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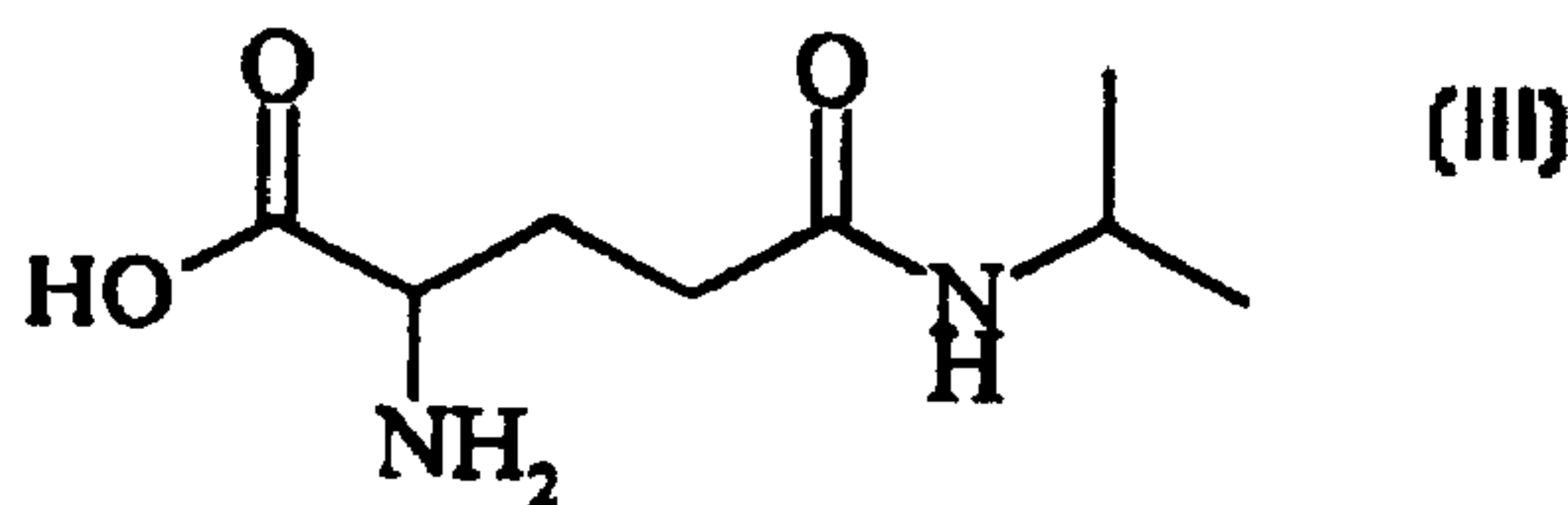
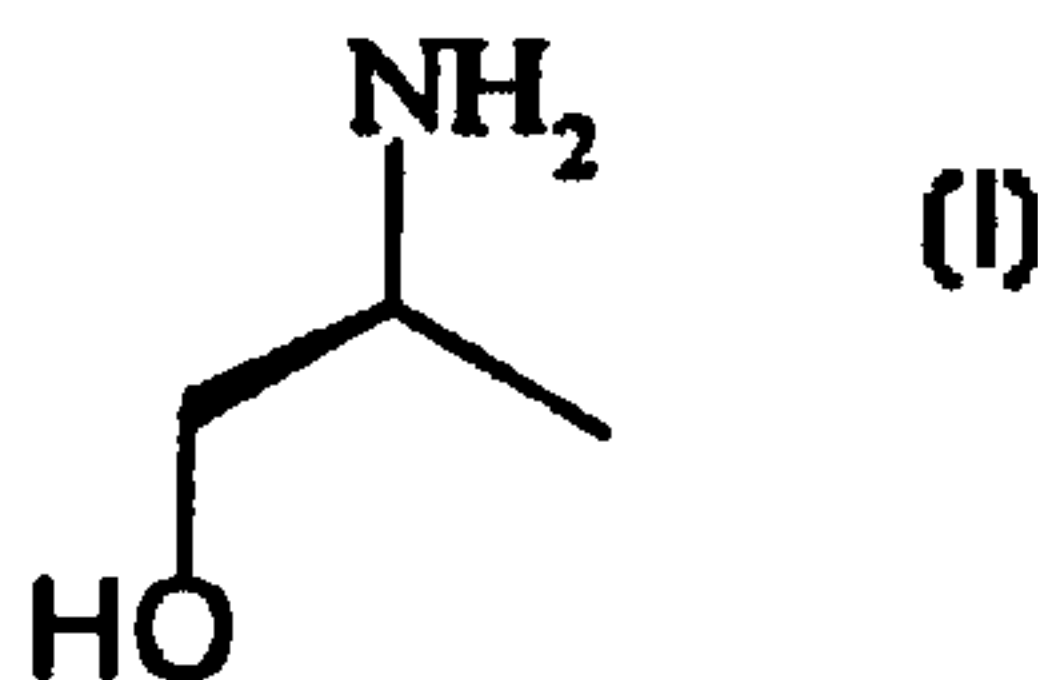
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(54) **METHODE DE PREPARATION DE L-ALAMINOL ET D'AMIDE
DE GAMMA-GLUTANYLISOPROPYL ET D'UNE SOUCHE DE
MICROORGANISME DU GENRE PSEUDOMONAS**

(54) **METHOD FOR PRODUCING L-ALANINOL AND GAMMA-
GLUTAMYL ISOPROPYLAMIDE AND A MICROBIAL
STRAIN OF THE GENUS PSEUDOMONAS**



(57) L'invention concerne un procédé biotechnologique qui permet de produire du L-alaninol, de formule (I), et un procédé qui permet de produire du γ -glutamylisopropylamide, de formule (III), en utilisant des nouveaux micro-organismes capables soit de produire du L-alaninol à partir d'isopropylamine, soit d'utiliser l'isopropylamine comme unique source de C et de N.

