having the amino acid sequence of GDH derived from *Bacillus subtilis* (GenBank Accession No.NP_388275) with the exception that the 96th amino acid residue, glutamic acid, is substituted with alanine, primers for amplifying the full-length sequence of the gdh gene, gdh_F (SEQ ID NO:13) and gdh_R(SEQ ID NO:14), were designed and synthesized. PCR was carried out according to a conventional method, to obtain a DNA fragment of about 0.8 kbp.

Based on a gene sequence of kr (SEQ ID NO:7) encoding KR derived from *P. putida* (GenBank Accession No. NP_745855, SEQ ID NO:8), primers for amplifying the full-length sequence of the kr gene, kr_F (SEQ ID NO:15) and kr_R (SEQ ID NO:16), were designed and synthesized. PCR was carried out using chromosomal DNA of *P. putida* as a template according to a conventional method, to obtain a DNA fragment of about 1.2 kbp.

[0060]

(2) Preparation of Expression Plasmid

Each of the DNA fragments obtained in (1) was digested with restriction enzymes *Eco*RI and *Xba*I, and introduced downstream of the trc promoter in a *Mun*I/*Xba*I digest of a plasmid pKW32, which is described in WO 2012/029819, using a Ligation-Convenience Kit (manufactured by Nippon Gene Co., Ltd.), to obtain pKW32dpkA, pKW32aip, pKW32gdh, and pKW32kr, respectively.

Subsequently, pKW32aip was digested with *SpeI* and *NdeI* to obtain a DNA fragment of about 2.4 kbp containing *aip*, and the resulting DNA fragment was introduced downstream of *dpkA* in the ring-opened (linear) plasmid of about 4.2 kbp obtained by digesting pKW32dpkA with *XbaI* and *NdeI*, to obtain pKW32 (*dpkA*, *aip*).

pKW32gdh was digested with *SpeI* and *NdeI* to obtain a DNA fragment of about 1.7 kbp containing *gdh*, and the resulting DNA fragment was introduced downstream of *aip* in the ring-opened (linear) plasmid of about 5.7 kbp obtained by digesting pKW32 (*dpkA*, *aip*) with *XbaI* and *NdeI*, to obtain pKW32 (*dpkA*, *aip*, *gdh*).

Finally, pKW32kr was digested with *SpeI* and *NdeI* to obtain a DNA fragment of about 2.1 kbp containing *kr*, and the resulting DNA fragment was introduced downstream of *gdh* in the ring-opened (linear) plasmid of about 6.5 kbp obtained by digesting pKW32 (*dpkA*, *aip*, *gdh*) with *XbaI* and *NdeI*, to obtain pKW32 (*dpkA*, *aip*, *gdh*, *kr*).

[0061]

(3) Preparation of Expressing Strain

Using the plasmid pKW32 (*dpkA*, *aip*, *gdh*, *kr*) obtained in (2), *E. coli* JM109 (manufactured by Takara Bio Inc.) was transformed according to a conventional method, to obtain recombinant *E. coli* JM109/pKW32 (*dpkA*, *aip*, *gdh*, *kr*).

[0062]

[Reference Example 2]

[Preparation Example of 5-Hydroxy-2-piperidinecarboxylic Acid Reaction Mixture]

To a 1-L jar fermenter (manufactured by ABLE Corporation, type BMJ), 45 g of 5-hydroxylysine hydrochloride (230 mmol, prepared according to a method described in Bull. Chem. Soc. Jpn., 1962, 35, 2006), 2.27 mL of Adekanol LG-109,

and 67.34 g (374 mmol) of glucose were added, and dissolved in water. Thereafter, 20 wt% aqueous sodium hydroxide solution was added dropwise thereto until the pH became 8. To the resulting liquid, bovine-liver-derived catalase (manufactured by Wako Pure Chemical Industries, Ltd.) was added at 2000 U/L; NADP⁺ (manufactured by Oriental Yeast Co., Ltd.) was added at 0.2 mmol/L; and wet cells of the recombinant *E. coli* JM109/pKW32 (dpkA, aip, gdh, kr) prepared in Reference Example 1 were added at 25 g/L; followed by adding water to the resulting mixture to a liquid volume of 566 mL. The reaction was allowed to proceed at 30°C, a stirring rate of 500 rpm, and an aeration rate of about 1 L/min for 43 hours. During the reaction, the pH was kept at 8 by adding 20 wt% aqueous sodium hydroxide solution dropwise. Exactly the same operations were carried out twice thereafter, and the reaction mixtures obtained by the 3 rounds of operations (using 135 g of 5-hydroxylysine hydrochloride, 690 mmol) were combined. The pH of the reaction mixture was adjusted to 2.5 using 6 mol/L sulfuric acid. Thereafter, 20 wt% aqueous sodium hydroxide solution was added dropwise to adjust the pH to 4, and the resulting liquid was centrifuged at 10,000 rpm for 20 minutes to remove insoluble impurities. The obtained supernatant was passed through a microfiltration membrane (manufactured by Asahi Kasei Corporation), and then through an ultrafiltration membrane (manufactured by Asahi Kasei Corporation), to remove impurities. Analysis of 5-hydroxy-2-piperidinecarboxylic acid contained in the resulting solution was carried out by HPLC under the conditions of <HPLC-1>. As a result, the amount of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid produced was 43.9g (302 mmol; yield, 44%), and the amount of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid produced was 29.7g (205 mmol; yield, 30%).

Example 1

[0063]

<1-1> Production of Benzyl (1*S*,4*S*)-5-Aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate

To 1153 g of a reaction mixture containing 20.7 g (143 mmol) of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and 18.0 g (124 mmol) of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid [(2*S*,5*S*):(2*S*,5*R*) = 53.6:46.4 (molar ratio)] obtained according to the method of Reference Example 2, 10 mol/L aqueous sodium hydroxide solution (60 mL) was added to adjust the pH from 3.65 to 10.81. The resulting liquid was concentrated under reduced pressure, to obtain 422.4 g of a slurry solution. In each of 2 flasks, 211.2 g of the obtained slurry was placed, and the internal temperature was decreased to 5°C to 7°C, followed by carrying out the following operations for each flask.

[The following is description of the operations for one of the flasks. Each flask contained 10.4 g (72 mmol) of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and 9.0 g (62 mmol) of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid.]

[0064]

To the flask, 18.8 mL (133 mmol) of benzyloxycarbonyl chloride was added dropwise, and 13.3 mL (133 mmol) of 10 mol/L aqueous sodium hydroxide solution was then added to adjust the pH from about 9 to 10, followed by allowing the reaction to proceed at a temperature within the range of 10°C to 20°C for 10 minutes. To the resulting liquid, 18.8 mL (133 mmol) of benzyloxycarbonyl chloride was added dropwise, and 13.5 mL (135 mmol) of 10 mol/L aqueous sodium hydroxide solution was then added to adjust the pH from 9 to 11, followed by allowing the reaction to proceed at a temperature within the range of 10°C to 20°C for 3 hours. To the resulting liquid, 19 mL of water was added, and 6.2 mL (44 mmol) of benzyloxycarbonyl chloride was then added dropwise, followed by allowing the liquid to gradually warm to room temperature. To the resulting liquid, 200 mL of toluene was added, and 4 mL of 10 mol/L aqueous sodium hydroxide solution (40

mmol) was then added to adjust the pH to 11, followed by separating the organic layer. To the aqueous layer, 100 mL of toluene was added to perform re-extraction, and the aqueous layer was separated to obtain the organic layer. This organic layer was combined with the previously obtained organic layer. The resulting mixture
 5 was washed with 10 mL of water, followed by separating the resulting organic layer. From the organic layer obtained from each of the two flasks, the solvent was removed by distillation under reduced pressure at 35°C, to obtain 64.9 g of a yellow oily substance.

