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(71) Applicant (for all designated States except US): **LONZA LTD** [CH/CH]; Lenzastrasse, CH-3930 Visp (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **AVI, Manuela** [IT/CH]; Jesuitenweg 168, CH-3902 Brig-Glis (CH).
KLEGRAF, Ellen [DE/CH]; Apollostrasse 8, CH-3902 Brig-Glis (CH).

(74) Agent: **BANSE & STEGLICH PATENTANWALT-SKANZLEI**; Herzog-Heinrich-Strasse 23, 80336 Munich (DE).

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(54) Title: PROCESS FOR PRODUCING L-CARNITINE FROM BETA-LACTONES EMPLOYING LIPASES

(57) Abstract: Subject of the invention is a process for the production of L-carnitine, wherein a β -lactone, which is a 4-(halomethyl)oxetane-2-one, is converted into L-carnitine, wherein the process comprises an enzymatic conversion of the β -lactone into (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester.

PROCESS FOR PRODUCING L - CARNITINE FROM BETA - LACTONES EMPLOYING LIPASES

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The invention relates to methods for the production of L-carnitine.

Background of the invention

10 Carnitine (vitamin B₁; 3-hydroxy-4-trimethylammonio-butanoate) is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. It is used as a nutritional supplement. Carnitine exists in two stereoisomers. The 15 biologically active form is L-carnitine, whilst its enantiomer, D-carnitine, is biologically inactive. When producing L-carnitine in an industrial process, it is desirable to produce the biologically active L-form in high purity.

Various methods were described for the industrial production of L-carnitine.

20 Microbiological processes are known, in which L-carnitine is produced directly by bacteria. In other processes, a racemate is produced by organic synthesis and separated subsequently into enantiomers.

25 Further, attempts have been made to synthesize L-carnitine directly from chiral precursors. A group of potential precursors are chiral cyclic lactones. Since methods for obtaining chiral lactones are known in principle, L-carnitine is available upon hydrolysis of the lactone ring.

US 5,473,104 discloses a process for the preparation of L-carnitine from (S)-3-30 hydroxybutyrolactone. The process is a two-step process, wherein in a first step (S)-3-hydroxybutyrolactone is converted into the corresponding hydroxy-activated lactone, whilst maintaining the ring structure. In a second step, the ring of the

activated lactone is opened and the trimethylammonium group is introduced with trimethylamine. Altogether, the reaction is relatively complicated because it requires the activation of an intermediate with harsh chemicals.

5 CH 680 588 A5 discloses a process for producing L-carnitine from a β -lactone precursor, wherein a chiral 2-oxetanone is converted into L-carnitine in a two-step process. In a first step, 4-(chloromethyl)-2-oxetanone is subjected to a hydrolysis step, in which the ring is opened and 4-chloro-3-hydroxybutyric acid is obtained. In a subsequent step, the acid is converted into L-carnitine with trimethylamine. However,
10 the production of chiral β -lactones requires relatively complicated heavy metal catalysts and the yields are often not sufficient.

Nelson & Spencer (J. Org. Chem. 2000, 65, 1227-1230) disclose a process for obtaining enantiomerically enriched β -lactones from β -lactone racemates by
15 enzymatic resolution with lipases. The substrates used are various alkyl- and aryl- β -lactone racemates. As summarized in Table 1, the yields are only sufficient for a limited number of reactions. The enantioselectivity depends strongly on the substituents of the β -lactone and the enantiomeric yield for the small substituent methyl is very low (table 1). The reactions require organic solvents, such as benzyl alcohol, which is not desirable for industrial applications for environmental reasons.
20 The reaction times are relatively long (mostly 72 hours).