(57) The invention relates to a biotechnological method for producing L-alaninol of the formula (I) and a method for producing γ -glutamylisopropylamide of the formula (III), using novel microorganisms which are capable either of producing L-alaninol from isopropylamine or of using isopropylamine as only source of C and N.



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(21) Internationales Aktenzeichen: PCT/EP98/05029 (22) Internationales Anmeldedatum: 7. August 1998 (07.08.98) (30) Prioritätsdaten: <table style="width: 100%;"> <tr> <td style="width: 30%;">1876/97</td> <td style="width: 40%;">8. August 1997 (08.08.97)</td> <td style="width: 30%;">CH</td> </tr> <tr> <td>1897/97</td> <td>12. August 1997 (12.08.97)</td> <td>CH</td> </tr> </table> (71) Anmelder (für alle Bestimmungsstaaten ausser US): LONZA AG [CH/CH]; Münchensteinerstrasse 38, CH-4052 Basel (CH). (72) Erfinder; und (75) Erfinder/Anmelder (nur für US): DE AZEVEDO WÄSCH, Susana, Ivone [DE/CH]; Culmannstrasse 59, CH-8006 Zürich (CH). VAN DER PLOEG, Jan, Rudolf [NL/CH]; Culmannstrasse 67, CH-8006 Zürich (CH). LEISINGER, Thomas [CH/CH]; Zürichbergstrasse 7, CH-8032 Zürich (CH). KIENER, Andreas [CH/CH]; Meisenweg 5, CH-3930 Visp (CH). HEINZMANN, Klaus [CH/CH]; Unterstalden, CH-3932 Visperterminen (CH). GILLIGAN, Thomas [US/US]; 130 Woodside Avenue, Ridgewood, NJ 07450 (US).	1876/97	8. August 1997 (08.08.97)	CH	1897/97	12. August 1997 (12.08.97)	CH	(74) Anwälte: RITTHALER, Wolfgang usw.; Winter, Brandl & Partner, Alois-Steinecker-Strasse 22, D-85354 Freising (DE). (81) Bestimmungsstaaten: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO Patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Veröffentlicht Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.	
1876/97	8. August 1997 (08.08.97)	CH						
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(54) Title: PROCESS FOR THE PRODUCTION OF L-ALANINOL AND GAMMA-GLUTAMYL ISOPROPYLAMIDE AND A MICROORGANISM STRAIN FO THE GENUS PSENDOMONAS (54) Bezeichnung: VERFAHREN ZUR HERSTELLUNG VON L-ALANINOL UND γGLUTAMYLISOPROPYLAMID UND MIKROORGANISMUSSTAMM DES GENUS PRESENDOMONAS (57) Abstract The invention relates to a biotechnological method for producing L-alaninol of the formula (I) and a method for producing γ-glutamylisopropylamide of the formula (III), using novel microorganisms which are capable either of producing L-alaninol from isopropylamine or of using isopropylamine as only source of C and N. (57) Zusammenfassung Beschrieben wird ein biotechnologisches Verfahren zur Herstellung von L-Alaninol der Formel (I), und ein Verfahren zur Herstellung von γ-Glutamylisopropylamid der Formel (III), unter Verwendung neuer Mikroorganismen, die entweder befähigt sind, aus Isopropylamin L-Alaninol zu produzieren oder befähigt sind, Isopropylamin als einzige C- und N-Quelle zu verwerten.								
<chem>CC(N)CO</chem> (I) <chem>CC(C)NC(=O)CCC(N)C(=O)O</chem> (III)								

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**Microbiological process for preparing L-alaninol and
 γ -glutamylisopropylamide**

5 The invention relates to novel microorganisms
which are able to grow using isopropylamine as sole C
and/or N source, and also to microorganisms which
produce L-alaninol from isopropylamine and do not
catabolize the former. The latter microorganisms are
10 used for a novel process for preparing L-alaninol
(S (+)-2-amino-1-propanol) starting from
isopropylamine.

L-Alaninol is an important intermediate for
preparing pharmaceuticals, for example for preparing
15 ofloxacin (J. Med. Chem 1997, 30, 2283-2286).

To date, only chemical processes for preparing
L-alaninol are known.

The object of the present invention was to
provide a simple and inexpensive process for preparing
20 L-alaninol.

This object is achieved using the micro-
organisms according to Claim 1 and the process
according to Claim 6.

The microorganisms of the invention are obtainable by
25 the following steps:

- a) Mutagenesis of microorganisms which are able to
grow using isopropylamine and L-alaninol as sole C
source,
- b) Selection of mutants which are able to grow using
30 L-alanine but not L-alaninol and isopropylamine as
sole C source.

Microorganisms which are able to grow using L-
alaninol and isopropylamine as sole C and/or N source
can be isolated from soil samples, sludge or
35 wastewater, in particular from contaminated soil and
sewage sludge, using customary microbiological
techniques.