As a result of ¹H-NMR analysis, this oily substance was found to be a mixture
 10 containing 45 wt% benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (111 mmol; yield, 78% with respect to the (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid), 15 wt% (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid=benzyloxyformic acid=anhydride (23 mmol; yield, 19% with respect to the (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid), 36 wt% benzyl
 15 alcohol, and 5 wt% toluene. Thus, the conversion of the material 5-hydroxy-2-piperidinecarboxylic acid, which contained (2*S*,5*S*) and (2*S*:5*R*) at a ratio of (2*S*,5*S*):(2*S*:5*R*) = 53.6:46.4 (molar ratio), to benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate allowed effective removal of compounds having the stereochemistry of (2*S*,5*R*), and, by this, the purity of compounds having
 20 the stereochemistry of (2*S*,5*S*) could be increased to (2*S*,5*S*):(2*S*:5*R*) = 82.8:17.2 (molar ratio).

[0065]

The resulting crude product was cooled to 10°C to 15°C, and 36 mL of toluene and 36 mL of hexane were added thereto. As a result, precipitation of white
 25 solids occurred. The precipitated white solids were collected by filtration, and washed by sprinkling 100 mL of hexane thereon. The resulting white solids were dried under reduced pressure at room temperature, to obtain 20.7 g of white solids.

As a result of ^1H -NMR analysis, the white solids were found to be a mixture containing 92.5 wt% benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (yield, 51% with respect to the (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid) and 7.5 wt% (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid=benzyloxyformic acid=anhydride. By the crystallization, the purity of compounds having the stereochemistry of (2*S*,5*S*) could be increased to (2*S*,5*S*):(2*S*,5*R*) = 92.5:7.5 (molar ratio).

^1H -NMR (400 MHz, CDCl_3)

δ 1.77-1.85 (1H, m), 2.02-2.25 (3H, m), 3.52 (1H, d, $J=11.9$ Hz), 3.70 (1H, dt, $J=12.2, 3.3$ Hz), 4.70-4.86 (2H, m), 5.12-5.21 (2H, m), 7.32-7.40 (5H, m).

[0066]

<1-1'> Production of Benzyl (1*S*,4*S*)-5-Aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate

To 80 g of a reaction mixture containing 1.74 g (12.0 mmol) of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and 1.01 g (7.0 mmol) of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid [(2*S*,5*S*):(2*S*,5*R*)=63.2:36.8 (molar ratio)] obtained according to the method of Reference Example 2, 10 mol/L aqueous sodium hydroxide solution was added to adjust the pH from 3.65 to 9.8. The resulting liquid was concentrated under reduced pressure, and 38 g of a solution was obtained. To the solution, 3.1 mL of 2-propanol was added. The internal temperature of the obtained solution was set to about 25°C. To the solution, 3.2 g (18.8 mmol) of benzyloxycarbonyl chloride was added dropwise, and 2.3 g (22.6 mmol) of 40 wt% aqueous sodium hydroxide solution was further added, followed by allowing the reaction to proceed at a temperature within the range of 20°C to 25°C for 30 minutes. To the resulting liquid, 3.2 g (18.8 mmol) of benzyloxycarbonyl chloride was added dropwise, and 2.3 g (22.6 mmol) of 40 wt% aqueous sodium hydroxide solution was further added, followed by allowing the reaction to proceed at a temperature within

the range of 20°C to 25°C for 30 minutes. Thereafter, the operation of adding 1.6 g (9.4 mmol) of benzyloxycarbonyl chloride dropwise to the resulting liquid, adding 1.1 g (11.3 mmol) of 40 wt% aqueous sodium hydroxide solution thereto, and then allowing the reaction to proceed at a temperature within the range of 20°C to 25°C for 30 minutes was repeated 3 times. Subsequently, the reaction mixture was cooled to 0°C to 5°C, and crystals precipitated in the reaction system were collected by filtration. The obtained crystals were dried under reduced pressure at 40°C to 45°C to obtain 2 g of white crystals.

As a result of ¹H-NMR analysis, the obtained crystals were found to be benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (80 wt%; yield, 56% with respect to the (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid).

[0067]

<1-1''> Production of Benzyl (1*S*,4*S*)-5-Aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate

To 160 g of a reaction mixture containing 3.26 g (22.5 mmol) of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and 2.24 g (15.4 mmol) of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid [(2*S*,5*S*): (2*S*,5*R*)=59.4:40.6 (molar ratio)] obtained according to the method of Reference Example 2, 10 mol/L aqueous sodium hydroxide solution was added to adjust the pH from 3.65 to 9.5. The resulting liquid was concentrated under reduced pressure, and 74 g of a solution was obtained. To the solution, 6.0 mL of 2-propanol was added. The internal temperature of the obtained solution was set to about 25°C. To the solution, 6.4 g (37.6 mmol) of benzyloxycarbonyl chloride was added dropwise, and 4.5 g (45.2 mmol) of 40 wt% aqueous sodium hydroxide solution was further added, followed by allowing the reaction to proceed at a temperature within the range of 20°C to 25°C for 30 minutes. To the resulting liquid, 6.4 g (37.6 mmol) of benzyloxycarbonyl chloride was added dropwise, and 4.5 g (45.2 mmol) of 40 wt% aqueous sodium hydroxide solution was

further added, followed by allowing the reaction to proceed at a temperature within the range of 20°C to 25°C for additional 30 minutes. Thereafter, 3.2 g (18.8 mmol) of benzyloxycarbonyl chloride was added dropwise to the reaction mixture, and 2.3 g (22.6 mmol) of 40 wt% aqueous sodium hydroxide solution was further added dropwise thereto, followed by allowing the reaction to proceed at a temperature within the range of 20°C to 25°C for 30 minutes. Thereafter, 1.9 g (11.1 mmol) of benzyloxycarbonyl chloride was added dropwise to the reaction mixture, and 1.4 g (14.0 mmol) of 40 wt% aqueous sodium hydroxide solution was added dropwise thereto, followed by allowing the reaction to proceed at a temperature within the range of 20°C to 25°C for 30 minutes. To the resulting reaction mixture, 71 mL of toluene was added, and liquid separation was carried out. To the extracted organic layer, 13 mL of water and 6.2 g of 40 wt% aqueous sodium hydroxide solution were added, and liquid separation was carried out again. The obtained aqueous layer, in an amount of 32 g, was divided into two aliquots, and subjected to the purification methods A and B.

[0068]

Purification Method A: Addition of Acetic Anhydride

To 16 g of the aqueous layer obtained in <1-1'>, 9.1 g (89 mmol) of acetic anhydride was added. The obtained crystals were collected by filtration, and dried under reduced pressure at 40°C to 45°C. The dried crystals appeared white, and their weight was 1.5 g.

