



DESCRIPTION



G00002

OPTICALLY ACTIVE FLUOROPHENYLALANINE DERIVATIVE AND PROCESS FOR PREPARING THE SAME

5

TECHNICAL FIELD

The present invention relates to (*R*)-N-(aminocarbonyl)-fluorophenylalanine which is a useful intermediate for manufacturing a medicine and a process for preparing the same.

10

BACKGROUND ART

It is considered that the corresponding (*R*)-2-hydroxy-3-(fluorophenyl)propionic acids can be easily derived from (*R*)-N-(aminocarbonyl)-fluorophenylalanines of the invention. These (*R*)-fluorophenylalanine derivatives are amino acid derivatives which are important as the compound that can be easily transformed into a synthetic key intermediate for fluorine-containing medicines (refer to, for example, Journal of Medicinal Chemistry, Vol.38, pp.5023, 1995; JP-A-61-260099; JP-A-9-208541; and WO-A-97/38009).

20

As the process for preparing optically active N-(aminocarbonyl)-phenylalanine derivatives, especially (*R*)-configuration compounds thereof, there has been known a method in which 5-benzylhydantoin or 5-(substituted benzyl)hydantoin is treated with a microorganism having the ability to selectively hydrolyze (*R*)-isomer thereof to give the corresponding (*R*)-N-(aminocarbonyl)-phenylalanine derivative. For example, there are:

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(1) a case where (*R*)-N-(aminocarbonyl)-phenylalanine and (*R*)-N-

(aminocarbonyl)-tyrosine were obtained by treating 5-benzylhydantoin and 5-(4-hydroxybenzyl)hydantoin respectively with a microorganism belonging to the genus *Bacillus* (JP-A-56-58493),

(2) a case where (*R*)-N-(aminocarbonyl)-phenylalanine was obtained by
5 treating 5-benzylhydantoin with a microorganism belonging to the genus *Hansenula* (JP-A-61-71196), and

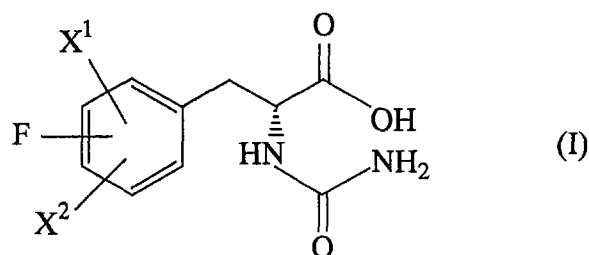
(3) a case where (*R*)-N-(aminocarbonyl)-phenylalanine and (*R*)-N-(aminocarbonyl)-tyrosine were obtained by treating 5-benzylhydantoin and 5-(4-hydroxybenzyl)hydantoin respectively with a microorganism
10 belonging to the genus *Agromyces*, *Chromobacterium* or *Cellulomonas* (JP-A-1-228489).

However, there is no report on preparation and isolation of (*R*)-N-(aminocarbonyl)-fluorophenylalanine of the present invention.

In the light of the foregoing, the object of the present
15 invention is to provide (*R*)-N-(aminocarbonyl)-fluorophenylalanine and the process for preparing the same.

DISCLOSURE OF INVENTION

As a result of our intensive investigations to resolve the
20 above-mentioned problem, we have found a microorganism having the ability to selectively hydrolyze (*R*)-isomer of 5-(fluorobenzyl)hydantoin, and have established the present invention. Namely, the present invention relates to (*R*)-N-(aminocarbonyl)-fluorophenylalanine of the general formula (I):



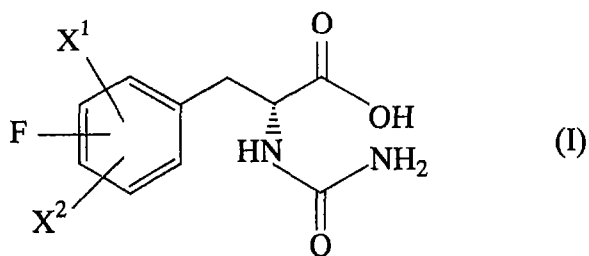
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wherein X¹ and X² are hydrogen atom or fluorine atom.

In the present invention, preferable example of (R)-N-(aminocarbonyl)-fluorophenylalanine includes (R)-N-(aminocarbonyl)-(2-fluorophenyl)alanine, (R)-N-(aminocarbonyl)-(3-fluorophenyl)alanine,
 10 (R)-N-(aminocarbonyl)-(4-fluorophenyl)alanine or (R)-N-(aminocarbonyl)-(3,4-difluorophenyl)alanine.

