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(54) **Title:** PURIFICATION PROCESS FOR CHENODEOXYCHOLIC ACID

(57) **Abstract:** The present invention relates to a process for purifying chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholic acid). In particular, the present invention relates to a process for purifying chenodeoxycholic acid from low grade of chenodeoxycholic acid mixture in swine bile solid, with high yield and purity.

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PURIFICATION PROCESS FOR CHENODEOXYCHOLIC ACID

TECHNICAL FIELD

The present invention relates to a process for purifying chenodeoxycholic acid ($3\alpha,7\alpha$-dihydroxy-5β-cholic acid). In particular, the present invention relates to a process for purifying chenodeoxycholic acid from low grade chenodeoxycholic acid mixture contained in swine bile solid, with high yield and purity.

BACKGROUND ART

Chenodeoxycholic acid is generally contained in bile of cow, swine, bear, or poultry such as chicken or goose, as well as in bile of human. Chenodeoxycholic acid is used as starting material for the preparation of ursodeoxycholic acid which is effective to alleviate biliary system diseases, hyperlipidemia, cholelithiasis, and chronic liver diseases, and a typical process for preparing ursodeoxycholic acid known in the art is as follows.

A typical process for preparing chenodeoxycholic acid comprises the steps of: esterifying cholic acid ($3\alpha,7\alpha,12\alpha$-trihydroxy cholic acid) with methyl; protecting the hydroxyl group of 3α and 7α position by acetylating them with anhydrous acetic acid;

oxidizing the hydroxyl group of 12α position to carbonyl group by using chromic acid, and then removing the carbonyl group by Wolff-kichner reduction reaction; hydrolyzing and deprotecting the obtained product to yield chenodeoxycholic acid. The above process requires the reaction to be maintained at a high temperature of more than 200℃, and the supply of raw material may be interrupted by bovine spongiform encephalopathy, etc.

Bile of poultry contains chenodeoxycholic acid, lithocholic acid, and a small amount of cholic acid. Thus, the process for separating chenodeoxycholic acid from poultry is well known in the art, but is not economically reasonable due to the supply decrease of raw material and low yield [see, Windhaus et al, I Physiol. Chem., 140, 177~185 (1924)].

US Patent No. 4,186,143 disclosed a process for purely separating and purifying chenodeoxycholic acid from chenodeoxycholic acid mixture derived from natural swine bile. This process comprises the major steps of: pre-treatment to remove 3α-hydroxy-6-oxo-5β-cholic acid by saponification of bile; esterification of bile acid; acetylation of bile acid ester; removal of intermediate product by using non-polar organic solvent;

crystallization of acetylated ester of formula I; deprotection; and production of the compound of formula I by using crystallization in organic solvent. However, this patent does not describe HPLC content for acetylated ester of formula I, and the purity of the final product is very low since the specific rotatory power is $[\alpha]_D^{25}$ +13.8° (c=1, $CHCl_3$), and the melting point is 119~121℃ [STD: $[\alpha]_D^{25}$ +15.2°(c=1, $CHCl_3$), melting point 127~129℃]. Also, the crystallization for purifying the final product requires a very long time (i.e., 16-48 hours), and the entire process is complex as eight (8) steps. Thus, when purifying the compound of formula I by using the above process, the yield of the final product becomes low, and the reaction time is as long as 12 days. Therefore, the process is not economically reasonable.

In particular, when purifying the acetylated ester of formula I from the swine bile solid having 5~35 wt% of chenodeoxycholic acid content used in the present invention by using the above process, despite two times of recrystallization in ethanol solvent, the content of the final product is as low as 80%.

To overcome the above problems, the object of the present invention is to provide a new process for purifying the compound of formula I in high purity and yield, with

reducing the time required for the entire process.

DISCLOSURE OF THE INVENTION

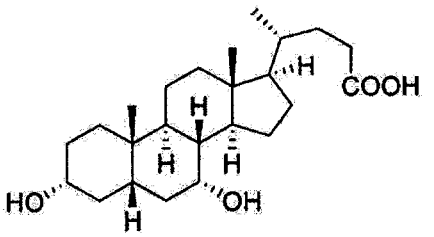
The present invention provides a process for purifying the compound of formula I, comprising the steps of pre-treatment of swine bile; esterification of bile acid; acetylation of bile acid ester; removal of intermediate product by using non-polar organic solvent; crystallization of acetylated bile acid ester; and deprotection; wherein the process is characterized in

1) dissolving swine bile solid having 5~35 wt% of chenodeoxycholic acid content in organic solvent containing salt, as pre-treatment step;

2) crystallizing the product obtained from the pre-treatment step in methanol or isopropanol, within the temperature range of 0 ~ 15℃, as crystallization step; and

3) deprotecting the product obtained from the crystallization step by adding base, and crystallizing the deprotected product in the presence of water by adding acid, as deprotection step.