The obtained crystals were analyzed by HPLC under the conditions of <HPLC-3>. As a result, the obtained crystals were found to be benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (100 wt%; yield, 50% with respect to the (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid).

[0069]

Purification Method B: Extraction under Acidic Conditions

To 16 g of the aqueous layer obtained in <1-1'>, 30 mL of water was added, and 5 mol/L hydrochloric acid was added thereto to adjust the pH in the system to 1.07. Thereafter, 55 mL of ethyl acetate was added to the resulting mixture, and liquid separation was carried out. Subsequently, 55 mL of ethyl acetate was added to the obtained aqueous layer, and liquid separation was carried out again. The obtained organic layers were combined, and concentrated under reduced pressure until the weight decreased to half of the original weight. To the obtained organic layer, 2.9 g (28.1 mmol) of triethylamine and 2.9 g (28.1 mmol) of acetic anhydride were added at 20°C to 25°C. After stirring the resulting mixture for 30 minutes, 0.57 g (5.6 mmol) of triethylamine and 0.57 g (5.6 mmol) of acetic anhydride were added thereto at 20°C to 25°C. After stirring the resulting reaction mixture for 30 minutes, 20 g of saturated aqueous sodium hydrogen carbonate solution was added thereto. After liquid separation, 71 mL of toluene was added to the obtained aqueous layer, and the operation of extraction was carried out again.

The obtained organic layers were combined, and concentrated under reduced pressure. Cooling of the obtained concentrate to 0°C to 5°C caused precipitation of crystals. The obtained crystals were collected by filtration, and then dried under reduced pressure at 40°C to 45°C. The dried crystals were white, and their weight was 2.4 g. The obtained crystals were analyzed by HPLC under the conditions of <HPLC-3>. As a result, the obtained crystals were found to be benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (98 wt%; yield, 70% with respect to the (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid).

[0070]

<1-2> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 20.0 g (92.5 wt%, 71 mmol) of the benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate obtained in <1-1> and 60 mL of ethanol were

added, and 76.5 mL of 1 mol/L aqueous sodium hydroxide solution was added thereto at room temperature, followed by stirring the mixture for 1 hour. The solvent was removed under reduced pressure at 35°C to 40°C, to obtain 61.2 g of a yellow oily substance. To the resulting substance, 100 mL of ethyl acetate was added, and the organic layer was separated. To the obtained aqueous layer, 14 mL of 5 mol/L hydrochloric acid was added to adjust the pH to 1.2. The aqueous layer was subjected to extraction using 200 mL of ethyl acetate twice, and the obtained organic layer was washed with 2 mL of water. After separation of the organic layer, the solvent was removed under reduced pressure at 35°C to 40°C, to obtain 24.0 g of a pale yellow oily substance.

As a result of ¹H-NMR analysis, this pale yellow oily substance was found to be (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (purity, 76 wt%; 65 mmol; yield, 92%). The peak for (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid was not found at all by ¹H-NMR.

¹H-NMR (400 MHz, CDCl₃, rotational isomer mixture)
 δ 1.27-1.39 (1H, m), 1.70-1.85 (1H, m), 1.98-2.06 (1H, m), 2.27-2.39 (1H, m), 2.80 (0.4H, t, *J*=11.8 Hz), 2.87 (0.6H, t, *J*=11.6 Hz), 3.61-3.72 (1H, m), 4.18-4.33 (1H, m), 4.81-4.88 (0.4H, m), 4.93-4.98 (0.6H, m), 5.12-5.21 (2H, m), 7.28-7.40 (5H, m).
 [0071]

<1-3> Production of (2*S*,5*S*)-5-Hydroxy-2-piperidinecarboxylic Acid

To a flask, 18.0 g (64.5 mmol) of the (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained in <1-2>, 90 mL of ethanol, and 2.5 g of 10% palladium carbon (manufactured by N. E. Chemcat Corporation, PE-type, 55.3%, containing water) were added, and hydrogenation was carried out under normal temperature and pressure. Three hours later, 22 mL of water was added thereto, and hydrogenation was carried out under normal temperature and pressure. Disappearance of the starting material could be confirmed 8.5 hours later. The

palladium carbon was removed by filtration, and 100 mL of water was sprinkled thereon for washing. From the obtained filtrate, the solvent was removed under reduced pressure at 45°C to 55°C, to obtain 11.6 g of a white slurry.

The obtained slurry was analyzed by HPLC under the conditions of <HPLC-1>. As a result, the slurry was found to contain 5-hydroxy-2-piperidinecarboxylic acid with a purity of 65 wt% in an amount of 64.5 mmol and yield of 81%. The isomer ratio was as follows: (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid:(2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid=98.8:1.2. The material used in <1-1> had a purity of as low as (2*S*,5*S*):(2*S*,5*R*)=53.7:46.3 (molar ratio), and the purity was increased to (2*S*,5*S*):(2*S*,5*R*)=98.8:1.2 (molar ratio) by the method of the present invention.

[0072]

<1-4> Activated Carbon Treatment, and Crystallization

To a flask, 0.38 g of the slurry obtained in <1-3> (purity, 65 wt%; 1.7 mmol), 2.5 mL of water, 0.025 g of an activated carbon manufactured by Calgon Mitsubishi Chemical Corporation, CAL, and 0.025 g of an activated carbon manufactured by Calgon Mitsubishi Chemical Corporation, 6ED, were added. The resulting mixture was stirred at room temperature for 1 hour, and the active carbons were then removed by filtration, followed by concentrating the aqueous solution to 0.5 mL. While the concentrate was stirred, 5.0 mL of ethanol was added dropwise thereto, and the resulting mixture was cooled to 5°C. As a result, crystals gradually precipitated. While the temperature was kept at 5°C, the stirring was continued for 1 hour, and 2.0 mL of acetone was added dropwise thereto at the constant temperature of 5°C, followed by stirring the resulting mixture for additional 0.5 hour. Thereafter, the precipitated crystals were collected by filtration, and 1.0 mL of an ice-cooled ethanol/acetone solution (=5/2 volume ratio) was sprinkled thereon for washing. The crystals were then dried under reduced pressure at 60°C, to obtain 0.25 g (purity,

98 wt%; yield, 99.2%) of crystals.

The obtained crystals were analyzed by HPLC under the conditions of <HPLC-1>. Based on the result, the isomer ratio of 5-hydroxy-2-piperidinecarboxylic acid contained in the obtained crystals was as follows: (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid:(2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid=99.5:0.5.