Further, the present invention relates to a process for preparing (R)-N-(aminocarbonyl)-fluorophenylalanine of the general formula (I) :

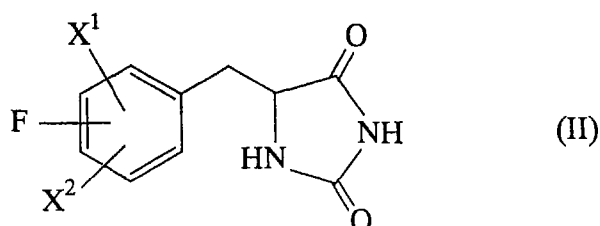
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wherein X¹ and X² are hydrogen atom or fluorine atom,
 by treating 5-(fluorobenzyl)hydantoin of the general formula (II):

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wherein X^1 and X^2 are as defined above,

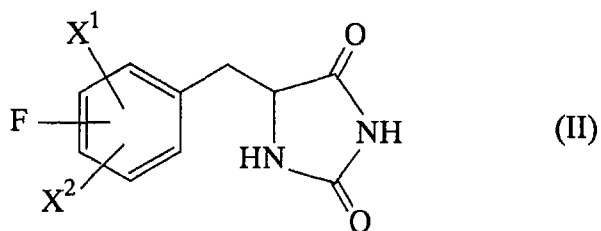
with an enzyme having the ability to selectively hydrolyze (*R*)-isomer thereof or a microorganism having the ability.

In the above-mentioned process, preferable example of the
5 enzyme or microorganism includes a microorganism belong to the genus *Bacillus*, *Alcaligenes* or *Pseudomonas*, or an enzyme derived from such microorganism.

In the above-mentioned process, more preferable example of
the enzyme or microorganism includes *Bacillus* sp., *Alcaligenes faecalis*
10 or *Pseudomonas putida*, or enzyme derived from the microorganism.

In the above-mentioned process, further more preferable
example of the enzyme or microorganism includes *Bacillus* sp. KNK108
(Depository authority: International Patent Organism Depository (IPOD),
National Institute of Advanced Industrial Science and Technology; AIST
15 Tsukuba Center 6, 1-1, Higashi 1-Chome Tsukuba-shi, Ibaraki-ken,
Japan; Date of deposit: July 2, 1981; Accession number: FERM BP-887),
Alcaligenes faecalis IFO 13111 or *Pseudomonas putida* IFO 12996, or an
enzyme derived from the microorganism.

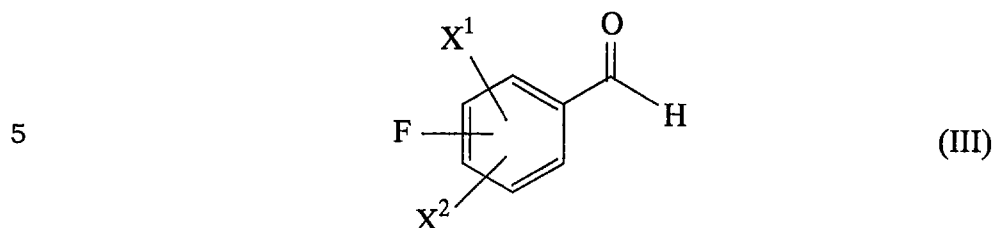
Further, in the above-mentioned process, preferable
20 compound of the general formula (II):



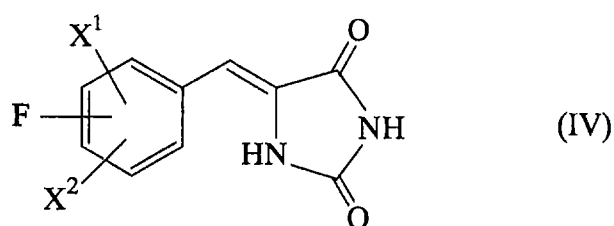
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wherein X^1 and X^2 are hydrogen atom or fluorine atom,

is one which is obtained by reacting a fluorobenzaldehyde of the general formula (III):

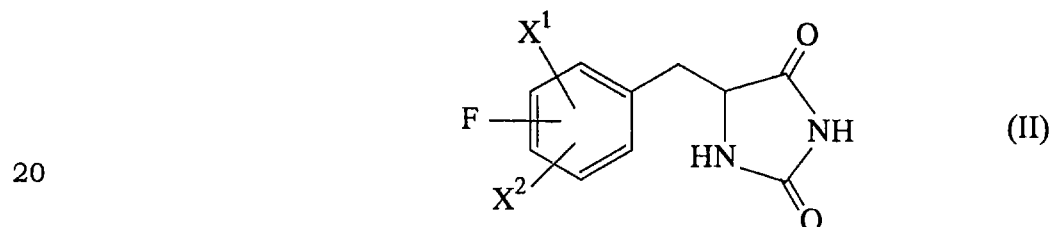


wherein X¹ and X² are as defined above,
with hydantoin in the presence of a base, and then reducing the
10 resulting 5-(fluorobenzylidene)hydantoin of the general formula (IV):



wherein X¹ and X² are as defined above,
in the presence of Raney nickel or palladium on activated carbon.