US 2006/0046286 discloses methods for obtaining chiral β -butyrolactones and 3-hydroxycarboxylic acid esters by enzymatic esterification from β -lactones. As for
25 Nelson & Spencer, the use of lipases is suggested. The reactions require organic solvents, such as toluene, and relatively long reaction times for about 16 hours. The substrate is β -butyrolactone. In most experiments, the substrate is not a racemate, but optically active (R)- β -butyrolactone. The specific reactions mostly yield (R)- β -butyrolactone at enhanced enantiomeric purity, whereas (S)-3-hydroxybutyric acid esters, if at all, are obtained only in relatively low amounts (examples 1, 6, 7).
30

Since enantiomerically pure L-carnitine is an important industrial product, it would be desirable to provide further efficient processes for its production. Specifically, it would be desirable to provide processes for the production of L-carnitine in a relatively simple manner at a high total yield and enantiomeric yield.

5

Problem underlying the invention

10 The problem underlying the invention is to provide a method for producing L-carnitine, which overcomes the above-mentioned drawbacks. Specifically, the problem is to provide an efficient and simple process for the production of L-carnitine.

15 The total yield as well as the chiral yield shall be high. The number of process steps shall be relatively low and the process shall not require complicated apparatuses. Overall, the process shall have a high be atom economy and shall be cost and labour efficient. The chemicals used shall be readily available and should not be too expensive.

20 It is a further problem underlying the invention to provide a mild process, which avoids harsh conditions and harsh chemicals. Chemicals, which affect the environment, such as organic solvents or heavy metals, or those which could affect the health of the workers, such as aromatic solvents, should be avoided. Heavy metal catalysts, which have to be removed, but nonetheless may remain as trace impurities in the product, shall be avoided. Specifically, no expensive catalysts comprising precious metals, such as platinum, should be used. The overall energy 25 input shall be low. Specifically, the reaction should be carried out at relatively low temperatures and for relatively low reaction times.

Disclosure of the invention

30 Surprisingly, the problem underlying the invention is solved by the process according to the claims. Further inventive embodiments are disclosed throughout the description.

Subject of the invention is a process for the production of L-carnitine, wherein a β -lactone, which is a 4-(halomethyl)oxetane-2-one, is converted into L-carnitine, wherein the process comprises an enzymatic conversion of the β -lactone into (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester.

The carnitine is L-carnitine ((R)-carnitine, levocarnitine). L-carnitine is the physiologically active stereoisomer of carnitine. The reaction product of the enzymatic conversion is an (R)-4-halo-3-hydroxybutyric acid or an ester thereof, depending on whether the enzymatic conversion is carried out in aqueous medium or in alcohol. This intermediate product can be converted into L-carnitine in a subsequent reaction step. The configuration of the chiral carbon of the acid or ester is (R), as for the corresponding L-carnitine. Thus, the subsequent conversion into L-carnitine can be carried out simply by replacing the halogen atom of the intermediate by a trimethylammonium group by means of a nucleophilic substitution, whilst the chirality remains unaffected.

In specific embodiments of the invention, the β -lactone is 4-(chloromethyl)oxetane-2-one, 4-(bromomethyl)oxetane-2-one or 4-(iodomethyl)oxetane-2-one. The use of 4-(chloromethyl)oxetane-2-one is preferred.

Preferably, the enzyme is stereoselective for the (R)-isomer of the β -lactone. In other words, preferably the enzyme converts exclusively, or at least predominantly, the (R)-lactone into the corresponding (R)-acid or acid ester. Thus, when the substrate is a β -lactone racemate, or at least comprises (S)- β -lactone and (R)-lactone, the enzyme selectively converts the (R)- β -lactone into the corresponding (R)-acid or acid ester, whereas the (S)- β -lactone remains unaffected or essentially unaffected. Thus the corresponding (S)- β -lactone remains and accumulates in the reaction mixture. Thus, the (R)-acid or acid ester is enriched in the product. Further, the (S)- β -lactone will be enriched in the product. Both compounds can be separated from each other due to their different solubilities in water and organic solvent. When starting from a β -lactone racemate, an ideal enzymatic reaction would yield a mixture of 50% Mol-% (R)-acid

or acid ester (with an optical purity of 100% ee) and 50 Mol-% of residual (S)- β -lactone, which subsequently would be removed from the mixture.