Example 2

[0073]

<2-1> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid and (2*S*,5*R*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To 124 mL of a reaction mixture containing 0.723 g (4.98 mmol) of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and 0.830 g (5.72 mmol) of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid obtained according to the method of Reference Example 2 [(2*S*,5*S*): (2*S*,5*R*)=46.5:53.5 (molar ratio)], 2.88 mL (36 mmol) of 50 wt% aqueous sodium hydroxide solution was added, and the resulting mixture was concentrated at a bath temperature of 40°C under a reduced pressure of 50 hPa, to obtain 132 g of a solution. In a water bath, 3.1 mL (22 mmol) of benzyloxycarbonyl chloride and 0.44 mL (5.5 mmol) of 50 wt% aqueous sodium hydroxide solution were dividedly added to the obtained solution while the pH was adjusted to about 9. After leaving the resulting solution to stand overnight, 60 mL of ethyl acetate and 0.88 mL (11 mmol) of 50 wt% aqueous sodium hydroxide solution were added thereto, followed by carrying out filtration through Celite. The organic layer was separated, and the aqueous layer was washed with ethyl acetate. To the aqueous layer, 2.9 mL of concentrated hydrochloric acid (33 mmol) was added to adjust the pH to 3. Thereafter, extraction with ethyl acetate was carried out 3 times. The solvent was removed from the resulting organic layer under reduced pressure at 35°C,

to obtain 2.44 g of a yellow oily substance.

As a result of $^1\text{H-NMR}$ analysis, this oily substance was found to be a mixture containing 80 wt% (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (6.91 mmol; yield, 65%; (2*S*,5*S*): (2*S*,5*R*)=4:6 (molar ratio)), 12 wt% ethyl acetate, and 8 wt% acetic acid.

[0074]

<2-2> Production of Benzyl (2*S*,5*S*)-3-Oxo-2-oxa-5-azabicyclo[2.2.2]octane-5-carboxylate

To a flask, 2.35 g of the (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained in <2-1> [6.72 mmol, (2*S*,5*S*): (2*S*,5*R*)=4:6 (molar ratio)], 10 mL of toluene, and 116 mg (0.67 mmol) of *p*-toluenesulfonic acid monohydrate were added, and the reaction was allowed to proceed at 60°C for 2 hours. After cooling the resulting reaction product to room temperature, 30 mL of ethyl acetate, 5 mL of water, and 7 mL of 1 mol/L sodium hydroxide solution were added thereto. As a result, the pH of the aqueous layer became 9. After separating the aqueous layer, re-extraction was carried out with ethyl acetate. The obtained organic layer was washed with saturated aqueous sodium bicarbonate solution and saturated brine. The resulting organic layer was concentrated to obtain 0.44 g of a brown oily substance.

As a result of $^1\text{H-NMR}$ analysis, this oily substance was found to be a mixture containing 75 wt% benzyl (2*S*,5*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (1.27 mmol; yield, 19%), 17 wt% ethyl acetate, and 9 wt% toluene.

In the material *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid, (2*S*,5*S*) and (2*S*,5*R*) were contained at (2*S*,5*S*): (2*S*,5*R*)=4:6 (molar ratio). By its conversion to benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate,

compounds having the stereochemistry of (2*S*,5*R*) could be effectively removed.

However, since the yield of benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate was 19%, the (2*S*,5*S*) isomer was obtained in a moderate yield of about 50%. It has been observed that a side reaction occurred between benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate generated by the reaction and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid left unreacted in the system, to cause esterification. This is assumed to be the cause of the moderate yield.

[0075]

10 <2-3> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 0.44 g (purity, 75 wt%; 1.27 mmol) of the benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate obtained in <2-2>, 2.6 mL of methanol, and 2.6 mL of 1 mol/L aqueous sodium hydroxide solution were added, and left to stand overnight. To the resulting reaction mixture, 1.3 mL of 2 mol/L hydrochloric acid was added to adjust the pH to 4, and extraction with ethyl acetate was then carried out. The resulting organic layer was concentrated to obtain 0.33 g of a brown oily substance. The substance was then purified by silica gel chromatography to obtain 0.25 g of (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (0.88 mmol; yield, 69%).

Example 3

[0076]

25 <3-1> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid and (2*S*,5*R*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 0.76 g of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid [5.24 mmol, (2*S*,5*S*): (2*S*,5*R*)=7:3

(molar ratio)], and 6.3 mL of 1 mol/L aqueous sodium hydroxide solution were added, and 0.93 mL (5.3 mmol) of benzyloxycarbonyl chloride was added to the resulting mixture under ice-cooling. The temperature of the mixture was then slowly increased to room temperature, and 2 mL of tetrahydrofuran and 5.2 mL of 1 mol/L aqueous sodium hydroxide solution were dividedly added thereto while the pH was adjusted to about 9. After leaving the resulting mixture to stand overnight, the organic layer was separated, and the aqueous layer was washed with ethyl acetate. To the aqueous layer, 3.2 mL of 2 mol/L hydrochloric acid was added to adjust the pH to 3, and the resulting mixture was extracted 3 times with ethyl acetate. The resulting organic layer was concentrated to obtain 0.79 g of a yellow oily substance.

As a result of ^1H -NMR analysis, this oily substance was found to be a mixture containing 76 wt% (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (2.15 mmol; yield, 41%; (2*S*,5*S*): (2*S*,5*R*)=7:3 (molar ratio)), and 24 wt% ethyl acetate.

[0077]

<3-2> Production of Benzyl (2*S*,5*S*)-5-Aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate

To a flask, 0.79 g of the (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained in <3-1> (purity, 76 wt%; 2.15 mmol; (2*S*,5*S*): (2*S*,5*R*)=7:3 (molar ratio)), 5 mL of toluene, and 49 mg (0.28 mmol) of *p*-toluenesulfonic acid monohydrate were added, and the reaction was allowed to proceed at 60°C for 2 hours. After cooling the resulting reaction product to room temperature, 10 mL of ethyl acetate, 3 mL of water, and 1.2 mL of 1 mol/L sodium hydroxide solution were added thereto. As a result, the pH of the aqueous layer became 7. After separating the aqueous layer, re-extraction was carried out with ethyl acetate. The obtained organic layer was washed with saturated aqueous

sodium bicarbonate solution and saturated brine. The resulting organic layer was concentrated to obtain 0.34 g of a brown oily substance.

As a result of ^1H -NMR analysis, this oily substance was found to be a mixture containing 62 wt% benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate (0.80 mmol; yield, 37%), 21 wt% (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (0.25 mmol; yield, 12%), and 17 wt% toluene.

In the material *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid, (2*S*,5*S*) and (2*S*,5*R*) were contained at (2*S*,5*S*):(2*S*,5*R*)=7:3 (molar ratio). By its conversion to benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate, compounds having the stereochemistry of (2*S*,5*R*) could be effectively removed. The total yield of benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate and (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid was 49%, and the (2*S*,5*S*) isomer was obtained in a moderate yield of about 70%.

Similarly to <2-1>, a side reaction occurred between benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate generated by the reaction and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid left unreacted in the system, to cause esterification. However, since the amount of (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid in the starting material was small, the (2*S*,5*S*) isomer could be selectively, and relatively efficiently recovered.

[0078]

<3-3> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 0.34 g of the oily substance obtained in <3-2> [containing 0.80 mmol benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate and 0.25 mmol (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid], 2 mL of methanol, and 1.9 mL of 1 mol/L aqueous sodium hydroxide solution were added,

and left to stand overnight. To the resulting reaction mixture, 2 mL of 2 mol/L hydrochloric acid was added to adjust the pH to 4, and extraction with ethyl acetate was then carried out. The resulting organic layer was concentrated to obtain 0.39 g of a brown oily substance.