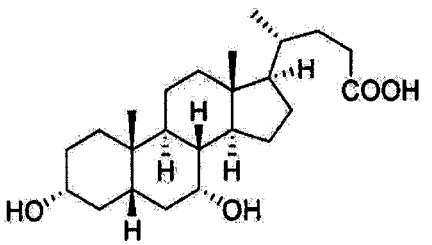
[Formula I]



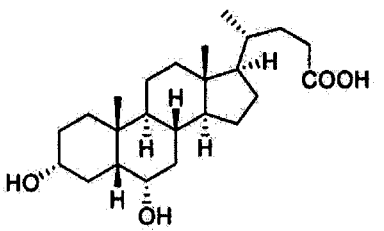
DETAILED DESCRIPTION OF THE INVENTION

In the present specification, the phrase "swine bile solid" represents solid derived from swine bile, and contains the mixture of chenodeoxycholic acids of Formulae I~IV.

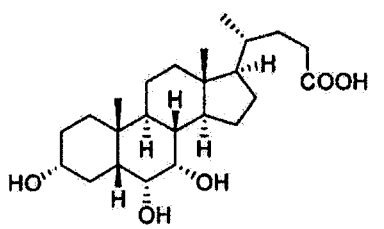
[Formula I]



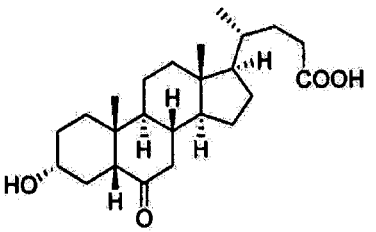
[Formula II]



[Formula III]



[Formula IV]



wherein, the compound of formula I represents chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholic acid, CDCA); the compound of formula II represents hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholic acid, HDCA); the compound of formula I represents hyocholic acid (3α,6α,7α-trihydroxy-5β-cholic acid, HCA); and the compound of formula IV represents 3α-hydroxy-6-oxo-5β-cholic acid(keto).

Hereinafter, each step for purifying chenodeoxycholic acid according to the present invention will be exemplified in detail.

Step 1: Pre-treatment of swine bile solid

To use swine bile solid having 5~35 wt% of chenodeoxycholic acid content in the purification step, all of the swine bile solid is stirred and dissolved in organic solvent with reflux. Then, the mixture is cooled to room temperature, and more stirred for 1~2 hours. Then, insoluble materials are removed from the mixture with using filter paper, preferably filter paper and diatomaceous earth. The organic solvent is removed under reduced pressure to obtain residues (CDCA, HDCA, HCA and keto), which are used in the next

step. Salt used in the present step can be optionally selected as long as it does not affect the compounds in the reactant. Preferably, the salt is at least one selected from the group consisting of sodium chloride, anhydrous magnesium sulfate ($MgSO_4$), and anhydrous sodium sulfate, more preferably sodium chloride. The amount of salt used in the present step is preferably 5~10 wt%, based on the amount of organic solvent. If the amount of salt is less than 5 wt%, water and insoluble materials (such as fatty acids, etc.) in the swine bile solid are not sufficiently removed, which makes the filtration difficult and reduces the yield and velocity of esterification reaction. If the amount of salt is more than 10 wt%, superfluous salt remains as impurity, which makes the purification difficult. Preferably, the organic solvent can be optionally selected from ones which can dissolve chenodeoxycholic acid of the swine bile solid and have no adverse effects thereto. More preferably, the solvent is ethyl acetate or acetone.

Step 2: Esterification of chenodeoxycholic acid

Alcohol is added to the chenodeoxycholic acid mixture residue obtained from the prior step, and then the solution is stirred with reflux before the residue is completely dissolved. Then, the solution is cooled to 0~5℃. Acid catalyst is added to the solution, which is stirred with reflux at room temperature until the esterificaton reaction of

chenodeoxycholic acid mixture is completed. When the reaction is completed, the solution is neutralized by adding base, and then filtered. The filtered material is washed with alcohol and concentrated under reduced pressure to obtain chenodeoxycholic acid ester mixture (CDCA-Me, HDCA-Me, HCA-Me and keto-Me) as residue. Alcohol used in the present step is not specifically limited, but preferably lower alcohol having 1~4 of carbon atoms, more preferably methanol, for easy esterification reaction. The acid catalyst used in the present step is preferably sulfuric acid or para-toluenesulfonic acid (PTSA), and the base is sodium bicarbonate, sodium carbonate or potassium carbonate.