15 Further, in the above-mentioned process, preferable example
of the compound of the general formula (II):



wherein X¹ and X² are hydrogen atom or fluorine atom,
includes 5-(2-fluorobenzyl)hydantoin, 5-(3-fluorobenzyl)hydantoin, 5-

(4-fluorobenzyl)hydantoin or 5-(3,4-difluorobenzyl)hydantoin.

BEST MODE FOR CARRYING OUT THE INVENTION

As the compound of the general formula (I) of the present
5 invention, there is (*R*)-N-(aminocarbonyl)-(2-fluorophenyl)alanine, (*R*)-
N-(aminocarbonyl)-(3-fluorophenyl)alanine, (*R*)-N-(aminocarbonyl)-(4-
fluorophenyl)alanine, (*R*)-N-(aminocarbonyl)-(3,4-difluorophenyl)alanine,
(*R*)-N-(aminocarbonyl)-(2,3-difluorophenyl)alanine, or (*R*)-N-
(aminocarbonyl)-(2,3,4-trifluorophenyl)alanine.

10 The compound (I) of the present invention, (*R*)-N-
(aminocarbonyl)-fluorophenylalanine, is a compound which can be used
as a starting material for an optically active (*R*)-fluorophenylalanine
derivative which is useful as a starting material for a medicine and the
like.

15 The compound (I) of the present invention can be obtained by
(a) reacting a fluorobenzaldehyde of the general formula (III) with
hydantoin in the presence of a base to give a 5-
(fluorobenzylidene)hydantoin of the general formula (IV), (b) then
reducing it in the presence of Raney nickel or palladium on activated
20 carbon to give a 5-(fluorobenzyl)hydantoin of the general formula (II),
(c) further treating the resulting 5-(fluorobenzyl)hydantoin with an
enzyme having the ability to selectively hydrolyze the (*R*)-isomer thereof
or a microorganism having the ability.

The above-mentioned steps will be explained in detail
25 hereinafter.

Step (a): This step is a condensation reaction of the
fluorobenzaldehyde of the general formula (III) with hydantoin, whereby

the 5-(fluorobenzylidene)hydantoin of the general formula (IV) is obtained. In this reaction, as the base for the condensation, acetic acid/sodium acetate, ammonium bicarbonate, ethanolamine or the like can be used in an aqueous solvent. The condensation reaction can be
5 carried out at about 10 - about 120°C, preferably about 70°C - about 120°C. The preferred molar ratio of the fluorobenzaldehyde to hydantoin is 0.8 - 1.2. After the reaction, the desired product 5-(fluorobenzylidene)hydantoin which is hardly soluble in water can be crystallized out by shifting the temperature to the room temperature.
10 The product can be used as the starting material in the following step as it is. In addition, the product also may be used after subjecting it to recrystallization or other purification procedure.

Step (b): This is a step in which the 5-(fluorobenzylidene)hydantoin of the general formula (IV) is reduced to
15 the 5-(fluorobenzyl)hydantoin of the general formula (II). The reduction can be carried out either by using hydrogen iodide/red phosphorus or hydrogen iodide in glacial acetic acid, or by catalytic hydrogenation, but the latter method is preferable from the viewpoint of yield. Raney nickel, palladium on activated carbon or platinum oxide in alcoholic solvent can
20 be used as the catalyst for the reduction by catalytic hydrogenation.

Step (c): This is a step in which the (*R*)-N-(aminocarbonyl)-fluorophenylalanine of the general formula (I) is prepared by treating the 5-(fluorobenzyl)hydantoin of the general formula (II) with the enzyme having the ability to selectively hydrolyze the (*R*) isomer of the above
25 compound or the microorganism having the ability.