5 As a result of ^1H -NMR analysis, this oily substance was found to be a mixture containing 76 wt% (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (1.05 mmol; yield, 100%; (2*S*,5*S*):(2*S*,5*R*)=87:13 (molar ratio)), 23 wt% ethyl acetate, and 1 wt% acetic acid.

[0079]

10 <3-4> Production of (2*S*,5*S*)-5-Hydroxy-2-piperidinecarboxylic acid

To a flask, 0.39g of the (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained in <3-3> (purity, 77 wt%; 1.06 mmol), 2 mL of methanol, and 61 mg of 10% palladium carbon (manufactured by N. E. Chemcat Corporation, PE-type, 55.3%, containing water) were added, and hydrogenation was

15 carried out under normal temperature and pressure for 3 hours. The palladium carbon was removed by filtration through Celite, and washing with methanol-water was carried. The obtained filtrate was concentrated to obtain 0.21 g of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid as a pale yellow oily substance.

Example 4

20 [0080]

<4-1> Production of Benzyl (1*S*,4*S*)-5-Aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate and Ethyl (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate

To a flask, 0.26 g of (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained according to the method of <2-1> (purity, 82 wt%; 0.76 mmol; (2*S*,5*S*):(2*S*,5*R*)=5:5 (molar ratio)), 1 mL of ethyl acetate, and 5 μL

25

(0.08 mmol) of methanesulfonic acid were added, and the reaction was allowed to proceed at 60°C for 3 hours. After cooling the resulting reaction product to room temperature, 10 mL of ethyl acetate and 1 mL of 1 mol/L sodium hydroxide solution were added thereto. As a result, the pH of the aqueous layer became about 10.

5 After separating the aqueous layer, re-extraction was carried out with ethyl acetate. The obtained organic layer was dried over magnesium sulfate. The resulting organic layer was concentrated to obtain 0.12 g of a pale brown oily substance.

As a result of ¹H-NMR analysis, this oily substance was found to be a mixture containing 26 wt% benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-
10 carboxylate (0.11 mmol; yield, 15%), 52 wt% ethyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (0.20 mmol; yield, 26%), 13 wt% ethyl acetate, and 8 wt% toluene.

It is thought that, in the present Example, a small amount of ethanol generated from ethyl acetate reacted with benzyl (1*S*,4*S*)-5-aza-2-oxa-3-
15 oxobicyclo[2.2.2]octane-5-carboxylate, to generate ethyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate. In the material *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid, (2*S*,5*S*) and (2*S*,5*R*) were contained at (2*S*,5*S*):(2*S*,5*R*)=5:5 (molar ratio). As a result of ¹H-NMR analysis and HPLC analysis (under the conditions of <HPLC-2>), it could be confirmed that the
20 isomer ratio of the ethyl *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate was as follows: (2*S*,5*S*):(2*S*,5*R*)>10:1 (molar ratio). Thus, compounds having the stereochemistry of (2*S*,5*R*) could be effectively removed. Since the total yield of benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate and ethyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate was 41%, the yield
25 of the (2*S*,5*S*) isomer was about 80%. Thus, the (2*S*,5*S*) isomer could be selectively and efficiently recovered.

[0081]

<4-2> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 0.12 g of the oily substance obtained in <4-1> (containing 0.11 mmol of benzyl (1*S*,4*S*)-5-aza-2-oxa-3-oxobicyclo[2.2.2]octane-5-carboxylate and
 5 0.20 mmol of ethyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate), 1 mL of ethanol, and 0.66 mL of 1 mol/L aqueous sodium hydroxide solution were added, and left to stand overnight. The reaction mixture was then concentrated, and 0.7 mL of 1 mol/L hydrochloric acid was added thereto to adjust the pH to 3, followed by carrying out extraction with ethyl acetate. The
 10 resulting organic layer was concentrated under reduced pressure to obtain 0.11 g of a brown oily substance.

As a result of ¹H-NMR analysis, this oily substance was found to be a mixture containing 84 wt% (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (0.33 mmol, quantitative, (2*S*,5*S*): (2*S*,5*R*)=10:1 (molar ratio)), 11 wt% ethyl
 15 acetate, and 5 wt% toluene.

[0082]

<4-3> Production of (2*S*,5*S*)-5-Hydroxy-2-piperidinecarboxylic Acid

To a flask, 0.11 g of the (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained in <4-2> (purity, 82 wt%, 0.33 mmol), 1 mL of
 20 methanol, and 18 mg of 10% palladium carbon (manufactured by N. E. Chemcat Corporation, PE-type, 55.3%, containing water) were added, and hydrogenation was carried out under normal temperature and pressure for 9 hours. The palladium carbon was removed by filtration through Celite, and washing with methanol-water was carried. The obtained filtrate was concentrated to obtain 58 mg of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid as a pale yellow oily substance
 25 ((2*S*,5*S*): (2*S*,5*R*)=10:1 (molar ratio), ¹H-NMR analysis).

Example 5

[0083]

<5-1> Production of Ethyl (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate

5 To a flask, 0.55 g of (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained according to the method of <2-1> (purity, 78 wt%; 1.52 mmol; (2*S*,5*S*): (2*S*,5*R*)=3:7 (molar ratio)), 2 mL of toluene, 0.5 mL of ethanol, and 10 μ L (0.15 mmol) of methanesulfonic acid were added, and the
10 reaction was allowed to proceed at 40°C for 5 hours.

The reaction mixture was analyzed by HPLC under the conditions of <HPLC-2>, and the following result was obtained: ethyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate:ethyl (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate:(2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-
15 piperidinecarboxylic acid:(2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid=24:8:7:57.

Under the assumption that the material and products have the same absorption coefficient, the degree of conversion of the (2*S*,5*S*) isomers was about 77%, and the degree of conversion of the (2*S*,5*R*) isomers was about 12%. Thus, the (2*S*,5*S*)
20 isomers showed a higher degree of esterification.

[0084]

<5-1'> Production of Isopropyl (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate

To a flask, 0.62 g of (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-
25 piperidinecarboxylic acid and (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained according to the method of <2-1> (purity, 78 wt%; 1.72 mmol; (2*S*,5*S*): (2*S*,5*R*)=3:7 (molar ratio)), 2 mL of toluene, 0.5 mL of 2-

propanol, and 11 μL (0.17 mmol) of methanesulfonic acid were added, and the reaction was allowed to proceed at 50°C for 5 hours. After cooling the resulting reaction product to room temperature, 3 mL of toluene and 1.2 mL of 1 mol/L sodium hydroxide solution were added thereto. As a result, the pH of the aqueous layer became about 9. After separating the aqueous layer, re-extraction was carried out with toluene. The resulting organic layer was concentrated to obtain 0.17 g of a pale brown oily substance.

As a result of ^1H -NMR analysis, this oily substance was found to be a mixture containing 91 wt% isopropyl *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate (0.47 mmol; yield, 27%; (2*S*,5*S*):(2*S*,5*R*)=10:1 (molar ratio)), and 9 wt% toluene.

