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(54) Title: OPTICAL RESOLUTION OF α -ALKYL- β -THIOLACTONES USING HYDROLYTIC ENZYME (57) Abstract The present invention relates to a process for preparing an (R)-α-alkyl-β-thiolactone or an (S)-α-alkyl-β-mercaptopropionic acid by stereo-selectively hydrolyzing a mixture of R and S enantiomers of α-alkyl-β-thiolactone using a hydrolytic enzyme.		

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OPTICAL RESOLUTION OF α -ALKYL- β -THIOLACTONES USING HYDROLYTIC ENZYME

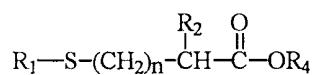
TECHNICAL FIELD

The present invention relates to a process for preparing an (R)- α -alkyl- β -thiolactone or an (S)- α -alkyl- β -mercaptopropionic acid by stereo-selectively hydrolyzing a mixture of R and S enantiomers of α -alkyl- β -thiolactone using a hydrolytic enzyme.

BACKGROUND ART

As the closely related prior art to the present invention, a process for preparing (S)- α -alkyl- β -mercaptopropionic acid using a hydrolytic enzyme such as esterase, lipase, etc. can be mentioned (see, USP 5,106,736). In this patented process, however, the substrate of the enzymatic reaction is not α -alkyl- β -thiolactone but α -alkyl- β -alkanoylmercaptopropionic acid ester represented by the following formula 1:

Formula 1



in which

n is 1 or 2,

R₁ represents $R_3-\overset{\overset{O}{||}}{C}-$, and

R₂, R₃ and R₄ independently of one another represent alkyl, cycloalkyl,

etc.

In addition, the reaction yield and optical purity of the product in the prior art process are 22-46% and 80-98%, respectively, which are somewhat insufficient to meet further application of this product. Furthermore, in order to prepare medicines such as captopril from (S)- α -methyl- β -mercaptopropionic acid, a product of the prior art process, some protection- deprotection reactions and complex condensation reactions should be carried out additionally.

DISCLOSURE OF INVENTION

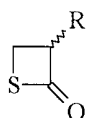
Thus, the present inventors have intensively studied to develop a novel process for preparing (R)- α -methyl- β -thiolactone in a high purity which can be used effectively in preparing medicines such as captopril due to its high reactivity. As a result, we have found that (R)- α -alkyl- β -thiolactone or (S)- α -alkyl- β -mercaptopropionic acid can be conveniently obtained from a mixture of R and S enantiomers of α -alkyl- β -thiolactone under mild conditions by using the stereo-selectivity of the hydrolytic enzymes, and then completed the present invention.

Therefore, it is an object of the present invention to provide a novel process for preparing (R)- α -alkyl- β -thiolactone or (S)- α -alkyl- β -mercapto- propionic acid which comprises stereoselectively hydrolyzing a mixture of R and S enantiomers of α -alkyl- β -thiolactone using a hydrolytic enzyme. Hereinafter, the constitution of the present invention will be explained in detail.

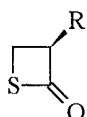
BEST MODE FOR CARRYING OUT THE INVENTION

The present invention uses a mixture of R and S enantiomers of α -alkyl- β -thiolactone, preferably α -alkyl- β -thiolactone racemate represented by the following formula 2 as a starting material, from which (R)- α -alkyl- β -thiolactone represented by the following formula 3 or (S)- α -alkyl- β -mercaptopropionic acid represented by the following formula 4 is obtained :

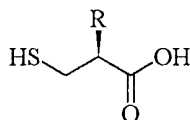
Formula 2



Formula 3



Formula 4



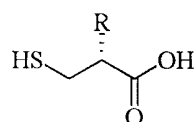
in the above formulas 2 to 4,

R represents C₁-C₆ alkyl.

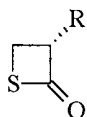
In the process for preparing (R)-form thiolactone of formula 3 from the mixture of R and S enantiomers of formula 2, (S)-form thiolactone is selectively hydrolyzed by a hydrolytic enzyme to produce

(R)- α -alkyl- β -mercaptopropionic acid represented by the following formula 5, which is removed during the subsequent separation procedure. The (S)- α -alkyl- β -mercaptopropionic acid is obtained when only the (R)-form thiolactone among the mixture is hydrolyzed due to the stereo-selectivity of the hydrolytic enzyme used. In this case, the remained (S)- α -alkyl- β -thiolactone represented by the following formula 6 is removed during the separation procedure.