The microorganism which selectively hydrolyze (*R*)-isomer of the 5-(fluorobenzyl)hydantoin of the general formula (II) can be found

out, for example, by the following method. Firstly, microbial cells are collected by centrifugation of 5 ml of the culture broth of a microorganism, and suspended in 2.5 ml of 100mM phosphate buffer (pH 7.0) containing 0.01 - 1 % of 5-(4-fluorobenzyl)hydantoin, and the
5 suspension is shaken in a test tube at 30°C for 2 - 3 days. The assay of the desired hydrolysis ability can be accomplished by properly diluting the reaction solution obtained after the reaction under shaking with tetrahydrofuran, centrifuging the diluted solution to remove insoluble materials such as microbial cells, and subjecting the solution to HPLC
10 analysis (column: YMC-Pack ODS-A (4.6 mm × 250 mm) made by YMC Inc., eluent: water/acetonitrile = 4/1, flow rate: 0.8 ml/min, detection: 215 nm, column temperature: 25°C, elution time: 5-(4-fluorobenzyl)hydantoin; 9.7 minutes, (R)-N-(aminocarbonyl)-(4-fluorophenyl)alanine; 8.4 minutes, these conditions are illustrative.) to
15 measure the amount of N-(aminocarbonyl)-(4-fluorophenyl)alanine produced in the reaction solution.

The microorganism to be used in the present invention is selected from among bacteria, actinomycetes, mold and yeast that passed the above-mentioned assay. For example, as the bacteria, there
20 are the microorganisms belonging to the genus *Achromobacter*, *Aerobacter*, *Aeromonas*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Enterobacter*, *Erwinia*, *Escherichia*, *Klebsiella*, *Microbacterium*, *Micrococcus*, *Protaminobacter*, *Proteus*, *Sarcina*, *Serratia* and *Xantomonas*. As the actinomycetes, there
25 are the microorganisms belonging to the genus *Actinomyces*, *Actinoplanes*, *Micobacterium*, *Rhodococcus* and *Streptomyces*. As the mold, there are the microorganisms belonging to the genus *Aspergillus*,

Paecyromyces and *Penicillium*. As the yeast, there are the microorganisms belonging to the genus *Candida*, *Hansenula*, *Pichia*, *Rhodotorula* and *Torulopsis*.

Above all, the microorganisms belonging to the genus
5 *Bacillus*, *Alcaligenes* and *Pseudomonas* are suitably used. Suitable examples are *Bacillus* sp., *Alcaligenes faecalis* and *Pseudomonas putida*, and more suitable examples are *Bacillus* sp. KNK108 (Accession Number FERM BP-887), *Alcaligenes faecalis* IFO 13111 and *Pseudomonas putida* IFO 12996.

10 For cultivation of these microorganisms, generally, any nutritious source can be used without limitation as long as these microorganisms can utilize. For example, carbon sources including sugars such as glucose, sucrose and maltose, organic acids such as lactic acid, acetic acid, citric acid and propionic acid, alcohols such as
15 ethanol and glycerin, carbon hydrides such as paraffin, oils and fats such as soy bean oil and colza oil, or a mixture thereof can be mixed, and nitrogen sources including ammonium sulfate, ammonium phosphate, urea, yeast extract, meat extract, peptone and corn steep liquor can be mixed. Further, nutrition sources such as other inorganic salts and
20 vitamins may be suitably mixed. Furthermore, it is preferred to adaptively enhance the desired enzyme by adding 0.05 - 0.8 % of various pyrimidine nucleic acid bases or their derivatives, or various hydantoins.

The pyrimidine nucleic acid bases having a high enzyme-
25 inducing effect include uracil, cytosine and thymine, and their derivatives include dihydrouracil, dihydrothymidine and the like. Among hydantoins, hydantoin, DL-5-methylhydantoin and the like are

suitable.

The cultivation of microorganism can be carried out under conventional conditions. For example, the cultivation can be carried out at a pH of 4 - 9 and at a temperature of 20°C - 50°C for 10 - 96 hours
5 under aerobic condition.

In the case where 5-(fluorobenzyl)hydantoin is reacted with the microorganism, generally, the culture broth of the above-mentioned microorganism as such as well as a concentrate thereof can be used for the reaction. Further, when an ingredient in the culture broth affects
10 the reaction unfavorably, it is preferred to use microbial cells obtained by treating the culture broth, for example, centrifuging the culture broth or a processed product of microbial cells.

The processed product of microbial cells of the above-mentioned microorganism is not particularly limited to specific one, but
15 includes dried cells obtained either by dehydration procedure using acetone or diphosphorus pentaoxide, or by drying procedure using desiccator or fan, microbial cells treated with surfactant, microbial cells treated with lytic enzyme, immobilized microbial cells, or cell-free extract sample obtained by disruption of microbial cells. Further, an enzyme
20 catalyzing hydrolysis purified from culture broth may be used.

In hydrolysis reaction, the substrate 5-(fluorobenzyl)hydantoin may be added in a lump at an early stage of the reaction, or may be stepwise added according to the progress of reaction.