Expediently, isolation is carried out by
enriching the microorganisms from a sample by selection

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with isopropylamine as sole C and/or N source. Starting from the microorganisms thus obtained, by selectively enriching with L-alaninol and isopropylamine as sole C and/or N source in the same manner, stable
5 microorganisms are obtained which are able to grow using L-alaninol and isopropylamine as sole C and N source.

The "wild-type" strains thus obtained are then subjected to mutagenesis, which mutagenesis can be
10 performed by known methods (J.H. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, 1972). Expedient mutation methods are the point mutation method, for example by mutagenic agents or UV irradiation, the frameshift method, the deletion method
15 or the transposon-insertion method.

By growth tests using each isopropylamine, L-alaninol and a further degradation product of L-alaninol as sole C and/or N source, from the culture of mutant microorganisms obtained by cultivation,
20 those microorganisms can be selected which produce L-alaninol from isopropylamine and do not catabolize the former. It was found that degradation of isopropylamine by the "wild type" strains via alaninol preferably results in L-alanine. Preferably, therefore,
25 selection of the microorganisms according to the invention is carried out each on isopropylamine, L-alaninol and L-alanine as sole C and/or N source. The desired microorganisms can be detected in that they are not able any more to grow using isopropylamine and
30 L-alaninol as sole C and/or N source but are able to grow using L-alanine as sole C and/or N source. These strains are termed hereinafter "alaninol producers".

Both the "wild-type" strains and the "alaninol producers" preferably belong to the genus *Pseudomonas*,
35 in particular to the species *Pseudomonas* sp. Kie 171, Kie 171-B (DSM 11521) and Kie 171-B1 (DSM 11629). This also includes their functionally equivalent variants and mutants. The microorganisms *Pseudomonas* sp. Kie 171-B (DSM 11521) were deposited on 23.04.1997, Kie-B1

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(DSM 11629) on 25.06.1997 and Kie 171 (DSM 12360) on 05.08.1998 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH [German collection of microorganisms and cell cultures], Mascheroderweg 1b, 5 D-38124 Braunschweig, under the Budapest Treaty.

For the purposes of the invention "functionally equivalent variants and mutants" are microorganisms derived from the parent microorganisms and have essentially the same properties and functions. Such 10 variants and mutants can be formed randomly, e.g. by UV irradiation.

15 Scientific description of strain Kie 171-B (DSM 11521)

Pseudomonas sp.

RNA Group 1

20 Properties of the strain

Cell shape	Rods
Width μm	0.7-0.8
Length μm	1.5-3.5
Motility	+
Flagella	monopolar, 1
Gram reaction	-
Lysis by 3% KOH	+
Aminopeptidase (Cerny)	+
Oxidase	+
Catalase	w
Fluorescence	-
Pyocyanin	-
ADH	+

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Urease -

Gelatin hydrolysis -

Nitrate reduction +

Denitrification -

Substrate utilization

Citrate +

Malate +

Phenylacetate +

D-Glucose +

Maltose -

Trehalose -

Mannitol -

Hippurate -

Arabinose -

Mannose +

m-Inositol -

Geraniol +

Azelate +

 α -Ketogluconate -

Lecithinase -

Abbreviations:

Laevan from sucrose - ADH: alcohol
dehydrogenase

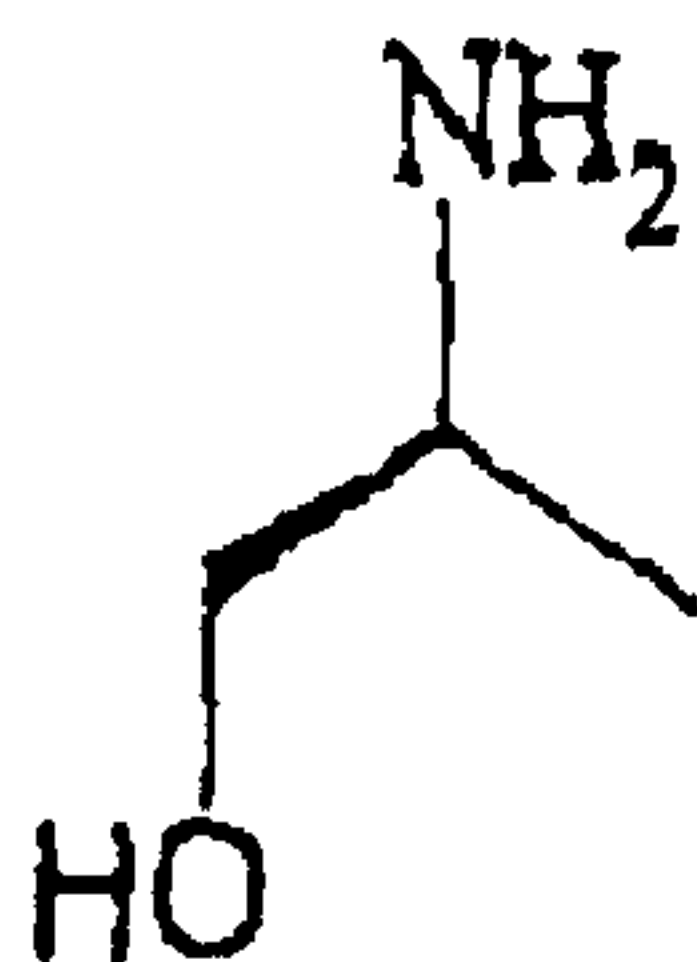
Growth at 41°C +

Analysis of the 16S DNA showed that this strain has a similarity with *Pseudomonas citronellolis*.