Formula 5



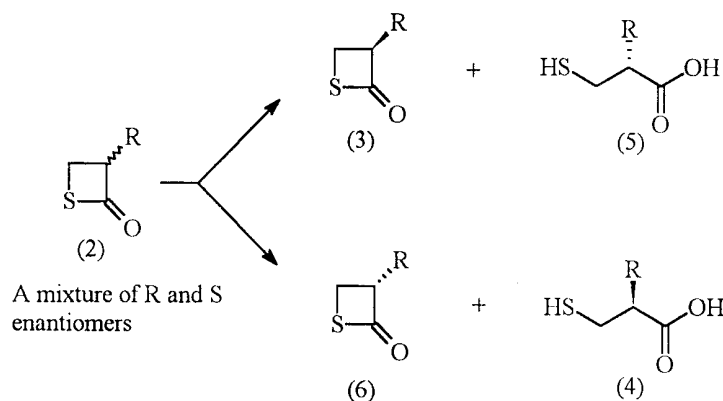
Formula 6



in the above formulas 5 and 6, R represents C₁-C₆ alkyl.

The hydrolysis procedure as explained above is summarized in the following reaction scheme 1 :

Reaction Scheme 1



Among the (R) - α -alkyl- β -thiolactone compounds obtained according to the present invention, (R) - α -methyl- β -thiolactone wherein methyl is attached to the α -position of thiolactone can be used as a useful and economic intermediate for synthesizing optically active medicines such as captopril, zofenopril, etc.

The reaction system according to the present invention consists essentially of a mixture of R and S enantiomers of α -alkyl- β -thiolactone, preferably α -alkyl- β -thiolactone racemate, as the starting material (in other words, substrate for the enzymatic reaction), a hydrolytic enzyme such as lipase, etc., an organic solvent and a small quantity of buffer solution to maintain the enzyme activity. If desired, surfactants may be added to the reaction solution in order to increase the enzyme activity. As the organic solvent, one or more selected from a group consisting of n -hexane, cyclohexane, n -heptane, cyclohexanone, n -octane, benzene, toluene, acetone, acetonitrile, chloroform, dichloromethane, 1,2-dichloroethane, dioxane, diethylether, dimethylformamide and tetrahydrofuran can be used in an amount of 10 to 500 times by weight with respect to the starting material. The reaction solution becomes so concentrated that the reaction rate is

lowered at the point near the reaction completion and that the optical purity of the product is deteriorated when the solvent is used in an amount of less than 10 times by weight. Also, it is uneconomic due to the use of excess solvent when the solvent is used in an amount of more than 500 times by weight. The most excellent stereo-selectivity may be resulted from using cyclohexane as the organic solvent. Buffer solutions which can be used in the present invention include phosphate buffer, bicarbonate buffer and Tris-HCl buffer having pHs ranging from 6.0 to 8.5. Since the reaction rate becomes very slow when the buffer solution is used in a less amount than that of the starting material, and the stereo-selectivity may be deteriorated when the buffer solution is used in an excess amount of more than 50 times by weight, it is preferable to use it in an amount of 1 to 50 times by weight with respect to the starting material. As the surfactant, one or more selected from a group consisting of Triton X-100[PEG(9-10) p-t-octylphenol, Sigma], Triton X-114[PEG(7-8) p-t-octylphenol, Sigma], Twin 80[PEG(20) sorbitan monooleate, Sigma], Twin 20[PEG(20) sorbitan monolaurate, Sigma], sodium dodecyl sulfate(SDS), lithium dodecyl sulfate(LDS), sodium cholate, sodium deoxy cholate, octyl glucoside[octyl- β -D-glucopyranoside, Aldrich], Brij 35[PEG(23) lauryl alcohol, Sigma], CHAPS[3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate, Sigma] and Xwittergent 3-14[N-alkyl-N,N-dimethyl-3-ammonio-1-propane sulfonate(14), Sigma], preferably one or more selected from a group consisting of Triton X-100, Triton X-114, Twin 80 and Twin 20 can be used in an amount of 1 to 10 times by weight with respect to the starting material. When the surfactant is used in a less amount than that of the starting material the reaction rate may be somewhat lowered and when used in an amount of more than 10 times by weight there may be some difficulties in the separation of the desired product.