Further, the reaction temperature is generally 10 - 70°C,
25 preferably 20 - 65°C, and the pH at the time of reaction is 7 - 11, preferably 7 - 10.

The concentration of microorganism in the reaction solution

may be suitably determined depending on its ability to reduce the substrate, and the concentration of the substrate in the reaction solution is preferably 0.01 - 50 % (W/V), more preferably 0.1 - 30 %.

Generally, the reaction is carried out with shaking or stirring.

5 The reaction time is suitably determined depending on substrate concentration, microorganism concentration and other reaction conditions. It is generally preferred to set each condition so that the reaction may be completed for 2 - 168 hours. Further, in order to increase solubility of the substrate, water-soluble organic solvent such
10 as methanol, ethanol, acetone, tetrahydrofuran, dimethylsulfoxide and the like can be added.

(*R*)-N-(aminocarbonyl)-fluorophenylalanine produced by hydrolysis reaction can be obtained by removing enzyme source such as microorganism from the reaction solution by centrifugation or filtration,
15 adjusting the pH of the reaction solution to 2 - 5, and filtering the reaction solution to collect the precipitated crystals. (*R*)-N-(aminocarbonyl)-fluorophenylalanine can be obtained in high purity by further purification, for example, by recrystallization procedure.

Next, the present invention will be explained in detail by
20 means of examples, but the present invention is not limited thereto.

EXAMPLE 1

To the mixture of 25 g (0.25 mole) of hydantoin, 34 g (0.27 mole) of 4-fluorobenzaldehyde and 125 ml of water was added 39.5 g (0.5
25 mole) of ammonium bicarbonate over 10 minutes. The resulting mixture was refluxed for 4 hours with heating and stirring. The reaction solution was cooled to the room temperature, and the product

was filtered off, washed with cold water and dried, whereby 43.5 g (yield: 84 %) of 5-(4-fluorobenzylidene)hydantoin was obtained.

EXAMPLE 2

5 5-(2-Fluorobenzylidene)hydantoin was obtained in an amount of 40 g (yield: 77 %) in the same procedure as in Example 1 except that the same amount of 2-fluorobenzaldehyde was used instead of 4-fluorobenzaldehyde.

EXAMPLE 3

10 5-(3-Fluorobenzylidene)hydantoin was obtained in an amount of 41 g (yield: 79 %) in the same procedure as in Example 1 except that the same amount of 3-fluorobenzaldehyde was used instead of 4-fluorobenzaldehyde.

EXAMPLE 4

15 5-(3,4-Difluorobenzylidene)hydantoin was obtained in an amount of 38 g (yield: 68 %) in the same procedure as in Example 1 except that 38 g (0.27 mole) of 3,4-difluorobenzaldehyde was used
20 instead of 4-fluorobenzaldehyde.

EXAMPLE 5

 5-(4-Fluorobenzylidene)hydantoin was obtained in an amount of 40 g (yield: 77 %) in the same procedure as in Example 1
25 except that 21 g of ethanolamine was used instead of ammonium bicarbonate.

EXAMPLE 6

5-(4-Fluorobenzylidene)hydantoin was dissolved in an amount of 20 g (0.097 mole) in 500 ml of tetrahydrofuran, and 8 g of 10 % palladium on activated carbon was added to the solution. The mixture was hydrogenated with stirring at 25°C under atmospheric pressure for 18 hours. The catalyst was filtered off and washed with tetrahydrofuran. The filtrate was concentrated under reduced pressure to crystallize out solid product. Thereto 1300 ml of water was added, and the mixture was heated up to 90°C to dissolve the solid product. The solution was cooled over one night to the room temperature, and 18 g (yield: 90 %) of the precipitated 5-(4-fluorobenzyl)hydantoin was obtained by filtration.

¹H-NMR (DMSO-d₆ δ ppm) : 10.48 (s, 1H), 7.91 (s, 1H), 7.22 (m, 2H), 7.10 (m, 2H), 4.32 (m, 1H), 2.92 (m, 2H)

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EXAMPLE 7

5-(2-Fluorobenzyl)hydantoin was obtained in an amount of 18 g (yield: 90 %) in the same procedure as in Example 6 except that 5-(2-fluorobenzylidene)hydantoin was used instead of 5-(4-fluorobenzylidene)hydantoin.

¹H-NMR (DMSO-d₆ δ ppm) : 10.50 (s, 1H), 7.93 (s, 1H), 7.30 (m, 2H), 7.15 (m, 2H), 4.30 (m, 1H), 3.05 (m, 1H), 2.88 (m, 1H)

EXAMPLE 8

5-(3-Fluorobenzyl)hydantoin was obtained in an amount of 18 g (yield: 90 %) in the same procedure as in Example 6 except that 5-(3-fluorobenzylidene)hydantoin was used instead of 5-(4-

25

fluorobenzylidene)hydantoin.