After selection in a medium customary to those skilled in the art, the microorganisms, before the actual biotransformation, are customarily cultured in a medium customary to those skilled in the art. The medium described in Table 1 can be used as selection and culture medium.

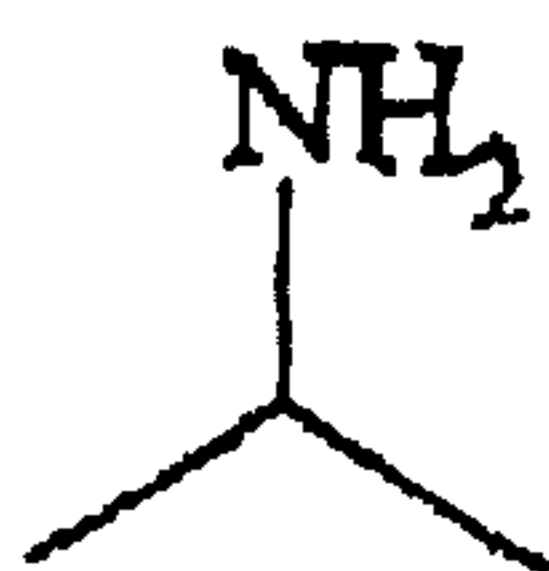
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The process of the invention for preparing L-alaninol of the formula



I

is carried out in such a manner that isopropylamine, abbreviated to IPA hereinafter, of the formula



II

is converted using the "alaninol producers".

Particularly suitable for the process are the above-described "alaninol producers" of the genus *Pseudomonas*, in particular the species *Pseudomonas* sp. Kie 171-B (DSM 11521) and Kie-B1 (DSM 11629) and their functionally equivalent variants and mutants.

The biotransformation can be carried out using resting cells (non-growing cells which no longer require C and energy sources) or using growing cells.

The media for the biotransformation which can be used are media customary to those skilled in the art, for example low-molarity phosphate buffers, Hepes buffer and complete media such as "Nutrient Yeast Broth" (NYB), or mineral salt media as described by Kulla et al., (Arch. Microbiol. 135, 1 (1983)) or in Table 1.

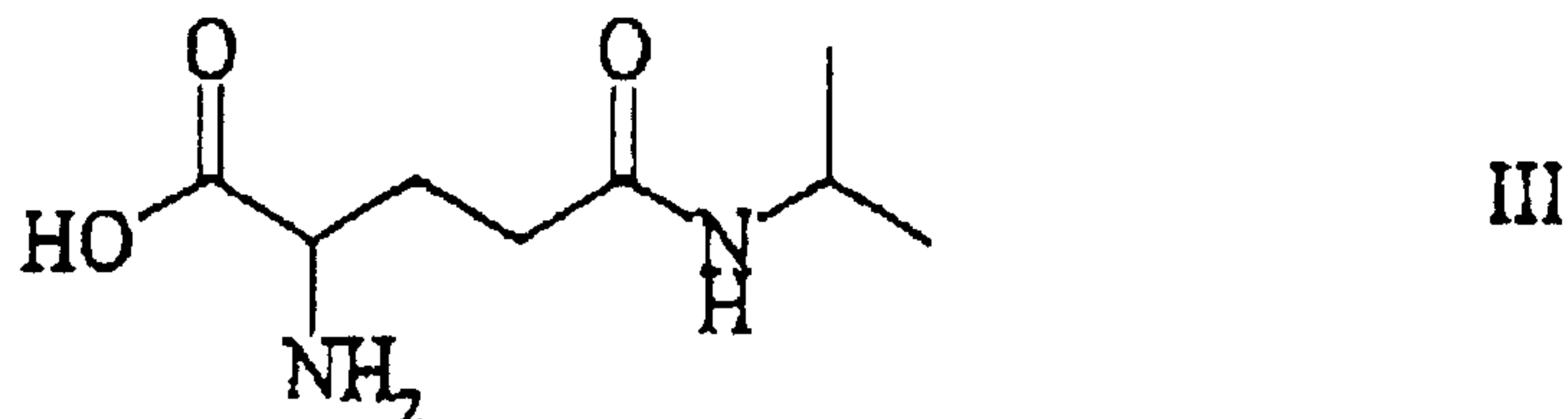
Expediently, the biotransformation is carried out with single or continuous addition of IPA, in such a manner that the concentration of IPA does not exceed 10% by weight, preferably 1% by weight.

The pH can be in a range from 4 to 10, preferably from 5 to 9. The biotransformation is expediently carried out at a temperature of from 10 to 50°C, preferably from 20 to 40°C.

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After a customary conversion time of from 1 to 100 h, L-alaninol can be isolated by customary work-up methods, for example by distillation of the basic, cell-free fermentation broth.

5 Surprisingly, it has now also been found that the "wild-type" strains and the "alaninol producers" produce γ -glutamylisopropylamide of the formula



γ -Glutamylisopropylamide is an important intermediate
10 for preparing glutamine transport inhibitors
(WO 91/12232).

The process of the invention for preparing γ -glutamylisopropylamide is carried out in such a manner that IPA of the formula



15 is converted using growing cells of the "wild-type" strains or the "alaninol producers".