In a preferred embodiment of the invention, the β -lactone is a racemate. Preferably,

5 an enantiomeric excess of the (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester is obtained. The enantiomeric excess (in relation to (S)-4-halo-3-hydroxybutyric acid or ester) is preferably higher than 80%, more preferably higher than 90%, 95% or 99% ee.

10 The enzymatic conversion is carried out in the presence of a solvent. Preferably, the solvent comprises water. Water may be present in a one-phase system or a two-phase system. In the presence of water, a (R)-4-halo-3-hydroxybutyric acid is obtained. The enzymatic conversion is then an enzymatic hydrolysis. As a result of the enzymatic hydrolysis, (R)-4-chloro-3-hydroxybutyric acid, (R)-4-bromo-3-15 hydroxybutyric acid or (R)-4-iodo-3-hydroxybutyric acid is obtained.

20 Preferably, the solvent is exclusively water. When using water as a solvent, the initial reaction mixture is a solution or suspension. In this embodiment, the reaction mixture is preferably a one-phase system, in which a clear phase separation between an aqueous phase and a liquid organic phase is not observed.

In another preferred embodiment of the invention, the enzymatic conversion is carried out in a two phase system, wherein (R)-4-halo-3-hydroxybutyric acid is enriched in the aqueous phase. In a two-phase system, the relatively non-polar β -lactone substrate is enriched in the organic phase, whereas the ionic acid product is enriched in the aqueous phase. Thus after finishing the reaction, the phases can be separated and the aqueous phase already comprises a relatively pure product. The organic phase may comprise any solvent, in which the β -lactone is highly soluble and the acid is poorly soluble. In comparison, the one-phase system in aqueous medium requires less organic solvent overall and is thus advantageous for economic and environmental reasons.

Examples of the solvent include hydrocarbons, such as aliphatic hydrocarbons such as pentane, hexane, heptane, octane, decane and cyclohexane, aromatic hydrocarbons such as toluene and xylene; halogenated hydrocarbons such as dichloromethane, 1,2-dichloroethane, chloroform, carbontetrachloride and o-dichlorobenzene; ethers such as diethyl ether, diisopropyl ether, tert-butyl methyl ether, dimethoxyethane, ethylene glycol diethyl ether, tetrahydrofuran, 1,4-dioxane and 1,3-dioxolane; amides such as formamide; sulfoxides such as dimethyl sulfoxide.

In another embodiment of the invention, the solvent is an alcohol, preferably an alcohol comprising 1 to 5 carbon atoms, more preferably methanol, ethanol or propanol. When carrying out the enzymatic conversion in an alcohol and in the absence of water, the product is a linear ester. Subsequently, when the ester is converted into L-carnitine, it is de-esterified.

In a preferred embodiment of the invention, after enzymatic conversion, residual β -lactone is removed from the solution. Preferably, the residual β -lactone is removed by extraction with a solvent. For example, when the solution is carried out in a one-phase system, the residual β -lactone may be extracted with an ester, such as ethylacetate, or with hexane. Preferably, multiple extractions with relatively low amounts of solvent are carried out.

In a preferred embodiment, the enzyme is a hydrolase (Enzyme Commission number EC 3). Hydrolases yields two products from a substrate by hydrolysis. Preferably, the hydrolase is an esterase (EC 3.1) or a peptidase (EC 3.4). An esterase is a hydrolase enzyme, such as a lipase, that hydrolyses esters into an acid and an alcohol. A peptidase (protease) is an enzyme that conducts proteolysis, that is, hydrolysis of a peptide bond.

In a preferred embodiment of the invention, the enzyme is a lipase. The lipase is selective for the (R)- β -lactone. Preferably, the lipase is from fungi, yeasts or bacteria. This means that the organism was the origin of the lipase. However, the lipase may

be an artificial, especially a recombinant, lipase. Such lipases are commercially available, for example from the companies Novozymes, Amano Enzyme or Nagase.

Preferably, the enzyme is a triacylglycerol lipase. Such lipases of enzyme class 5 3.1.1.3 catalyze hydrolysis of triglycerides into fatty acids and glycerols.