^1H -NMR (DMSO- d_6 δ ppm) : 10.48 (s, 1H), 7.92 (s, 1H), 7.31 (m, 1H), 7.00 (m, 3H), 4.35 (m, 1H), 2.95 (m, 2H)

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EXAMPLE 9

5-(3,4-Difluorobenzyl)hydantoin was obtained in an amount of 20 g (yield: 91.5 %) in the same procedure as in Example 6 except that 22 g (0.097 mole) of 5-(3,4-difluorobenzylidene)hydantoin was used instead of 5-(4-fluorobenzylidene)hydantoin.

10 ^1H -NMR (DMSO- d_6 δ ppm) : 10.55 (s, 1H), 7.92 (s, 1H), 7.35 (m, 1H), 7.25 (m, 1H), 7.05 (m, 1H), 4.35 (m, 1H), 2.95 (dd, 2H)

EXAMPLE 10

(1) Preparation of medium

15 The liquid medium containing the following composition was prepared, poured into each 500 ml Sakaguchi's flask in a volume of 100 ml and subjected to steam sterilization at 120°C for 20 minutes.

Medium composition:

	Meat extract	2.0 %
20	Proextract	2.0 %
	NaCl	0.3 %
	MnCl ₂ · 4H ₂ O	20 ppm
	Uracil	0.2 %
	pH	7.5

25 (2) Cultivation · harvest

Bacillus sp. KNK 108 (Accession number: FERM BP-887) was cultivated in 10 ml of the medium having the same composition as in (1)

in a cotton plugged large-sized test tube at 37°C for 18 hours. One ml of the culture broth was inoculated on the medium prepared in (1) and cultivated at 37°C for 24 hours. The obtained culture broth was centrifuged to harvest microbial cells, and the microbial cells were washed with 100 ml of physiological saline and then suspended in 100 ml of 50 mM phosphate buffer (pH 7.5).

(3) Hydrolysis reaction

The resulting suspension of microbial cells was poured into 200 ml 4-necked round bottom flask and 1 g of 5-(4-fluorobenzyl)hydantoin was added thereto. The mixture was subjected to reaction at 37°C for 20 hours during which the reaction solution was adjusted to pH 9.0 with 1N sodium hydroxide. After reaction, the reaction solution was adjusted to pH 7.0 with 1N hydrochloric acid, and centrifuged to remove insoluble materials such as microbial cells. The resulting supernatant was adjusted to pH 4.0 with 1N hydrochloric acid, and 0.87 g (Yield: 80 %) of the precipitated (*R*)-N-(aminocarbonyl)-(4-fluorophenyl)alanine was obtained by filtration.

$[\alpha]_D^{20} = -31.5$ (c=2, 1N NH₄OH)

¹H-NMR (DMSO-d₆ δ ppm) : 7.20 (s, 2H), 7.15 (m, 2H), 6.18 (d, 1H), 5.60 (s, 1H), 4.30 (m, 1H), 3.00 - 2.80 (dd, 2H)

A portion (20 mg) of the thus-obtained (*R*)-N-(aminocarbonyl)-(4-fluorophenyl)alanine was dissolved in 1 ml of 1N sulfuric acid, and 10 mg of sodium nitrite was added thereto. The mixture was subjected to reaction at the room temperature for one hour. The reaction solution was analyzed using HPLC, whereby the optical purity of the product was found to be at least 99 %ee.

HPLC analysis condition

Column: Crownpak-CR (made by DAICEL CHEMICAL INDUSTRIES,
LTD.)

Eluent: aqueous perchloric acid solution adjusted to pH 1.5

Flow rate: 0.5 ml/min

5 Column temperature: 0 - 5°C; Detection wave length, 210 nm

EXAMPLE 11

(*R*)-N-(aminocarbonyl)-(4-fluorophenyl)alanine having an optical purity of at least 99 %ee was obtained in an amount of 0.85 g
10 (yield: 78 %) in the same procedure as in Example 10 except that *Alcaligenes faecalis* IFO 13111 was used instead of *Bacillus* sp. KNK 108 (Accession number: FERM BP-887).

EXAMPLE 12

15 (*R*)-N-(aminocarbonyl)-(4-fluorophenyl)alanine having an optical purity of at least 99 %ee was obtained in an amount of 0.9 g (yield: 83 %) in the same procedure as in Example 10 except that *Pseudomonas putida* IFO 12996 was used instead of *Bacillus* sp. KNK 108 (Accession number: FERM BP-887).