Particularly suitable for the process are the above-described "wild-type" strains or "alaninol
20 producers" of the genus *Pseudomonas*, in particular the species *Pseudomonas* sp. Kie 171, Kie 171 B and their functionally equivalent variants and mutants.

Otherwise, the process for preparing γ -glutamylisopropylamide is carried out under the same conditions
25 as the process for preparing L-alaninol.

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Examples:**Example 1**

- 5 Isolation of microorganisms which are able to utilize isopropylamine as sole C and N source

10 Aerobic microorganisms which are able to utilize IPA as sole C and N source were enriched on minimal medium (Table 1) in the presence of 25 mM IPA as sole C source. For this 100 ml of this medium were introduced into 300 ml Erlenmeyer flasks and each was inoculated with different sewage sludge samples (2 ml) from the LONZA AG sewage treatment plant in Visp, Switzerland. The flasks were incubated for 5 days at 15 30°C without agitation. 1 ml of the medium was then used to inoculate a further flask containing fresh medium. This flask was in turn incubated under the same conditions. In total this enrichment cycle was repeated five times. The enriched bacteria were then streaked on 20 to minimal medium containing agar (15 g/l) and 25 mM IPA to form individual colonies.

The isolated IPA-utilizing microorganisms were further selected under the same conditions for the ability to utilize L-alaninol as sole C source. In this 25 manner, the strain *Pseudomonas* sp. Kie-171 (wild-type strain) was finally isolated.

Table 1:

Composition of minimal medium (MM medium)	
Constituents	Concentration [mg/l]
MgCl ₂ · 6H ₂ O	720
CaCl ₂ · 2H ₂ O	26.1
FeCl ₃ · 6H ₂ O	1.44
EDTA · Na ₂ · 2H ₂ O	10
FeSO ₄ · 7H ₂ O	4
ZnSO ₄ · H ₂ O	0.2
H ₃ BO ₄	0.6
CoCl ₂ · 6H ₂ O	0.4
MnCl ₂ · 4H ₂ O	0.045
CuCl ₂ · 2H ₂ O	0.02
NiCl ₂ · 6H ₂ O	0.04
Na ₂ MoO ₄ · 2H ₂ O	0.06
(NH ₄) ₂ SO ₄	2000
NaHPO ₄	2000
KH ₂ PO ₄	1000
NaCl	2000

Solution pH = 7.0

- 5 To determine the catabolic pathway of IPA, the growth of strain Kie 171 was tested on various C sources (each 20 mM, pH 7.0) in 25 ml of minimal medium. The growth was followed by measuring the OD₆₅₀. The results are described in Table 2. Growth occurred
- 10 on the following C sources: isopropylamine, L-alaninol, L-alanine, D-alanine, L-lactate, D,L-lactate, propionic acid, propane-1,2-diol, ethanolamine and propionaldehyde. No growth took place on the following C sources: D-alaninol, D,L-alaninol, acetone, 2-propanol,
- 15 1-propanol, propylamine, methylamine, 2-aminopentane, tert-butylamine, isopropanol, L-serine, L-2-amino-1-butanol, D,L-propane-1,2-diol, L-propane-1,2-diol, D-propane-1,2-diol, ethylamine, D-2-aminobutane, malonic acid, methylamine and hydroxyacetone.

Table 2:

No growth after 5 days		Growth within 2 days
Acetone	Propanol	Isopropylamine
Hydroxyacetone	2-Propanol	L-Alaninol
Isopropanol	D-Alaninol	Propionic acid
D,L-Alaninol	2-Amino-propanediol	L-Lactate
	D,L-Propane-1,2-diol	D,L-Lactate
L-Serine	L-Propane-1,2-diol	L-Alanine
tert-Butylamine	D-Propane-1,2-diol	D-Alanine
Ethylamine	Ethylamine	Propane-1,2-diol
L-2-Amino-1-	D-2-Aminobutane	Ethanolamine
butanol	Malonic acid	Propionaldehyde
Propylamine	Methylamine	
	(±)-2-Aminopentane	

Example 2

- 5 Isolation of mutants of strain Kie 171 whose IPA catabolic pathway is interrupted

a) Using the point mutation method

10 Mutants for producing L-alaninol from IPA must not be able to utilize either IPA or L-alaninol. They must transform IPA solely into L-alaninol.

15 To produce such mutants, Kie 171 was cultured on 20 mM IPA up to an OD₆₅₀ of 0.6 (exponential phase). As mutagen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was added to a suspension of strain Kie 171 [concentration of 0.5 µg/µl of cells (2.5 · 10⁹ cells/ml)] in a C-free medium. After incubation for 30 minutes, MNNG was extracted by washing with C-free minimal medium.