Hydrolytic enzymes which can be used in the present invention to carry out the optical resolution include lipases, proteases and esterases. Specifically, a lipase derived from *Pseudomonas cepacea*, a lipase derived from *Penicillium roqueforti*, a cholesterol esterase, a lipase derived from *Penicillium camembertii*, a lipase derived from *Aspergillus*, a lipase derived from *Rhizopus japonicus*, a lipase derived from calf tongue root and chymopapain can be mentioned. Free enzymes or immobilized enzymes on a support may be employed in the reaction solution. As the suitable support for the enzyme immobilization, one or more selected from a group consisting of ceramic, celite, silica gel, alumina, borosilicate, calcium carbonate and zeolite can be mentioned. When an immobilized enzyme is used, said enzyme may be suspended in the reaction system or may be filled into a column. In the latter case, the reaction can be proceeded by passing the reaction solution through the column filled with an enzyme immobilized on a support at an appropriate flow rate, then by recycling the eluant. The reaction may not be carried out smoothly and accordingly, the yield may be lowered when the enzyme is used in an amount of less than 0.1 times by weight with respect to the starting material. When the enzyme is used in an amount of more than 100 times by weight, the separation process may be cumbersome and uneconomic. Therefore, it is preferable to use the enzyme in the amount ranging from 0.1 to 100 times by weight with respect to the starting material. It is more preferable to use the enzyme in the amount ranging from 0.3 to 15 times by weight.

The present reaction is carried out at temperatures ranging from -20 to 70°C for 8 to 72 hours. By controlling the reaction temperature, time, the amount of solvent and buffer solution within those ranges as

set forth above, the stereo-selectivity of the hydrolytic enzyme and the reaction rate and yield can be improved.

After the reaction is completed, weak basic work-up may be carried out to separate the desired (R)- α -alkyl- β -thiolactone or (S)- α -alkyl- β -mercaptopropionic acid, which are then analyzed by HPLC. In the present invention, an efficient analysis can be achieved when ChiralPack AS HPLC column(manufactured by Daicel Co., Japan) is used with n-hexane as an eluent at 0.7_{ml}/min of gradient flow rate. However, the eluent and column type can be varied and easily selected by a person having ordinary skill in the art upon considering the kind of substituents.

The present invention will be more specifically explained by the following examples. However, it should be understood that the examples are intended to illustrate but not to in any manner limit the scope of the present invention.

Example 1

10_{mg} of α -ethyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 8.5) was added thereto. 10_{mg} of the lipase derived from *Pseudomonas cepacea* was added to the reaction solution and the reaction was started at 45 $^{\circ}$ C. The reaction was stopped after 20 hours and the reaction solution was filtered. The filtrate was washed with saturated aqueous sodium bicarbonate solution and concentrated to obtain (R)- α -ethyl- β -thiolactone with a yield of 46% (99.2% ee, purity by HPLC).

《HPLC condition》

Column : ChiralPack AS (manufactured by Daicel Co., Japan)

Elution rate : 0.7_{ml}/min

Eluent : n-hexane

Example 2

40_{mg} of α -ethyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 20 _{μ l} of phosphate buffer solution (1M, pH 7.5) was added thereto. To the reaction mixture was added 100 _{μ l} of Triton X-100 as a surfactant and then mixed. 20_{mg} of the lipase derived from *Penicillium camembertii* was added to the reaction solution and the reaction was started at 55°C. The reaction was stopped after 27 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -ethyl- β -thiolactone with a yield of 48% (99.1% ee, purity by HPLC).

Example 3

10_{mg} of α -ethyl- β -thiolactone racemate was dissolved in 2_{ml} of n-hexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 8.0) was added thereto. 10_{mg} of the lipase derived from *Penicillium roqueforti* was added to the reaction solution and the reaction was started at 10°C. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -ethyl- β -thiolactone with a yield of 46% (99.0% ee, purity by HPLC).

Example 4

10_{mg} of α -ethyl- β -thiolactone racemate was dissolved in 2_{ml} of

cyclohexane and then 100 μ l of phosphate buffer solution (1M, pH 6.0) was added thereto. 20 mg of the lipase derived from *Penicillium roqueforti* was added to the reaction solution and the reaction was started at 45 $^{\circ}\text{C}$. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -ethyl- β -thiolactone with a yield of 46% (99.2% ee, purity by HPLC).