Lipases from fungi, yeasts or bacteria are often synthesized *in vivo* as inactive precursors. These precursors are processed and secreted. Such lipases are typically 10 extracellular lipases. Mature proteins are generated by cleaving off N-terminal peptides, such as signal peptides or propeptides. According to the invention, the lipase is preferably a mature lipase. In other words, it is preferably in the active form. The inventive process is preferably an *in vitro* process.

15 The lipase may comprise a catalytic triad of amino acids Ser-His-Asp. For example, such lipases are found in yeast, such as *Candida*.

In preferred embodiments of the invention, the lipase is from *Candida*, *Pseudomonas*, *Aspergillus*, *Bacillus* or *Thermomyces*. The lipase may be a derivative 20 of such a lipase, preferably one having at least 75%, preferably at least 90%, sequence identity.

Preferably, the lipase is from *Candida*, *Pseudomonas* or *Aspergillus*. More 25 preferably, the lipase is from *Pseudomonas cepacia* (*Burkholderia cepacia*), *Pseudomonas fluorescens* or *Candida antarctica*. The lipase may be a derivative of such a lipase, preferably one having at least 75%, preferably at least 90%, sequence identity.

Derivatives are obtainable by amino acid substitution, deletion, insertion or 30 modification. Preferably, derivatives are obtained by recombinant DNA modification methods. Such recombinant lipases may have higher stabilities and efficiencies compared to their natural precursors.

Especially preferred are lipases from *Pseudomonas cepacia* and/or lipase B from *Candida antarctica*. It was found that these enzymes provide the desired products at high total and enantiomeric yield.

5 In a highly preferred embodiment, the enzyme is lipase from *Pseudomonas cepacia* of SEQ ID NO:1 (UniProt identifier P22088[45-364]; Joergensen et al., J. Bacteriol. 1991; 173, p559-567). The mature protein is a protein having 320 amino acids. It is derived from a 364 amino acid precursor comprising a signal peptide and a propeptide.

10

In another preferred embodiment, the enzyme is lipase from *Pseudomonas cepacia* having an amino acid sequence different from SEQ ID NO:1. *P. cepacia* comprises several lipases with amino acid sequences of high homology with SEQ ID NO:1. For example, the enzyme may be *P. cepacia* alkaline lipase (NCBI Acc. No: ABX71757.1; Dalal et al., J. Biotechnol. Appl. Biochem. 2008; 51, p23-31). This alkaline lipase has about 98% sequence identity with the lipase of SEQ ID NO:1. The enzyme may also be *P. cepacia* lipase (Genbank: ABN09945.1; Yang et al., J. Mol. Catal., B Enzym. 2007; 45, p91-96). This lipase has about 92% sequence identity with lipase of SEQ ID NO:1.

15

15 Lipase B from *Candida antarctica* of SEQ ID NO:2 (UniProt identifier P41365[26-342]) is a protein having 317 amino acids. The mature protein is derived from a 342 amino acid precursor comprising a signal peptide and a propeptide.

20

20 Preferably, the derivative is a derivative of lipase from *Pseudomonas cepacia* of SEQ ID NO:1 and/or from lipase B from *Candida antarctica* of SEQ ID NO:2, preferably one having at least 75%, preferably at least 90%, sequence identity compared to SEQ ID NO:1 and/or SEQ ID NO:2. As used herein, sequence identity is preferably determined by BLAST according to standard parameters. Derivatives of such lipases obtainable by amino acid substitution, deletion, insertion or modification, and methods for obtaining such derivatives, are known in the art. For example, derivatives of lipase B from *Pseudomonas cepacia* having increased catalytic activity

and methods for their production are disclosed in Puech-Guenot et al., J. Biomol. Screen. 2008; 13(1), p72-79. Derivatives of lipase B from *Candida antarctica* having increased catalytic activity and methods for their production are disclosed in Qian et al., J. Mol. Biol. 2009; 393; p191-201.