Step 3: Acetylation of chenodeoxycholic acid ester

All the hydroxy groups in chenodeoxycholic acid ester mixture are acetylated by adding anhydrous acetic and weak base to the residue obtained from the prior step with reflux. When the reaction is completed, toluene is added to the reaction solution with stirring at reflux. Then, the anhydrous acetic acid, acetic acid, and base remaining after the reaction are removed by concentrating the reaction solution under reduced pressure, to obtain a mixture of acetylated chenodeoxycholic acid ester (CDCA-diAc-Me, HDCA-diAc-Me, HCA-triAc-Me and keto-Ac-Me) as residue. The weak base used in the present step is preferably anhydrous sodium acetate or pyridine, more preferably

anhydrous sodium acetate.

Step 4: Removal of intermediate products of formulae III and IV

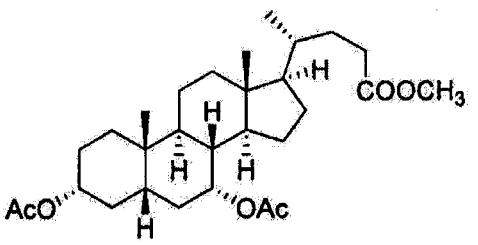
Non-polar solvent is added to the residue. The mixture is stirred with reflux until all the residue is dissolved and cooled to room temperature. With maintaining the temperature of solvent within 20~25℃, intermediate products of formulae III and IV; and a part of intermediate product of formula II (HCA-triAc-Me, keto-Ac-Me and part of HDCA-diAc-Me) are crystallized and removed by filtration. Thus filtered material is additionally washed with non-polar solvent, and then the filtered and washed solution is concentrated under reduced pressure, and dried in vacuum. The non-polar solvent used in the present step is preferably hexane, heptane, octane, isooctane and the like, more preferably hexane or heptane.

Step 5: Production of chenodeoxycholic acid-diacetate-methyl-ester

To produce chenodeoxycholic acid-diacetate-methyl-ester(CDCA-diAc-Me) of formula V which is an intermediate product for preparing the compound of formula I, alcohol solvent is added to the product obtained from the prior step, and then the compound of formula V is crystallized by standing the mixture for 2~3 hours at 0℃~15℃,

preferably 0℃~5℃.

[Formula V]



If the temperature is less than 0℃, the content for the compound of formula V decreases, and if the temperature is more than 15℃, the crystallization is not done sufficiently. When the compound of formula V is crystallized, the intermediate product of formula II is removed from the solvent by using filtration. Thus filtered material is washed with alcohol solvent, and dried in vacuum to obtain chenodeoxycholic acid-diacetate-methyl-ester of formula V as crude product. To purify the compound of formula V in high purity, recrystallization is performed until the content for the compound of formula V becomes 98.5% or more, preferably 99% or more, under the same conditions. To obtain the content of 99% or more, it is preferable to perform the recrystallizaton three (3) times or more. The alcohol used in the crystallization is preferably lower alcohol, more preferably methanol or isopropanol, most preferably methanol, considering the content for the compound of formula V. The amount of alcohol used in the crystallization is 0.5~3 times, preferably 1.5~3 times, to the amount of residue. If the

amount is less than 0.5 times, the filtration is difficult since crystals coagulate each other. If the amount becomes more than 3 times, the content for the compound of formula V is not affected by the amount.

Step 6: Deprotection and crystallization of chenodeoxycholic acid

The compound of formula V obtained from the prior step is deprotected in the presence of base, and the pH of the reaction solution is adjusted to 4 or lower, preferably 2~3 in acid condition, to form chenodeoxycholic acid of formula I. Simultaneously, the reaction solution stands at 35~45℃, preferably 35~40℃, to crystallize the compound of formula I in the presence of water. The reaction solution is filtered, washed with water, and dried in vacuum to refine chenodeoxycholic acid purely. The base used for the deprotection is not specifically limited, but sodium hydroxide or potassium hydroxide is preferred for the post-treatment step. If the pH is more than 4, crystals are not formed, and if the pH is less than 2, the purity of the final product is reduced due to superfluous acid. If the crystallization temperature in water is less than 35℃, the purity of the compound of formula I decreases, and if the temperature is more than 45℃, the filtration is difficult since crystals derived from the compound of formula I coagulate each other. The acid used in neutralizing the reaction solution is also not specifically limited, but