In the present Example, the material *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid contained (2*S*,5*S*) and (2*S*,5*R*) at (2*S*,5*S*):(2*S*,5*R*)=3:7 (molar ratio). Based on the result of ^1H -NMR analysis, the isomer ratio of the isopropyl *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate was follows: (2*S*,5*S*):(2*S*,5*R*)=10:1 (molar ratio). Thus, compounds having the stereochemistry of (2*S*,5*R*) could be effectively removed. Since the yield of the isopropyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate was 27%, the yield of the (2*S*,5*S*) isomer was about 80%. Thus, the (2*S*,5*S*) isomer could be selectively and efficiently recovered.

[0085]

<5-2> Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 0.17 g of the isopropyl (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylate obtained in <5-1'> (purity, 91 wt%; 0.47 mmol), 0.7 mL methanol, and 0.71 mL of 1 mol/L aqueous sodium hydroxide solution were added, and the reaction was allowed to proceed at 60°C for 4 hours. After washing the

reaction mixture with toluene, 0.45 mL of 2 mol/L hydrochloric acid was added thereto to adjust the pH to 3, and extraction with ethyl acetate was carried out. The resulting organic layer was concentrated to obtain 0.17 g of a brown oily substance. As a result of ¹H-NMR analysis, this oily substance was found to be a mixture
 5 containing 70 wt% *N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (0.41 mmol; yield, 88%; (2*S*,5*S*):(2*S*,5*R*)=7:1 (molar ratio)), 26 wt% ethyl acetate, and 4 wt% toluene.

[0086]

<5-3> Production of (2*S*,5*S*)-5-Hydroxy-2-piperidinecarboxylic Acid

10 To a flask, 0.17 g of the (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid obtained in <5-2> (purity, 70 wt%; 0.41 mmol; (2*S*,5*S*):(2*S*,5*R*)=7:1 (molar ratio)), 1 mL of methanol, and 22 mg of 10% palladium carbon (manufactured by N. E. Chemcat Corporation, PE-type, 55.3%, containing water) were added, and hydrogenation was carried out under normal temperature and
 15 pressure for 3 hours. The palladium carbon was removed by filtration through Celite, and washing with methanol-water was carried. The obtained filtrate was concentrated to obtain 89 mg of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid as a pale yellow oily substance.

20 [0087]

[Reference Example 3]

<3-1> Cloning of Lysine Hydroxylase Gene

Based on a gene sequence (hyl-1, SEQ ID NO:17) encoding an L-arginine-β hydroxylase VioC homologue Hyl-1 derived from the *Flavobacterium johnsoniae*
 25 NBRC14942 strain (GenBank Accession No. ABQ06186, SEQ ID NO:18), primers for amplifying the full-length sequence of the hyl-1 gene, hyl1_F (SEQ ID NO:29) and hyl1_R (SEQ ID NO:30), were designed and synthesized. Using chromosomal

DNA of *Flavobacterium johnsoniae* as a template, PCR was carried out according to a conventional method, to obtain a DNA fragment of about 1.0 kbp.

In addition, VioC homologues derived from the *Kineococcus radiotolerans* NBRC101839 strain, *Chitinophaga pinensis* NBRC15968 strain, *Chryseobacterium*
 5 *gleum* NBRC15054 strain, and *Niastella koreensis* NBRC106392 strain were designated Hyl-2 (GenBank Accession No.ABS05421, SEQ ID NO:20), Hyl-3 (GenBank Accession No.ACU60313, SEQ ID NO:22), Hyl-4 (GenBank Accession No.EFK34737, SEQ ID NO:24), and Hyl-5 (GenBank Accession No.AEV99100, SEQ ID NO:26), respectively. Based on gene sequences encoding the enzymes,
 10 (hyl-2 (SEQ ID NO:19), hyl-3 (SEQ ID NO:21), hyl-4 (SEQ ID NO:23), and hyl-5 (SEQ ID NO:25)), primers for amplifying the full-length sequence of each gene were designed and synthesized. Primers hyl2_f (SEQ ID NO:31) and hyl2_r (SEQ ID NO:32) for hyl-2, primers hyl3_f (SEQ ID NO:33) and hyl3_r (SEQ ID NO:34) for hyl-3, primers hyl4_f (SEQ ID NO:35) and hyl4_r (SEQ ID NO:36) for hyl-4, and
 15 primers hyl5_f (SEQ ID NO:37) and hyl5_r (SEQ ID NO:38) for hyl-5 were synthesized, and PCR was carried out using chromosomal DNA of each strain according to a conventional method. Each reaction produced a DNA fragment of about 1.0 kbp.

Each of the 5 kinds of DNA fragments obtained was digested with restriction
 20 enzymes *NdeI* and *XhoI*, and ligated into *NdeI/XhoI*-digested pET21a (Novagen) according to a conventional method, to obtain pEHYL1, pEHYL2, pEHYL3, pEHYL4, and pEHYL5, respectively.

A gene sequence (hyl-6, SEQ ID NO:27) encoding Hyl-6 (GenBank Accession No. ABS05421, SEQ ID NO:28), which was derived from a marine
 25 actinobacterium PHSC20C1, was artificially synthesized, and inserted into pJExpress401 (DNA2.0) to prepare a plasmid pJHYL6.

Subsequently, *E. coli* (*Escherichia coli*) BL21(DE3) (manufactured by

Invitrogen) was transformed with each of the resulting plasmids according to a conventional method, to obtain recombinant *E. coli* BL21(DE3)/pEHYL1, BL21(DE3)/pEHYL2, BL21(DE3)/pEHYL3, BL21(DE3)/pEHYL4, BL21(DE3)/pEHYL5, and BL21(DE3)/pJHYL6. In order to obtain bacterial cells expressing the genes, each type of recombinant *E. coli* was cultured at 30°C using liquid LB medium supplemented with ampicillin and a lac promoter inducer, and collected after 20 hours of the culture.

[0088]

<3-2> Confirmation of Lysine Hydroxylase Activity by Resting-cell Reaction

In a plastic tube, 5 mmol/L L-lysine, 10 mmol/L 2-oxoglutaric acid, 1 mmol/L L-ascorbic acid, 0.1 mmol/L iron sulfate, and recombinant *E. coli* obtained by the method according to Reference Example <3-1> were mixed to provide a reaction mixture such that the turbidity OD₆₀₀ was 10. The reaction was allowed to proceed in 0.5 mL of the prepared mixture at 30°C at pH 7.0 for 3 hours. The reaction product was derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA), and hydroxylysine was analyzed by HPLC under the conditions of <HPLC-4>. As a result, as shown in Fig. 2 and Fig. 3, it could be confirmed that BL21(DE3)/pEHYL2 and BL21(DE3)/pJHYL6 produced a compound corresponding to the retention time of a standard product of 3-hydroxylysine, 8.04 minutes. It could also be confirmed that BL21(DE3)/pEHYL1, BL21(DE3)/pEHYL3, BL21(DE3)/pEHYL4, and BL21(DE3)/pEHYL5 produced a compound corresponding to the retention time of a standard product of 4-hydroxylysine, 8.16 minutes.