5

In a preferred embodiment of the invention, the enzymatic conversion is carried out at a temperature between 0°C and 50°C, preferably between 20°C and 40°C, specifically about 25 °C or about 30°C. The temperature is adjusted in view of the specific enzyme used.

10

In a preferred embodiment of the invention, the enzymatic conversion is carried out in aqueous solution comprising 0.1 to 50 weight% β -lactone, more preferably between 0.1 and 10 weight% or between 0.5 and 3 weight% β -lactone.

15 The reaction is carried out in an appropriate buffer. The buffer is adapted to the specific enzyme used. Mostly, the pH will be approximately neutral, for example between 5 and 9, or between 7 and 8. If necessary, the pH may be adapted during the reaction to remain stable by the addition of a base or an acid.

20 In a preferred embodiment, the reaction mixture consists of the buffer, enzyme and β -lactone. Additives might be added, for example those which enhance the stability or the turnover of the enzyme, such as salts, metal ions or co-factors. Overall, the reaction requires only a low number of chemicals and can be carried out in a relatively simple manner at mild temperatures.

25

When a desired amount of the acid or acid ester is obtained, the reaction is terminated. The reaction may be terminated by removing the enzyme, for example by filtration. When using a β -lactone racemate as a substrate and a highly selective enzyme, up to 50% of the β -lactone may be converted into the corresponding (R)-30 acid or (R)-acid ester. After removal and isolation, residual (S)- β -lactone may be used for other reactions.

The reaction time may be between between 1 to 50 hours, preferably between 2 to 20 hours. It was found that with some enzymes an efficient turnover can be achieved within 10 hours, specifically within 8 hours.

5 Mono-halogenated β -lactones for carrying out the inventive ring opening reaction are known in the art. In view of the stereoselectivity of the enzyme, a β -lactone racemate can be used in the inventive process. Mono-halogenated β -lactones are obtainable e.g. by low pressure carbonylation of the corresponding epoxides as disclosed in US2007/0213524 A1 or starting from unsaturated acid using Lewis acid and

10 hypochlorite as described by Lopez-Lopez, Jose; Tetrahedron Letters 2007, 48(10), 1749-1752.

In a preferred embodiment of the invention, chiral 4-(halomethyl)oxetane-2-ones are obtained by a [2+2] cycloaddition reaction in the presence of a catalyst. In a specific 15 embodiment of the invention, a chiral β -lactone is used. This may enhance the enantiomeric yield in the inventive reaction. A chiral β -lactone can be obtained by a [2+2] cycloaddition of ketene with an aldehyde $X\text{-CH}_2\text{-CHO}$, wherein X is selected from Cl, Br and I, in the presence of a chiral catalyst. Ketene (ethenone, formula $\text{C}_2\text{H}_2\text{O}$) is a colorless gas, which is highly reactive due to two adjacent double bonds 20 in the molecule.

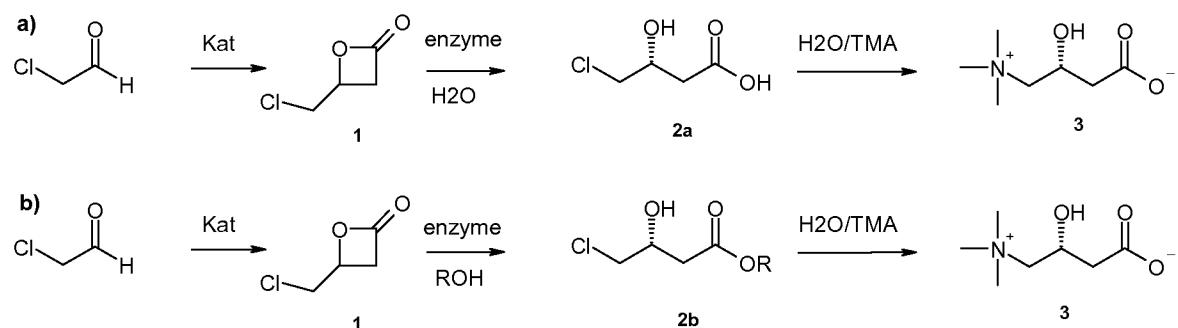
In a preferred embodiment of the invention, the 4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester obtained in the enzymatic conversion is subsequently converted into L-carnitine with trimethylamine (TMA). Preferably, the 25 halogen atom is substituted by a trimethylamine group in a nucleophilic substitution reaction. The TMA can be brought into contact with the β -lactone in the presence of a base. The base might be added after bringing the β -lactone in contact with the TMA.