hydrochloric acid or sulfuric acid is preferred for the post-treatment step. Since the residue obtained from the prior step contains 98.5% or more of the compound of formula V, preferably 99% or more, the compound of formula I can be purely refined without additional crystallization using organic solvent since the content of impurities is low. The product containing the compound of formula I crystallized in water according to the present invention is suitable for industrial manufacturing process since its melting point is about 20℃ higher, and has lower volume, than crystallized compound of formula I in organic solvent.

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

Analytical method

HPLC was used to confirm the intermediate products separated form each step, and the test conditions are as follows:

Column: Capcell pak UG120 C18 (4.6 X 250mm, Shiseido)

Mobile phase: acetonitrile/water (85:15)

Detector: ultraviolet spectrometer (210nm)

Flow rate: 1.0ml/min

Insertion: 20㎕

Example

Step 1: Pre-treatment of swine bile solid

150g of swine bile solid having 30~35 wt% of chenodeoxycholic acid content, and 60g of sodium chloride in 600 ml of ethyl acetate were stirred with reflux for 1 hour, to dissolve all the swine bile solid. Then, the mixture was cooled to 20~25℃, stirred for 1 hour, and filtered through diatomaceous earth, and thus filtered material was washed with 60 ml of ethyl acetate. Organic solvent was removed by concentrating the filtered material under reduced pressure to obtain chenodeoxycholic acid mixture (CDCA, HDCA, HCA and keto) as residue.

Step 2: Esterification of chenodeoxycholic acid

To the residue obtained from the prior step was added 375ml of methanol, and the mixed solution was stirred with reflux for 30 minutes until the residue was completely

dissolved. This solution was cooled to 0~10℃, 4.88ml of sulfuric acid was added to the solution with stirring, and then the esterification reaction of chenodeoxycholic acid mixture was completed by stirring at 20~25℃ for 2 hours. When the esterification reaction is completed, the solution was neutralized with 53.9g of sodium bicarbonate, and then filtered. Thus filtered material was washed with 150ml of methanol, and concentrated under reduced pressure to obtain 134g of chenodeoxycholic acid ester mixture (CDCA-Me, HDCA-Me, HCA-Me and keto-Me) as residue.

Step 3: Acetylation of chenodeoxycholic acid ester

To 134g of chenodeoxycholic acid ester mixture obtained from the prior step were added 20g of anhydrous sodium acetate and 200ml of anhydrous acetic acid. The mixed solution was refluxed at 120~140℃ for 5 hours, and then immediately concentrated under reduced pressure. Anhydrous acetic acid and acetic acid were completely removed by adding 25ml of toluene to the reaction solution, stirring with reflux for 15 minutes, and concentrating under reduced pressure, to obtain acetylated chenodeoxycholic acid ester mixture (CDCA-diAc-Me, HDCA-diAc-Me, HCA-triAc-Me and keto-Ac-Me) as residue. HPLC result for the residue (RT): HCA-triAc-Me (8.76min), keto-Ac-Me (9.05min), CDCA-diAc-Me (12.21min), and HDCA-diAc-Me (12.81min)

Step 4: Removal of intermediate products of formulae III and IV

To the residue obtained from the prior step was added non-polar solvent (400ml of hexane), and then the mixed solution was stirred with reflux for 30 minutes. Then, hexane solvent was cooled to 25~35℃, stirred for 3 hours, and then filtered. Thus filtered material (HCA-triAc-Me, keto-Ac-Me and part of HDCA-diAc-Me) was additionally washed with 65ml of hexane, and the filtered and washed solution was concentrated under reduced pressure to obtain the intermediate products of formulae I and II (CDCA-diAc-Me, HDCA-diAc-Me) as residue. HPLC result for the residue (RT): CDCA-diAc-Me (12.21min) and HDCA-diAc-Me (12.81min).