20 Dilution series were prepared in C-free minimal medium (Table 1) and the resultant preparations were spread out on solid L-glutamate medium (minimal medium, 20 mM L-glutamate and 15 g/l of

- 10 -

agar) and incubated at 30°C. The colonies then obtained were tested for growth on various C sources (IPA, L-alaninol, L-alanine and L-propionic acid in a concentration of 20 mM). This took place firstly on solid medium and then in liquid culture. Mutants which could not utilize IPA and L-alaninol were studied further. The mutant *Pseudomonas* sp. Kie 171-B (DSM 11521) was isolated, which grew neither on IPA nor on L-alaninol, but could still utilize L-alanine, propionic acid and L-glutamate.

b) Using the transposon-insertion method

To produce the transposon mutants, essentially the protocol of Lorenzo, de V. and Timmis K.N. "Analysis and construction of stable phenotypes in Gram-negative bacteria with Tn5 and Tn 10-derived minitransposons" Meth. Enzymol. Volume 235, pages 386-405, 1994 was followed:

E. coli S17-1 λ pir, which contains the plasmid pUT mini-Tn5 having the kanamycin (Km) resistance gene, was conjugated with *Pseudomonas* sp. Kie 171. The donor strain *E. coli* S17-1 λ pir was grown for this on a fresh Luria broth (LB) plate containing 200 μ g of ampicillin per ml at 37°C. *Pseudomonas* sp. Kie 171 was cultured at 30°C on an MM (minimal medium) plate (Table 1) containing 20 mM IPA. An *E. coli* S17-1 λ pir colony was used to inoculate 25 ml of LB medium which contained 200 μ g/ml of ampicillin and 50 μ g/ml of Km. This culture was cultured overnight at 150 rpm and 37°C. *Pseudomonas* sp. Kie 171 was likewise shaken overnight in 25 ml of MM containing 20 mM IPA at 150 rpm and 30°C.

The cells of *Pseudomonas* sp. Kie 171 and of *E. coli* S17-1 λ pir were then harvested by centrifuging for 15 minutes at 4000 rpm and washed

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twice with 5 ml of 0.9% NaCl. After the final washing, the cells were taken up in 500 μ l of 0.9% NaCl. 500 μ l of *E. coli* and 500 μ l of *Pseudomonas* sp. Kie 171 were mixed with one another on an LB plate without Km. The *E. coli* cell suspension was applied as one drop to the centre of the plate in this case. *Pseudomonas* sp. Kie 171 was pipetted shortly thereafter onto the same point so that the organisms are in close contact for successful conjugation. This plate was incubated at 30°C for a period of 8 h. The cells were then taken up in 2 ml of 0.9% NaCl. 300 μ l aliquots of the cell suspension were plated out directly onto MM plates containing 50 μ g/ml of Km, 10 mM L-lactate and 10 mM L-alanine and incubated at 30°C. The colonies then obtained were tested for growth on various C sources (IPA, L-alaninol, L-alanine, L-lactate, L-alanine and L-glutamate) in the presence of 50 μ g/ml of Km. This took place firstly on solid medium and then in liquid culture. Mutants which could not utilize IPA or L-alaninol were studied further. The mutant Kie 171-B1 was isolated which grew neither on IPA nor on L-alaninol, but could still utilize L-alanine, L-lactate and L-alanine and L-glutamate.

Example 3:

30 Biotransformation of IPA to L-alaninol

For the biotransformation of IPA to L-alaninol, a 25 ml overnight preculture of Kie 171-B (DSM 11521) was used. This was cultured on minimal medium containing 20 mM L-glutamate and used to inoculate a culture of 250 ml of the same medium in a 1 l flask. After culturing Kie 171-B up to the start of the exponential phase (OD_{650} from 0.4 to 0.6), IPA (10 mM) was added. After an OD_{650} of 1-1.3 was reached, the culture was centrifuged at 4000 rpm for 15 minutes and

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the sediment was washed twice with half the amount of culture medium without C source. The cells could then be taken up in the desired volume of minimal medium (Table 1) without C source, so that 5 ml of a concentrated cell suspension ($O D_{650} \sim 13$) were obtained. After adding 10 or 20 mM IPA this culture of resting cells was shaken at 150 rpm and 30°C. The samples were withdrawn at various time points (16 h, 25 h and 40 h). With both of the initial IPA concentrations (10 mM and 20 mM), a final concentration of 7 mM L-alaninol was reached after the biotransformation. This corresponded to a yield of 37% or 59%. The remainder was unmetabolized IPA.

15 Detection of alaninol by GC-MS:

Alaninol which had been prepared by biotransformation was detected in the cell-free solution by GC-MS. The fragmentation pattern was identical to that of the reference compound.

20

Detection of L-alaninol by HPLC:

L-Alaninol was derivatized by 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl isothiocyanate. For the analysis, a Macherey-Nagel NUCLEOSIL 120 3 C₁₈ AB column was used at 40°C. Detection was at 250 nm.

25

Mobile phase: Solution A H₂O

Solution B Acetonitrile

Solution C 250 mM KH₂PO₄ (pH 2.3)

Time (min.)	%B	%C
1.00	22.5	20.0
6.50	34.0	20.0
8.50	50.0	10.0
11.00	50.0	10.0
13.00	22.5	20.0

30

Retention times: L-Alaninol 5.46 min.
D-Alaninol 5.69 min.

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Example 4:**Biotransformation of IPA to L-alaninol by Pseudomonas sp. Kie 171-B1**

5 For biotransformation of IPA to L-alaninol, an overnight preculture of Pseudomonas sp. Kie 171-B1 was used. This was cultured on MM medium containing 20 mM L-glutamate and 50 mg/ml of Km, and used to inoculate a culture of 250 ml of the same medium in a 1 l flask.
10 After culturing Kie 171-B1 to the exponential phase (OD₆₅₀ of 0.4-0.6), 10 mM IPA was added. This culture was shaken further at 30°C and 150 rpm. After 72 h, 60 ml of the culture medium were withdrawn. The cells were centrifuged off at 4000 rpm for 20 minutes. 30 ml
15 of the cell-free supernatant were, after freezing in liquid nitrogen, lyophilized to dryness without further treatment. A 20-fold concentrated solution was produced from the lyophilisate. Alaninol was detected therein by GC measurements.