Example 5

10 mg of α -ethyl- β -thiolactone racemate was dissolved in 2 ml of cyclohexane and then 100 μ l of Tris-HCl buffer solution (1M, pH 7.0) was added thereto. 5 mg of the lipase derived from *Penicillium camembertii* was added to the reaction solution and the reaction was started at 45 $^{\circ}\text{C}$. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -ethyl- β -thiolactone with a yield of 47% (99.0% ee, purity by HPLC).

Example 6

10 mg of α -ethyl- β -thiolactone racemate was dissolved in 2 ml of cyclohexane and then 100 μ l of phosphate buffer solution (1M, pH 8.5) was added thereto. 20 mg of cholesterol esterase was added to the reaction solution and the reaction was started at 50 $^{\circ}\text{C}$. The reaction was stopped after 12 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -ethyl- β -thiolactone with a yield of 45% (99.1% ee, purity by HPLC).

Example 7

10_{mg} of α -ethyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 500 _{μ l} of phosphate buffer solution (1M, pH 7.5) was added thereto. 5_{mg} of the lipase derived from *Pseudomonas cepacea* was added to the reaction solution and the reaction was started at 45°C. The reaction was stopped after 8 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -ethyl- β -thiolactone with a yield of 45% (99.0% ee, purity by HPLC).

Example 8

10_{mg} of α -propyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 8.5) was added thereto. 20_{mg} of the lipase derived from *Rhizopus japonicus* was added to the reaction solution and the reaction was started at 45°C. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -propyl- β -thiolactone with a yield of 45% (99.0% ee, purity by HPLC).

Example 9

40_{mg} of α -propyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 20 _{μ l} of phosphate buffer solution (1M, pH 8.0) was added thereto. To the reaction mixture was added 100 _{μ l} of Triton X-100 as a surfactant and then mixed. 20_{mg} of the lipase derived from *Penicillium camembertii* was added to the reaction solution and the reaction was started at 37°C. The reaction was stopped after 27 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -propyl- β -thiolactone with a yield of 46% (99.2% ee,

purity by HPLC).

Example 10

10_{mg} of α -propyl- β -thiolactone racemate was dissolved in 2_{ml} of n-hexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 8.5) was added thereto. 10_{mg} of chymopapain was added to the reaction solution and the reaction was started at 10°C. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -propyl- β -thiolactone with a yield of 45% (99.1% ee, purity by HPLC).

Example 11

10_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 7.5) was added thereto. 10_{mg} of the lipase derived from *Pseudomonas cepacea* was added to the reaction solution and the reaction was started at 45°C. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -methyl- β -thiolactone with a yield of 48% (99.6% ee, purity by HPLC).

Example 12

40_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 20 _{μ l} of phosphate buffer solution (1M, pH 7.0) was added thereto. To the reaction mixture was added 100 _{μ l} of Triton X-100 as a surfactant and then mixed. 20_{mg} of the lipase derived from *Pseudomonas cepacea* was added to the reaction solution and the

reaction was started at 37°C. The reaction was stopped after 27 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -methyl- β -thiolactone with a yield of 49% (99.4% ee, purity by HPLC).

Example 13

10_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml} of n-hexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 7.5) was added thereto. 10_{mg} of the lipase derived from *Penicillium roqueforti* was added to the reaction solution and the reaction was started at 10°C. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -methyl- β -thiolactone with a yield of 46% (99.1% ee, purity by HPLC).

Example 14

10_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 100 _{μ l} of phosphate buffer solution (1M, pH 7.0) was added thereto. 20_{mg} of the lipase derived from *Penicillium camembertii* was added to the reaction solution and the reaction was started at 45°C. The reaction was stopped after 20 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -methyl- β -thiolactone with a yield of 47% (99.2% ee, purity by HPLC).

Example 15

10_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml}

of cyclohexane and then 100 μ l of phosphate buffer solution (1M, pH 8.5) was added thereto. 20_{mg} of the lipase derived from *Pseudomonas cepacea* was added to the reaction solution and the reaction was started at 4°C. The reaction was stopped after 40 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -methyl- β -thiolactone with a yield of 46% (99.0% ee, purity by HPLC).

Example 16

10_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 100 μ l of phosphate buffer solution (1M, pH 7.0) was added thereto. 20_{mg} of the lipase derived from calf tongue root was added to the reaction solution and the reaction was started at 25°C. The reaction was stopped after 24 hours and the reaction solution was filtered. The filtrate was treated with saturated aqueous sodium carbonate solution to separate the aqueous layer containing the desired product. The aqueous layer was adjusted to pH 1.5 by using 10% aqueous hydrochloric acid solution and the desired product was extracted with dichloromethane. The extract was concentrated to obtain (S)- α -methyl- β -mercaptopropionic acid with a yield of 45% (99.0% ee, purity by HPLC).