In a preferred embodiment of the inventive process, the β -lactone is converted into L-30 carnitine in a two-step process. In a first step, a 4-(chloromethyl)-2-oxetanone is subjected to the enzymatic hydrolysis, in which the β -lactone ring is opened and 4-

chloro-3-hydroxybutyric acid is obtained. In a subsequent step, the acid, ester is converted into L-carnitine with trimethylamine.

An exemplified inventive process for the synthesis of L-carnitine is shown in scheme 5 below. The process comprises a [2+2] cycloaddition of ketene and an aldehyde of the formula $\text{Cl}-\text{CH}_2-\text{CHO}$. The resulting β -lactone racemate **1** is subjected to the enzymatic ring cleavage reaction to yield the corresponding (R)-acid **2a** (in water as a solvent) or (R)-ester **2b** (in alcohol). The acid **2a** or ester **2b** is then converted into L-carnitine with TMA.

10



15

Scheme 1: Synthesis of L-carnitine

In a preferred embodiment of the invention, the TMA is recycled during the process. Since TMA is available in gaseous form, it can be led through the reaction fluid, 20 collected and recycled. In the reaction medium, dissolved TMA can be separated from the mixture after reaction is finished (e. g by distillation) and reintroduced in the process. Preferably, the TMA is reintroduced into the reaction pathway in a cyclic process. TMA is commercially available in the form of a pure gas (Fluka Chemicals) or in the form of an aqueous solution of 10 to 40 wt.%. The amount of TMA in the 25 reaction mixture may be between 1 and 3 equivalents, preferably between 1 and 2.5 equivalents. However, the amount and excess of TMA is less critical than the amount of metal hydroxide, because it can be recycled during the reaction and reintroduced into the reaction chamber.

Another subject of the invention is a process for the production of a (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester, wherein the process comprises an enzymatic conversion of a β -lactone, which is a 4-(halomethyl)oxetane-2-one, into (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester.

5 These reaction products are valuable intermediates for the production of L-carnitine. They may be converted into L-carnitine in a simple nucleophilic substitution, wherein the chirality remains unaffected. In addition, they might be used for the synthesis of other organic compounds or derivatives of L-carnitine.

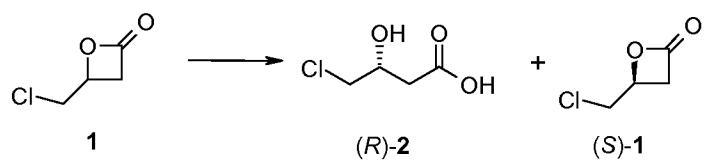
10 In a preferred embodiment of the invention, in the enzymatic conversion the total yield of (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester is between 40% and 50%, based on the total initial amount of β -lactone, and/or the enantiomeric purity of the (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester is at least 90%, more preferably at least 95%.

15 The inventive process solves the problems underlying the invention. The process is relatively simple and economical and requires only a low number of process steps. Thus side reactions are avoided and the total yield and enantiomeric yield are high. Since the enzymatic conversion may use enzymatic resolution, it is possible to start 20 from a β -lactone racemate. Thus, it is not necessary to use expensive chiral reactants.

25 The reaction conditions are mild, because neither harsh chemicals, such as heavy metal catalysts, nor high amounts of organic solvents are necessary. The process requires relatively low amounts of energy for heating. In principle, only enzymes are necessary as additives, which can be removed easily and are generally non-hazardous. The intermediate is thus especially applicable for producing the food additive L-carnitine.