[0089]

<3-3> Synthesis of (2*S*,3*S*)-3-Hydroxylysine

To a 1-L jar fermenter, 35 mL of 1 mol/L potassium phosphate buffer (pH 7.0), 304 mL of desalted water, 1.28 g of L-lysine hydrochloride, 2.05 g of 2-oxoglutaric acid, 0.14 g of sodium L-ascorbate, 0.02 g of iron sulfate, 0.35 g of

Adekanol LG109, and 8 g of wet cells of recombinant *E. coli* BL21(DE3)/pEHYL2 obtained by the method according to Reference Example <3-1> were mixed together, and the reaction was allowed to proceed at 30°C, pH 7.0, a stirring rate of 500 rpm, and an aeration rate of 2.0 vvm for 17 hours. Completion of the reaction was
 5 judged by confirming disappearance of the peak for L-lysine by carrying out HPLC analysis under the conditions of <HPLC-5>. From the liquid after the completion of the reaction, bacterial cells and bacterial debris were removed by centrifugation and microfiltration, to obtain 390 g of a filtrate.

After passing 390 g of the filtrate through an ion-exchange resin column
 10 (DIAION (registered trademark) SK-1B (Type H), 60.0 g), washing was carried out with water, followed by elution with an aqueous solution containing 150 mmol of ammonia. The ammonia eluate was concentrated to obtain 1.0 g of (2*S*,3*S*)-3-hydroxylysine (6.17 mmol; yield, 88%).

¹H-NMR (400MHz, D₂O) δ, 1.45-1.58 (2H, m), 1.63-1.73 (1H, m), 1.74-1.88 (1H,
 15 m), 2.93-3.04 (2H, m), 3.47 (1H, d, *J*=4.3 Hz), 3.89 (1H, dt, *J*=8.4, 4.5 Hz)
 [0090]

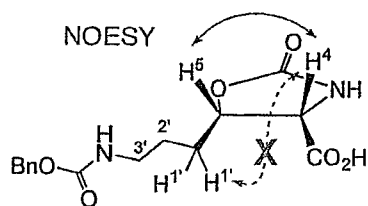
<3-4> Determination of Stereochemistry of (2*S*,3*S*)-3-Hydroxylysine

To a flask, 8.3 mg (0.051 mmol) of (2*S*,3*S*)-3-hydroxylysine obtained by the method according to Reference Example <3-3>, 0.26 ml of 1 mol/L aqueous sodium
 20 hydroxide solution, and 18 μL (0.13 mmol) of benzyloxycarbonyl chloride were added, and the resulting mixture was stirred at room temperature for 1 hour. To the mixture, 0.26 mL of 1 mol/L aqueous sodium hydroxide solution and 18 μL (0.13 mmol) of benzyloxycarbonyl chloride were further added, and the reaction was allowed to proceed overnight at room temperature. Subsequently, 0.5 mL of
 25 tetrahydrofuran was added to the reaction mixture, and the reaction was allowed to proceed at 60°C for additional 2 hours. After cooling the reaction mixture to room temperature, 95 mg of sodium hydroxide was added thereto, and the reaction was

allowed to proceed overnight at room temperature. The reaction mixture was washed twice with toluene-tetrahydrofuran (1:1), and 250 μ L of concentrated hydrochloric acid was added to the reaction mixture to make the reaction mixture strongly acidic. After 3 times of washing with ethyl acetate, the aqueous layer was extracted 4 times with 1-butanol. The 1-butanol layer was dried over anhydrous magnesium sulfate, and then concentrated, to obtain 14.6 mg of (4*S*,5*S*)-5-(3-benzyloxycarbonylamino-
 5
 propyl)-2-oxo-4-oxazolidinecarboxylic acid (0.045 mmol; yield, 89%).

The stereochemistry of the obtained (4*S*,5*S*)-5-(3-benzyloxycarbonylamino-
 10
 propyl)-2-oxo-4-oxazolidinecarboxylic acid was determined by NOESY measurement. The NOESY measurement was carried out using an AVANCE DRX500 spectrometer manufactured by Bruker (equipped with a CryoProbe) at 25°C with a mixing time of 0.8 msec. As a chemical shift reference, 3.31 ppm for methanol was used. The result of NOESY measurement was as
 15
 follows.

[0091]



[0092]

Since a cross peak was found between the 4-position hydrogen atom (H⁴) and
 20
 5-position hydrogen atom (H⁵), but was not found between the 4-position and the 1'-position, the substituents at the 4-position and the 5-position could be confirmed to have the *cis* configuration. Since the absolute configuration of the lysine used in the enzymatic reaction was *S*, it could be confirmed that the 5-(3-benzyloxycarbonylamino-
 propyl)-2-oxo-4-oxazolidinecarboxylic acid obtained in the

present Reference Example has the stereochemistry of (4*S*,5*S*), and that 3-hydroxylysine as its material has the stereochemistry of (2*S*,3*S*).

¹H-NMR (400 MHz, MeOH-d₄) δ, 1.39-1.53 (3H, m, H^{1'}, H^{2'} x2), 1.59-1.68 (1H, m, H^{1'}), 3.02-3.08 (2H, m, H^{3'}), 3.60-3.64 (1H, m, H⁴), 3.93-4.00 (1H, m, H⁵), 4.90-5.02 (2H, m, Bn), 7.18-7.28 (5H, m, Bn).

[0093]

[Reference Example 4]

Production of (2*S*,3*S*)-3-Hydroxypipicollic Acid [(2*S*,3*S*)-3-Hydroxy-2-piperidinecarboxylic Acid]

In a plastic tube, 0.75 mL of 1 mol/L tris hydroxymethyl aminomethane buffer (pH 8.0), 9.21 mL of desalted water, 86 mg of the (2*S*,3*S*)-3-hydroxylysine obtained in Reference Example <3-3>, 0.083 mL of 50 mmol/L NADPH, 0.7 mL of 1.0 mol/L glucose, and 1.25 mL of a 100-g/L suspension of the recombinant *E. coli* JM109/pKW32 (dpkA, aip, gdh, kr) obtained in Reference Example 1 were mixed together, and the reaction was allowed to proceed at 30°C at pH 8.0 at a stirring rate of 1000 rpm for 20 hours. Completion of the reaction was judged by confirming disappearance of the peak for (2*S*,3*S*)-3-hydroxylysine by HPLC analysis under the conditions of <HPLC-6>. From the liquid after the completion of the reaction, bacterial cells and bacterial debris were removed by centrifugation, to obtain 10.5 g of a supernatant.

After passing 10.5 g of the supernatant through an ion-exchange resin column (DIAION (registered trademark) SK-1B (Type H), 4.0g), washing was carried out with water, followed by elution with an aqueous solution containing 16.4 mmol of ammonia. The ammonia eluate was concentrated to obtain 255 mg of a solid brown substance. As a result of NMR analysis, this solid substance was found to be a mixture containing 20 wt% (2*S*,3*R*)-3-hydroxypipicollic acid (0.35 mmol; yield,

66.3%) and 80 wt% tris hydroxymethyl aminomethane.

¹H-NMR (400 MHz, D₂O) δ , 1.38-1.56 (2H, m), 1.73-1.85 (2H, m), 2.71-2.79 (1H, m), 3.04-3.11 (1H, m), 3.23 (1H, d, $J=7.6$ Hz), 3.79-3.86 (1H, m).