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EXAMPLE 13

(*R*)-N-(aminocarbonyl)-(2-fluorophenyl)alanine having an optical purity of at least 99 %ee was obtained in an amount of 0.8 g (yield: 74 %) in the same procedure as in Example 10 except that 5-(2-
25 fluorobenzyl)hydantoin was used as the substrate instead of 5-(4-fluorobenzyl)hydantoin.

$[\alpha]_D^{20} = -24.0$ (c=2, 1N NH₄OH)

¹H-NMR (DMSO-d₆ δ ppm) : 7.23 (s, 2H), 7.10 (m, 2H), 6.18 (d, 1H), 5.55 (s, 1H), 4.35 (m, 1H), 3.20 - 2.80 (dd, 2H)

EXAMPLE 14

5 (*R*)-N-(aminocarbonyl)-(3-fluorophenyl)alanine having an optical purity of at least 99 %ee was obtained in an amount of 0.85 g (yield: 78 %) in the same procedure as in Example 10 except that 5-(3-fluorobenzyl)hydantoin was used as the substrate instead of 5-(4-fluorobenzyl)hydantoin.

10 $[\alpha]_D^{20} = -25.2$ (c=2, 1N NH₄OH)

¹H-NMR (DMSO-d₆ δ ppm) : 7.20 (m, 1H), 6.95 (m, 3H), 5.95 (d, 1H), 5.55 (s, 1H), 3.95 (m, 1H), 3.10 - 2.85 (dd, 2H)

EXAMPLE 15

15 (*R*)-N-(aminocarbonyl)-(3,4-difluorophenyl)alanine having an optical purity of at least 99 %ee was obtained in an amount of 0.8 g (yield: 74 %) in the same procedure as in Example 10 except that 1 g of 5-(3,4-difluorobenzyl)hydantoin was used as the substrate instead of 5-(4-fluorobenzyl)hydantoin.

20 $[\alpha]_D^{20} = -28.2$ (c=2, 1N NH₄OH)

¹H-NMR (DMSO-d₆ δ ppm) : 12.70 (br, 1H), 7.31 (m, 1H), 7.20 (m, 1H), 7.00 (m, 1H), 6.20 (d, 1H), 5.55 (s, 1H), 4.30 (m, 1H), 3.10 - 2.75 (dd, 2H)

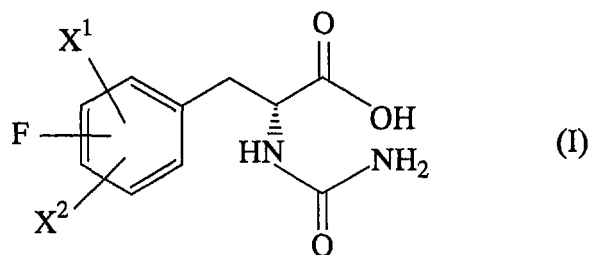
INDUSTRIAL APPLICABILITY

25 According to the present invention, (*R*)-N-(aminocarbonyl)-fluorophenylalanine, which is useful as a starting material for an optically active (*R*)-fluorophenylalanine derivative which is useful as a

starting material for a medicine and the like, can be obtained.

CLAIMS

1. (*R*)-N-(aminocarbonyl)-fluorophenylalanine of the general formula (I):



10 wherein X¹ and X² are hydrogen atom or fluorine atom.

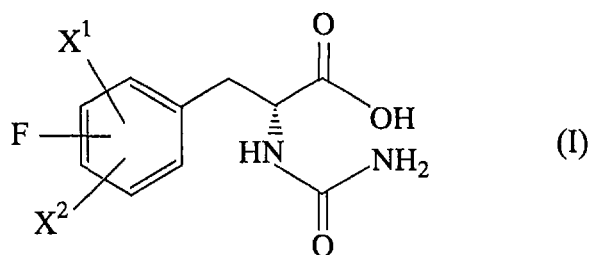
2. (*R*)-N-(aminocarbonyl)-(2-fluorophenyl)alanine

15 3. (*R*)-N-(aminocarbonyl)-(3-fluorophenyl)alanine

4. (*R*)-N-(aminocarbonyl)-(4-fluorophenyl)alanine

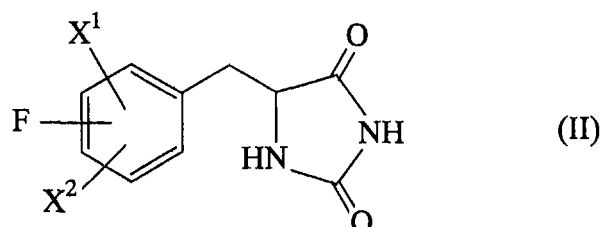
5. (*R*)-N-(aminocarbonyl)-(3,4-difluorophenyl)alanine