Examples

An enzymatic resolution was carried out in a one-phase aqueous system. The 5 substrate was a 4-(chloromethyl)oxetane-2-one racemate, which was converted into a mixture of (R)-4-chloro-3-hydroxybutyric acid and residual (S)- β -lactone as illustrated by scheme 2 below.



10

Scheme 2: Enzymatic resolution of a β -lactone racemate according to the examples.

Analytics

15

The reaction is monitored by gas chromatography (GC) on a Lipodex E column. 4-(Chloromethyl)-2-oxetanone **1** is analyzed by GC on a Lipodex E column. 4-Chloro-3-hydroxybutyric acid **2** is derivatized using a chiral, fluorescent reagent. The reaction mixture is analyzed by HPLC using an ODS-column and flourimetric detection.

20

Examples 1 to 5

To a solution or suspension of 12.5 mg enzyme in 5.0 mL potassium phosphate buffer (0.1 M, pH 7.5), 50 mg 4-(chloromethyl)-2-oxetanone **1** is added and the 25 reaction mixture is stirred at 30°C. The pH is continually adjusted to 7.5 with 0.5 M KOH. At regular intervals, 200 μ L samples are taken, extracted with 400 μ L ethyl acetate, filtered and analyzed by chiral GC. After complete hydrolysis of the (R)-enantiomer, the enzyme is centrifuged off and the reaction mixture is extracted twice with 5.0 mL ethyl acetate. (R)-4-chloro-3-hydroxy butyric acid **2** is obtained as 30 aqueous solution. Reactions were carried out with different lipases as summarized in

table 1 below. Conversion and enantiomeric purities are shown in table 1. **(S)-1** and **(R)-2** are both obtained at high enantiomeric purity. The reaction time at 30°C was only between 4 to 8 hours.

Ex .	Enzyme, Source	Commercial Name, Supplier	Reactio n Time [h]	Convers. [%]	ee [%]	
					(S)-1	(R)-2
1	lipase <i>Pseudomonas cepacia</i>	Lipase PS-C Amano	6	52	99.9	99.7
2	lipase <i>Pseudomonas cepacia</i>	Lipase PS Amano	4	44	99.9	96.0
3	lipase <i>Pseudomonas fluorescens</i>	Lipomod L056P	6	55	99.9	91.5
4	lipase B <i>Candida antarctica</i>	Novozyme 435	7.5	48	99.9	99.8
5	lipase	Lipase P Nagase	8	41	99.9	85.0

5

Table 1: Summary of conditions and results of examples 1 to 5.

CLAIMS

1. A process for the production of L-carnitine, wherein a β -lactone, which is a 4-(halomethyl)oxetane-2-one, is converted into L-carnitine, wherein the process comprises an enzymatic conversion of the β -lactone into (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester.

2. The process of at least one of the preceding claims, wherein the β -lactone is a racemate.

3. The process of claim 2, wherein an enantiomeric excess of the (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester is obtained.

4. The process of at least one of the preceding claims, wherein the enzymatic conversion is carried out in an aqueous medium and (R)-4-halo-3-hydroxybutyric acid is obtained.

5. The process of at least one of the preceding claims, wherein the enzymatic conversion is carried out in a two phase solution, wherein (R)-4-halo-3-hydroxybutyric acid is enriched in the aqueous phase.

6. The process of at least one of the preceding claims, wherein, after enzymatic conversion, residual β -lactone is removed from the solution.

7. The process of claim 6, wherein the residual β -lactone is removed by extraction with a solvent.

8. The process of at least one of the preceding claims, wherein the enzyme is a lipase.

9. The process of at least one of the preceding claims, wherein the lipase is from *Candida*, *Pseudomonas*, *Aspergillus*, *Bacillus* or *Thermomyces*, or a derivative of such a lipase having at least 75%, preferably at least 90%, sequence identity.

5 10. The process of at least one of the preceding claims, wherein the lipase is from *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Candida Antarctica*, the lipase preferably having the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2, or a derivative of such a lipase having at least 75%, preferably at least 90%, sequence identity.