5 Example 6

[0094]

Production of (2*S*,5*S*)-*N*-Benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic Acid

To a flask, 78.7 mg (0.542 mmol) of (2*S*,5*S*)-5-hydroxy-2-piperidinecarboxylic acid and 2.8 mg (0.019 mmol) of (2*S*,5*R*)-5-hydroxy-2-piperidinecarboxylic acid obtained according to the method of Example 1, and 98.5 mg of (2*S*,3*S*)-3-hydroxy-2-piperidinecarboxylic acid obtained according to the method of Reference Example 4 (purity, 20 wt%, 0.136 mmol; containing 78.8 mg of tris hydroxymethyl aminomethane) were added, and 0.200 mL of water and 0.674 mL of 2 mol/L aqueous sodium hydroxide solution were added thereto at room temperature. After adding 0.190 mL (1.35 mmol) of benzyloxycarbonyl chloride dropwise to the resulting mixture, 0.500 mL of 2 mol/L aqueous sodium hydroxide solution was added thereto to adjust the pH from 9 to 10. The reaction was allowed to proceed at room temperature for 25 minutes, and 0.098 mL (0.697 mmol) of benzyloxycarbonyl chloride was then added dropwise to the resulting reaction mixture, followed by adding 0.400 mL of 2 mol/L aqueous sodium hydroxide solution thereto to adjust the pH from 10 to 11. The reaction was further allowed to proceed at room temperature for 40 minutes, and 0.098 mL (0.697 mmol) of benzyloxycarbonyl chloride was added dropwise thereto, followed by allowing the reaction to proceed at room temperature for 2 hours. To the resulting reaction mixture, 3 mL of toluene was added, and liquid separation was then carried out. To the extracted organic layer, 0.500 mL of water was added, and liquid separation was carried out again. The resulting organic layer was concentrated to obtain 330 mg of

a pale yellow oily substance. To this oily substance, 2 mL of ethanol, 1.1 mL of 1 mol/L aqueous sodium hydroxide solution, and 0.3 mL of water were added, and the reaction was allowed to proceed at room temperature for about 1 hour. The resulting reaction mixture was concentrated, and ethanol was evaporated.

5 Thereafter, 1 mL of ethanol, 0.3 mL of water, 0.1 mL of 1 mol/L aqueous sodium hydroxide solution, and 0.1 mL of 2 mol/L aqueous sodium hydroxide solution were added thereto, and the reaction was allowed to proceed at room temperature for 2 hours. The reaction mixture was concentrated, and ethanol was evaporated, followed by carrying out liquid separation using 3 mL of toluene. To the obtained
10 aqueous layer, 1 mL of water and 0.75 mL of 1 mol/L hydrochloric acid were added to adjust the pH of the aqueous solution to 1. The aqueous layer was subjected twice to extraction with 4 mL of ethyl acetate, and the resulting organic layer was concentrated to obtain 92.0 mg of a pale yellow oily substance.

As a result of ^1H -NMR analysis, this pale yellow oily substance was found to
15 contain 80.5 wt% (2*S*,5*S*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid (0.265 mmol; yield, 49%) and 19.5 wt% ethyl acetate. No peak was found at all for either (2*S*,5*R*)-*N*-benzyloxycarbonyl-5-hydroxy-2-piperidinecarboxylic acid or (2*S*,3*S*)-*N*-benzyloxycarbonyl-3-hydroxy-2-piperidinecarboxylic acid by ^1H -NMR.

20 INDUSTRIAL APPLICABILITY

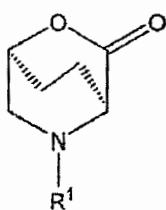
[0095]

The present invention can be used as a method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid, which is useful as an intermediate for pharmaceuticals, and can also be used as a method for producing its derivatives.

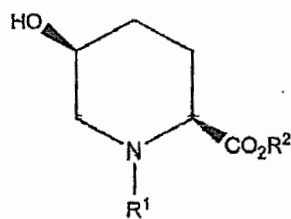
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Claims

1. A method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative, (wherein R^1 represents a protective group for an amino group, and R^2 represents a C_1 - C_6 alkyl group).



(1)



(2)

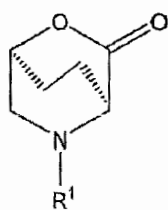
comprising a step of reacting *cis*-5-hydroxy-2-piperidinecarboxylic acid with an acid halide and/or acid anhydride to convert said *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound of Formula (1); or

comprising a step of reacting *cis*-5-hydroxy-2-piperidinecarboxylic acid with an acid halide and/or acid anhydride, and then with an alcohol in the presence of an acid catalyst, to convert said *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound of Formula (2).

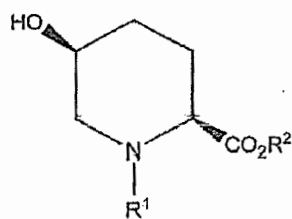
2. A method for regenerating *cis*-5-hydroxy-2-piperidinecarboxylic acid, said method comprising the steps of:

converting *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2) according to the method of claim 1 (wherein R^1 represents a protective group for an amino group, and R^2 represents a C_1 - C_6 alkyl group); and

converting the compound(s) of Formula (1) and/or Formula (2) into *cis*-5-hydroxy-2-piperidinecarboxylic acid.



(1)

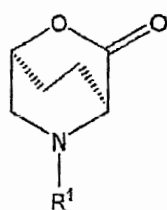


(2)

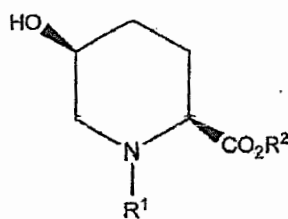
3. The method for producing a *cis*-5-hydroxy-2-piperidinecarboxylic acid derivative according to claim 1, wherein said *cis*-5-hydroxy-2-piperidinecarboxylic acid is *cis*-5-hydroxy-2-piperidinecarboxylic acid synthesized by bacterial reaction and/or enzymatic reaction.

4. The method for regenerating *cis*-5-hydroxy-2-piperidinecarboxylic acid according to claim 2, wherein said *cis*-5-hydroxy-2-piperidinecarboxylic acid is *cis*-5-hydroxy-2-piperidinecarboxylic acid synthesized by bacterial reaction and/or enzymatic reaction.

5. A method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid, said method comprising a step of reacting a mixture containing *cis*-5-hydroxy-2-piperidinecarboxylic acid and an impurity with an acid halide and/or acid anhydride, or with an acid halide and/or acid anhydride and an alcohol, to convert the *cis*-5-hydroxy-2-piperidinecarboxylic acid into a compound(s) of Formula (1) and/or Formula (2), separating said compound(s) and then converting the separated compound(s) into *cis*-5-hydroxy-2-piperidinecarboxylic acid.



(1)



(2)

6. The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to claim 5, wherein said impurity is 2-piperidinecarboxylic acid or an analogue thereof.

7. The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to claim 6, wherein said 2-piperidinecarboxylic acid or an analogue thereof is *trans*-5-hydroxy-2-piperidinecarboxylic acid.

8. The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to any one of claims 5 to 7, wherein said step of separating the compound(s) of Formula (1) and/or Formula (2) is carried out by crystallization or solvent extraction.

9. The method for purifying *cis*-5-hydroxy-2-piperidinecarboxylic acid according to any one of claims 5 to 8, wherein said mixture containing

cis-5-hydroxy-2-piperidinecarboxylic acid and an impurity is a mixture synthesized by bacterial reaction and/or enzymatic reaction.