Step 5: Production of chenodeoxycholic acid-diacetate-methyl-ester

To the residue obtained from the prior step was added 270ml of methanol, and the mixed solution is stirred with reflux for 30 minutes, cooled to 0~10℃, more stirred for 2 hours, and then filtered. The filtered material (CDCA-diAc-Me) was washed with methanol 70ml, and dried in vacuum at 60℃ to obtain 85% content of crude product. Then, to the crude product was added 72ml of methanol, and recrystallization was performed to the mixture at 0~5℃ for 2 hours. Recrystallization is additionally

performed to the mixture one more time to obtain 99% content of chenodeoxycholic acid-diacetate-ester. The yield is 24.5g (19.5g+mother liquor 5g). m.p.: 128~129℃. HPLC result for the residue (RT): CDCA-diAc-Me (12.21 min).

Step 6: Deprotection and crystallization of chenodeoxycholic acid

To 220ml of water were added 24.5g of chenodeoxycholic acid-diacetate-ester and 29.5g of sodium hydroxide, and then the solution was stirred with reflux for 4 hours. To the solution was added 370ml of water. The solution's pH is adjusted to 2.0~3.0 by using 59ml of hydrochloric acid. Then, the solution was stirred at 35~45℃ for 1 hour, and then filtered. The filtered material was washed with 24.5ml of water and dried in vacuum at 70℃ to obtain 19.5g of pure chenodeoxycholic acid. m.p.: 160~161℃, $[\alpha]_D^{25}$ +13.0°(c=1, $CHCl_3$).

Comparative Example

Step 1: Pre-treatment of bile

150g of concentrated swine bile was dissolved in 1000ml of hot water. Then, 100g of sodium hydroxide was added, and the solution was stirred with reflux for 20 hours. This solution was cooled to 25℃. 1500ml of water was added to the solution, which was

kept cool for one day. 10g of diatomaceous earth was added to the reaction solution, which was then stirred and filtered to remove precipitated sodium 3α-hydroxy-6-ketocholate of formula IV. The filtrate was adjusted to pH 8 by using conc. sulfuric acid, and then stirred for 15 minutes after adding 5g of sodium hydrosulfite. Then, 400ml of ethyl acetate was added to the solution, which was then adjusted to pH 5 by using diluted sulfuric acid. The solution was stirred for 30 minutes, and aqueous layer was removed therefrom by layer separation. To the organic layer were added 7g of diatomaceous earth and 7g of active carbon, which was then stirred for 30 minutes and filtered. Thus filtered material was washed with 50ml of ethyl acetate, and concentrated under reduced pressure.

Step 2: Esterfication of bile acid

The residue obtained from the prior step was dissolved in 300ml of methanol. Then, the solution was neutralized with sodium bicarbonate (pH 7), filtered, and then concentrated under reduced pressure.

Step 3: Removal of methyl ester of formula II

The residue obtained from the prior step was dissolved in 320ml of hot benzene, and the mixed solution was concentrated to 225ml, and kept cold for one day. Then, the

solution was filtered; thus filtered material (methyl ester benzene adduct of formula II) was washed with benzene, and the benzene-filtered and washed solution was concentrated under reduced pressure.

Step 4: Acetylation of bile acid ester

To the residue obtained from the prior step were added 75ml of anhydrous acetic acid and 7.5g of anhydrous sodium acetate, and then the mixed solution was stirred with reflux for 5 hours. The remaining anhydrous acetic acid was removed by distilling anhydrous acetic acid, stirring with reflux for 15 minutes after adding 35ml of methanol, and then distilling under reduced pressure.

Step 5: Removal of acetylated ester of formula III

The residue obtained form the prior step was refluxed in 200ml of hexane solvent, and the solution was stored at 20℃ for one day, and filtered. Thus filtered material (crude crystal of HDCA-triAc-Me) was washed with hexane, and the filtered and washed solution was distilled under reduced pressure.

Step 6: Separation of the compound of formula V

The residue obtained form the prior step was dissolved in 46ml of hot ethanol, and then kept cool for one day. This solution was filtered, and thus filtered material was washed with 27ml of cold ethanol, and dried in vacuum at 60℃. 21.5g of the compound of formula V was recrystallized 3 times by using ethanol to obtain 18.5g of product. m.p. 119~121°C; $[\alpha]_D^{25}$ +10.4°(c=1, Dioxane); $[\alpha]_D^{25}$ +13.8°(c=1, $CHCl_3$).

Step 7: Saponification and neutralization

To 185ml of water were added 18.5g of chenodeoxycholic acid-diacetate-methyl-ester and 18.5g of sodium hydroxide, and then the mixed solution was stirred with reflux for 14 hours. Then, the solution's pH was adjusted to 4.5 by using conc. sulfuric acid.