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11. The process of at least one of the preceding claims, wherein the enzymatic conversion is carried out at a temperature between 0°C and 50°C, preferably between 20°C and 40°C, and/or wherein the enzymatic conversion is carried out in aqueous solution comprising 0.1 to 10 weight% β -lactone.

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12. The process of at least one of the preceding claims, in which the β -lactone is synthesized in a preceding step in a [2+2] cycloaddition of ketene and an aldehyde of the formula X-CH₂-CHO, wherein X is selected from Cl, Br and I.

20 13. The process of at least one of the preceding claims, wherein the 4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester obtained in the enzymatic conversion is converted into L-carnitine with trimethylamine.

25 14. The process of at least one of the preceding claims, wherein the total yield of (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester in the enzymatic conversion is between 40% and 50%, based on the total initial amount of β -lactone, and/or wherein the enantiomeric purity of the (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester is at least 90%.

30 15. A process for the production of a (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester, wherein the process comprises an enzymatic

conversion of a β -lactone, which is a 4-(halomethyl)oxetane-2-one, into (R)-4-halo-3-hydroxybutyric acid or (R)-4-halo-3-hydroxybutyric acid ester.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/064575

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P13/00 C12P7/42 C12P7/62 C12P41/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, COMPENDEX, FSTA, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CH 680 588 A5 (LONZA AG) 30 September 1992 (1992-09-30) cited in the application page 2, line 14 - line 21 page 2, line 23 - line 46 page 4; claim 3 -----	1-14
A	DATABASE CAPLUS [Online] CHEMICAL ABSTRACTS SERVICE; 2009, LEE, H. ET AL.: "Method for preparing optically active alkyl 3-hydroxybutanoate derivative", XP002666098, Database accession no. 2009:278677 the whole document	15
X	DATABASE CAPLUS [Online] CHEMICAL ABSTRACTS SERVICE; 2009, LEE, H. ET AL.: "Method for preparing optically active alkyl 3-hydroxybutanoate derivative", XP002666098, Database accession no. 2009:278677 the whole document	15
A	-/-	1-14

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 30 August 2012	Date of mailing of the international search report 12/09/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Fuchs, Ulrike

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/064575

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>& KR 2009 0022032 A (KOREA RESEARCH INSTITUTE OF CHEMICAL TECHNOLOGY) 4 March 2009 (2009-03-04)</p> <p>-----</p> <p>SONG, C.E. ET AL.: "New Method for the Preparation of (R)-Carnitine", TETRAHEDRON ASYMMETRY, vol. 6, no. 5, May 1995 (1995-05), pages 1063-1066, XP004048201, abstract page 1063, line 10 - page 1064, line 26 scheme: 1</p> <p>-----</p> <p>WO 2009/062731 A1 (LONZA LTD) 22 May 2009 (2009-05-22) page 2, line 4 - line 14 page 3 - page 5; examples 1-5</p> <p>-----</p> <p>DATABASE Geneseq [Online]</p> <p>8 January 2009 (2009-01-08), "Burkholderia cepacia triacylglycerol lipase sequence, SEQ ID 177", XP002682343, Database accession no. AUR75024 SEQ ID NO: 177 of W02009005647 A2; the whole document</p> <p>-----</p> <p>DATABASE Geneseq [Online]</p> <p>14 January 2010 (2010-01-14), "Candida antarctica lipase B protein SEQ ID:1", XP002682344, Database accession no. AXW34320 SEQ ID NO: 1 of W02010005235 A2; the whole document</p> <p>-----</p>	1-15
A		1-15
A		1-15
A		1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/064575

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
CH 680588	A5	30-09-1992	NONE	

WO 2009062731	A1	22-05-2009	AR 071060 A1 AT 523483 T CN 101861298 A EP 2231587 A1 ES 2372723 T3 JP 2011503132 A KR 20100090285 A TW 200922912 A US 2010267986 A1 WO 2009062731 A1	26-05-2010 15-09-2011 13-10-2010 29-09-2010 25-01-2012 27-01-2011 13-08-2010 01-06-2009 21-10-2010 22-05-2009