20

Example 5:**Formation of γ -glutamylisopropylamide (γ -GIPA)**

25 **Pseudomonas Kie 171 and Pseudomonas sp. Kie 171-B excrete γ -GIPA during growth on IPA.**

During growth of Pseudomonas sp. Kie 171 on IPA, and of its mutant Pseudomonas sp. Kie 171-B on L-glutamate in the presence of IPA, γ -GIPA was excreted
30 into the medium. By culturing a relatively large amount of biomass, 100 mg of this compound were isolated and its structure was identified using ¹H-NMR, ¹³C-NMR and MS. When IPA was transformed to L-alaninol by resting cells of Pseudomonas sp. Kie 171-B, no γ -GIPA was
35 formed.

This was demonstrated by HPLC measurements. γ -GIPA is therefore only formed by cells during growth.

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^1H -NMR (D_2O ; 400 MHz $\delta_{\text{TSP}} = 0$): 3.91 ppm (sept, 1H, H-6);
 3.75 ppm (t, 1H, H-2);
 2.38 ppm (m, 2H, CH_2 -4);
 2.13 ppm (m, 2H, CH_2 -3);
 1.13 ppm (d, 6H, CH_3 -7 and CH_3 -8).

^{13}C -NMR (D_2O ; 100 MHz; $\delta_{\text{dioxane}} = 67.4$ ppm) 174.7 and 174.2 ppm (2 x s, C-1 and C-5);
 55.1 ppm (d, C-2);
 42.6 ppm (d, C-6);
 32.6 ppm (t, C-4);
 27.4 ppm (t, C-3);
 22.2 and 22.1 ppm (2 x q, C-7 and C-8).

Example 6:

5 Biotransformation of IPA to L-alaninol in the presence of $^{18}\text{O}_2$

For this experiment, *Pseudomonas* sp. Kie 171-B1 was used. This strain was cultured in a similar manner to Example 4 on MM medium containing glutamate and kanamycin. After culturing up to the exponential phase, 10 MM IPA was added for enzyme induction. This culture was shaken for a further 3 h at 30°C and 150 rpm. The cells were then centrifuged off, washed with MM medium and concentrated to an OD546 $\approx$ 13. 25 ml of this cell suspension were then charged into 100 ml serum bottles, which were closed with rubber stoppers. With Sample 1, the atmosphere was left untreated. With Sample 2, all of the air was exchanged twice for $^{18}\text{O}_2$ (96%). At the start of the biotransformation, 0.5 ml of 1M IPA solution (pH 7.0) was added to both samples (IPA concentration in the solution 20 mM).

The samples were shaken for a further 8 h at 30°C and 150 rpm. In Sample 1, 6.9 mM alaninol was detected and in Sample 2 0.48 mM alaninol was detected.

- 15 -

The cells were then centrifuged off and the water in the cell-free supernatant was removed on the rotary evaporator. Trifluoroacetic anhydride was added to the residue to form N-trifluoroacetylalaninol. From this compound, the rate of incorporation of ^{18}O was determined by GC/MS.

The following peaks of the trifluoroacetylated derivative were used to determine the rate of incorporation:

m/e	Assignment	
174	M+ + 1	protonated M+ containing one ^{18}O
172	M+ + 1	protonated M+
158	M+ - 15	Loss of methyl from an M+ containing one ^{18}O
156	M+ - 15	Loss of methyl

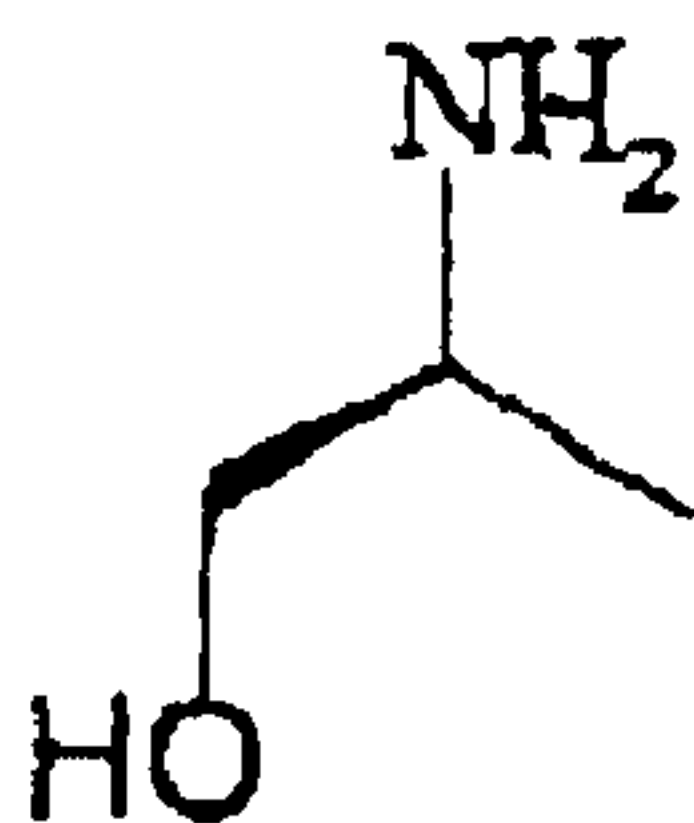
The evaluation of the SIM spectrum showed a rate of incorporation of ^{18}O of 37.2% for Sample 2.