Example 17

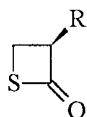
10_{mg} of α -methyl- β -thiolactone racemate was dissolved in 2_{ml} of cyclohexane and then 100 μ l of phosphate buffer solution (1M, pH 7.5) was added thereto. To the reaction mixture was added 100_{mg} of the lipase derived from *Pseudomonas cepacea* immobilized on ceramic (the amount of the lipase itself is 10_{mg}) and the reaction was started at

45°C. The reaction was stopped after 12 hours and the desired product was separated in the same procedure as Example 1 to obtain (R)- α -methyl- β -thiolactone with a yield of 47% (99.0% ee, purity by HPLC).

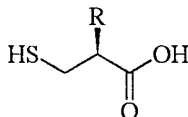
WHAT IS CLAIMED IS :

1. A process for preparing an (R)- α -alkyl- β -thiolactone represented by the following formula 3 or an (S)- α -alkyl- β -mercaptopropionic acid represented by the following formula 4 which comprises stereoselectively hydrolyzing a mixture of R and S enantiomers of α -alkyl- β -thiolactone using a hydrolytic enzyme in an organic solvent in the presence of a buffer solution.

Formula 3



Formula 4



in the above formulas 3 and 4,

R represents C₁-C₆ alkyl.

2. The process of claim 1, wherein the mixture of R and S enantiomers of α -alkyl- β -thiolactone is α -alkyl- β -thiolactone racemate.

3. The process of claim 1, wherein a hydrolytic enzyme selected from a group consisting of lipase, esterase and protease is used in an amount of 0.1 to 100 times by weight with respect to the mixture of R and S enantiomers of α -alkyl- β -thiolactone.

4. The process of claim 1, wherein one or more organic solvents selected from a group consisting of n-hexane, cyclohexane, n-heptane, cyclohexanone, n-octane, benzene, toluene, acetone, acetonitrile, chloroform, dichloromethane, 1,2-dichloroethane, dioxane, diethylether, dimethylformamide and tetrahydrofuran are used in an amount of 10 to 500 times by weight with respect to the mixture of R and S enantiomers of α -alkyl- β -thiolactone.

5. The process of claim 1, wherein one or more buffer solutions selected from a group consisting of phosphate buffer, bicarbonate buffer and Tris-HCl buffer having pHs ranging from 6.0 to 8.5 are used in an amount of 1 to 50 times by weight with respect to the mixture of R and S enantiomers of α -alkyl- β -thiolactone.

6. The process of claim 1, wherein the hydrolysis reaction is carried out in the presence of a surfactant.

7. The process of claim 6, wherein one or more surfactants selected from a group consisting of Triton X-100, Triton X-114, Twin 80, Twin 20, sodium dodecyl sulfate (SDS), lithium dodecyl sulfate (LDS), sodium cholate, sodium deoxy cholate, octyl glucoside, Brij 35, CHAPS and Xwittergent 3-14 are used in an amount of 1 to 10 times by weight with respect to the mixture of R and S enantiomers of α -alkyl- β -thiolactone.

8. The process of claim 1, wherein the hydrolytic enzyme is employed in the state immobilized on one or more supports selected from a group consisting of ceramic, celite, silica gel, alumina, borosilicate, calcium carbonate and zeolite.

9. The process of claim 8, wherein the immobilized hydrolytic enzyme is suspended in the reaction solution or is filled into a column.
10. The process of claim 1, wherein the reaction is carried out at temperatures ranging from -20 to 70°C for 8 to 72 hours.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 98/00040

A. CLASSIFICATION OF SUBJECT MATTER IPC ⁶ : C 12 P 41/00 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) IPC ⁶ : C 12 P 41/00 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) WPI		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 289 804 A2 (TOYO JOZO CO., LTD.) 09 November 1988 (09.11.88), claim 1.	1
A	US 5 106 736 A (PATEL et al.) 21 April 1992 (21.04.92), claim 1. -----	1
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
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Date of the actual completion of the international search 04 December 1998 (04.12.98)		Date of mailing of the international search report 16 December 1998 (16.12.98)
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