Step 8: Production of the compound of formula I

The reaction solution was extracted by using ethyl acetate, and aqueous layer was discarded therefrom. Ethyl acetate layer in the solution was washed with 6% saline, and the solution was distilled to about 90ml. This solution was cooled, kept cool for one day after adding 90ml of hexane, and filtered. Thus filtered material was washed with 20ml of hexane, and dried in vacuum at 60℃ to produce 12.7g of chenodeoxycholic acid. m.p. 142~145°C; $[\alpha]_D^{25}$ +13.0°(c=1, $CHCl_3$).

INDUSTRIAL APPLICABILITY

The present invention can purify chenodeoxycholic acid of formula I from swine bile solid in high yield and purity. Also, the present invention is suitable for industrial purification by reducing the purification time.

CLAIMS

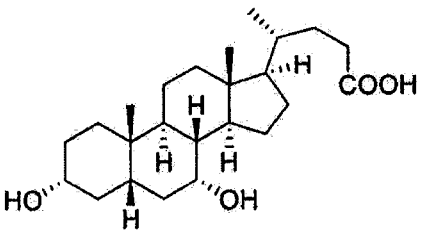
1. A process for purifying the compound of formula I, comprising the steps of pre-treatment of swine bile; esterification of bile acid; acetylation of bile acid ester; removal of intermediate product by using non-polar organic solvent; crystallization of acetylated bile acid ester; and deprotection; wherein the process is characterized in,

1) dissolving swine bile solid having 5~35 wt% of chenodeoxycholic acid content in organic solvent containing salt, as pre-treatment step;

2) crystallizing the product obtained from the pre-treatment step in methanol or isopropanol, within the temperature range of 0 ~ 15 ℃, as crystallization step; and

3) deprotecting the product obtained from the crystallization step by adding base, and crystallizing the deprotected product in the presence of water by adding acid, as deprotection step.

【Formula I】



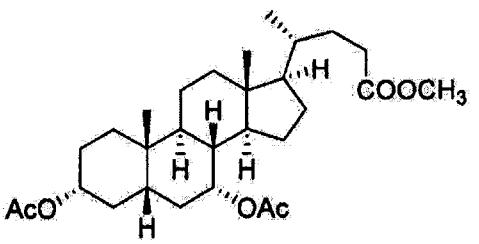
2. The process according to claim 1, wherein the salt used in the pre-treatment step is at least one selected from the group consisting of sodium chloride, anhydrous

magnesium sulfate, and anhydrous sodium sulfate.

3. The process according to claim 2, wherein the amount of salt is 5~10 wt%, based on the total weight of organic solvent.

4. The process according to claim 1, wherein the product obtained from the crystallization step contains 98.5% or more of the compound of formula V.

【Formula V】



5. The process according to claim 1, wherein the crystallization step is carried out in the temperature range of 0~5℃.

6. The process according to claim 1, wherein the amount of methanol or isopropanol used in the crystallization step is 0.5~3 times the amount of residue obtained from the step of removal of intermediate product.

7. The process according to claim 1, wherein the pH of the deprotection step is 4 or less.

8. The process according to claim 1, wherein the crystallization in the presence of water is carried out in the temperature range of 35~45℃.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/002972

A. CLASSIFICATION OF SUBJECT MATTER

C07J 9/00(2006.01)i

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)

C07J 9/00

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

Korean Patents and applications for inventions since 1975.

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

STN(CASLINK)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 1528779 A (HUADONG TECHNOLOGY UNIV.) 15 SEP. 2004, see entire document.	1 - 8
A	JP 03227998 A2 (SHOWA DENKO K. K.) 08 OCT. 1991, see entire document.	1 - 8
A	ZHANG et. al. `Isolation and purification of chenodeoxycholic acid from pig bile` In: Shengwu Huaxue Yu Shengwu Wuli Jinzhan, 1987, (4), p.68-71	1 - 8
A	JP 60181096 A2 (TOKYO TANABE CO., LTD.) 14 SEP. 1985, see entire document.	1 - 8
A	FR 2429224 A1 (CANADA PACKERS LTD., CAN.) 18 JAN. 1980, see entire document.	1 - 8

☐ 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
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"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	Date of mailing of the international search report
25 OCTOBER 2006 (25.10.2006)	**25 OCTOBER 2006 (25.10.2006)**

Name and mailing address of the ISA/KR	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2006/002972

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 1528779 A	15-09-2004	None	
JP 03227998 A2	08-10-1991	None	
JP 60181096 A2	14-09-1985	None	
FR 2429224 A1	18-01-1980	None	