The incorporation of ^{18}O during the biotransformation of IPA to alaninol can therefore be explained by the fact that an oxygenase catalyses this reaction. The relatively low incorporation of ^{18}O in Sample 2 can be explained by the fact that ^{16}O -labelled alaninol was carried over during the incomplete washing of the cells.

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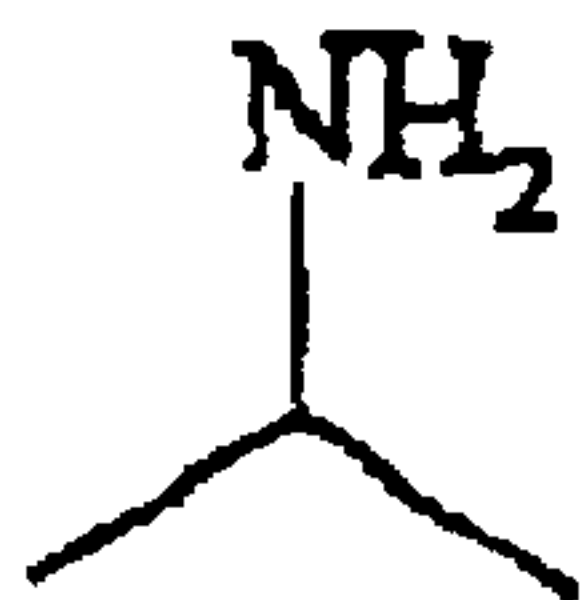
Patent claims:

1. Microorganisms, characterized in that they are able to convert isopropylamine to L-alaninol and do not catabolize the latter.
2. Microorganisms according to claim 1 obtainable by:
- a) Mutagenesis of microorganisms which are able to grow using isopropylamine and L-alaninol as sole C source,
- b) selection of mutants which are able to grow using L-alanine but not L-alaninol and isopropylamine as sole C source.
3. Microorganisms according to Claim 2 or 3 of the genus *Pseudomonas*.
4. Microorganisms according to any one of Claims 1 to 3 of the species *Pseudomonas* sp. Kie 171-B (DSM 11521) and Kie 171-B1 (DSM 11629), and also their functionally equivalent variants and mutants.
5. Microorganisms, characterized in that they are able growing using isopropylamine as sole C source.
6. Process for preparing L-alaninol of the formula



I

- characterized in that isopropylamine of the formula



II

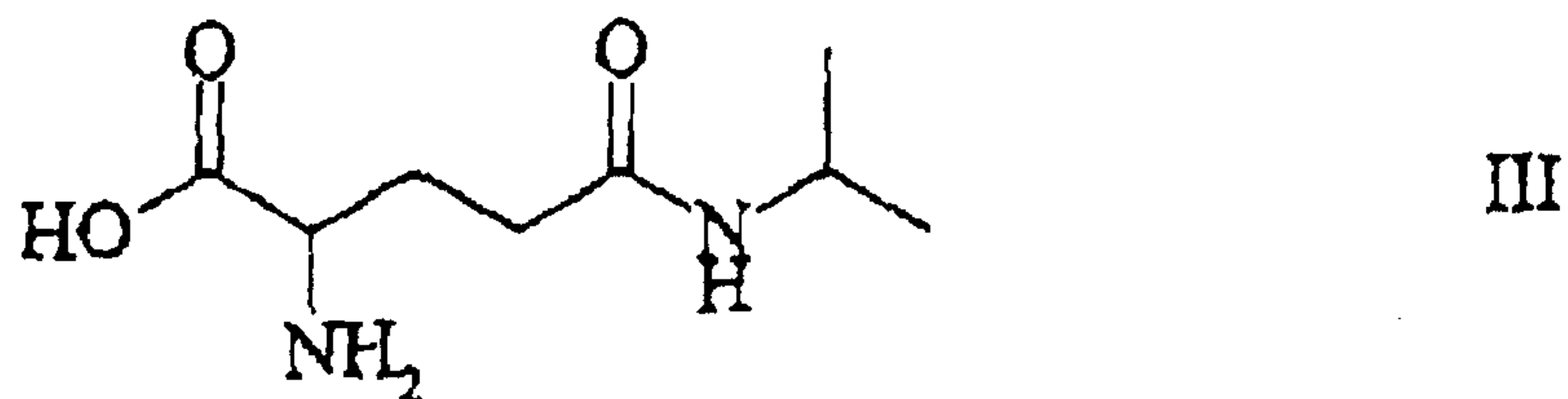
- is converted to L-alaninol using the microorganisms according to any one of Claims 1 to 4.
7. Process according to Claim 6, characterized in that the biotransformation is carried out using microorganisms of the genus *Pseudomonas*.

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8. Process according to any one of Claims 6 and 7, characterized in that the biotransformation is carried out with addition of isopropylamine in such a manner that the concentration does not exceed 10% by weight.

5 9. Process according to any one of Claims 6 to 8, characterized in that the biotransformation is carried out at a temperature of from 10 to 50°C and a pH of from 4 to 10.

10 10. Process for preparing γ -glutamylisopropylamide of the formula



characterized in that isopropylamine of the formula



15 is converted into the product of formula III using growing cells of the microorganisms according to any one of Claims 1 to 6.