20 6. A process for preparing (*R*)-N-(aminocarbonyl)-fluorophenylalanine of the general formula (I):



wherein X^1 and X^2 are hydrogen atom or fluorine atom,
which comprises treating 5-(fluorobenzyl)hydantoin of the general
formula (II):

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wherein X^1 and X^2 are as defined above,
10 with an enzyme having the ability to selectively hydrolyze (*R*)-isomer
thereof or a microorganism having said ability.

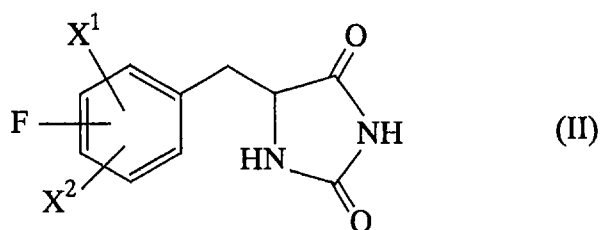
7. The process of Claim 6, wherein said enzyme or
microorganism is a microorganism belonging to the genus *Bacillus*,
15 *Alcaligenes* or *Pseudomonas*, or an enzyme derived from these
microorganisms.

8. The process of Claim 6, wherein said enzyme or
microorganism is *Bacillus* sp., *Alcaligenes faecalis* or *Pseudomonas*
20 *putida*, or an enzyme derived from these microorganisms.

9. The process of Claim 6, wherein said enzyme or
microorganism is *Bacillus* sp. KNK108 (Depository authority:
International Patent Organism Depositary (IPOD), National Institute of
25 Advanced Industrial Science and Technology; AIST Tsukuba Center 6,
1-1, Higashi 1-Chome Tsukuba-shi, Ibaraki-ken, Japan; Date of deposit:
July 2, 1981; Accession number: FERM BP-887), *Alcaligenes faecalis*

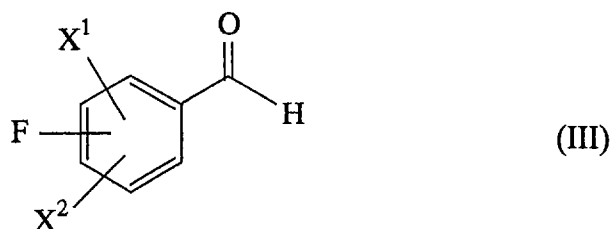
IFO 13111 or *Pseudomonas putida* IFO 12996, or an enzyme derived from these microorganisms.

10. The process of Claim 6, wherein the compound of the
5 general formula (II):



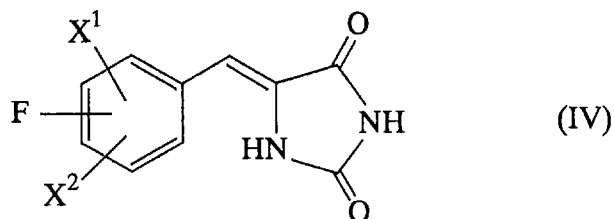
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wherein X¹ and X² are hydrogen atom or fluorine atom,
is one which is obtained by reacting a fluorobenzaldehyde of the general
formula (III):



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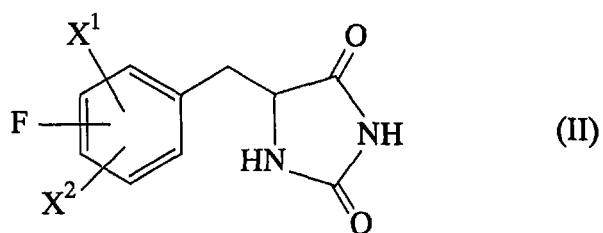
wherein X¹ and X² are as defined above,
20 with hydantoin in the presence of a base, and then reducing the
resulting 5-(fluorobenzylidene)hydantoin of the general formula (IV):



wherein X^1 and X^2 are as defined above,

in the presence of Raney nickel or palladium on activated carbon.

11. The process of Claim 6, 7, 8, 9 or 10, wherein the
5 compound of the general formula (II):



10

wherein X^1 and X^2 are hydrogen atom or fluorine atom,

is 5-(2-fluorobenzyl)hydantoin, 5-(3-fluorobenzyl)hydantoin, 5-(4-fluorobenzyl)hydantoin or 5-(3,4-difluorobenzyl)hydantoin.