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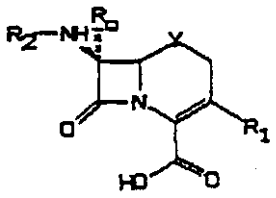


(I)

(57) Abstract

A method for the recovery of an N-substituted cephalosporanic acid compound of general formula (I), wherein R_2 is selected from the group consisting of adipyl (1,4-dicarboxybutane), succinyl, glutaryl, adipyl, pimelyl, suberyl, 2-(carboxyethylthio)acetyl, 3-(carboxyethylthio)propionyl, higher alkyl saturated and higher alkyl unsaturated dicarboxylic acids, from a complex mixture comprising in addition to the compound of general formula (I) 6-aminopenicillanic acid (6-APA) and optionally one or more N-substituted penicillanic acid compounds, comprising the steps of: (a) acidifying the complex mixture to a pH below 6.5 and maintaining the mixture below said pH at a temperature of between 10 °C and 150 °C; and/or (b) contacting the complex mixture with a carbon dioxide source; and (c) recovering the cephalosporanic acid compound of formula (I) from the mixture obtained after steps (a) and/or (b).

NOVEL PROCESS FOR THE FERMENTATIVE PRODUCTION OF CEPHALOSPORIN

Field of the invention

The present invention relates to a process for the preparation of cephalosporins and cephalosporin derivatives. More in particular, the present invention relates to the recovery of cephalosporins and derivatives thereof from complex mixtures of cephalosporins and other beta-lactam compounds. The invention is also concerned with recovery of deacylated cephalosporins from mixtures of beta-lactam compounds and side chains, such as those obtainable by enzymatic side-chain removal.

Background of the invention

Semi-synthetic routes to prepare cephalosporins mostly start from fermentation products such as penicillin G, penicillin V and Cephalosporin C, which are converted to the corresponding β-lactam nuclei, for instance in a manner as is disclosed in K. Matsumoto, Bioprocess. Techn., 16, (1993), 67-88, J.G. Shewale & H. Sivaraman, Process Biochemistry, August 1989, 146-154, T.A. Savidge, Biotechnology of Industrial Antibiotics (Ed. E.J. Vandamme) Marcel Dekker, New York, 1984, or J.G. Shewale et al., Process Biochemistry International, June 1990, 97-103. The obtained β-lactam nuclei are subsequently converted to the desired antibiotic by coupling to a suitable side chain, as has been described in *inter alia* EP 0 339 751, JP-A-53005185 and CH-A-640 240. By making different combinations of side chains and β-lactam nuclei, a variety of penicillin and cephalosporin antibiotics may be obtained.

7-Amino desacetoxy cephalosporanic acid (7-ADCA) and 7-aminocephalosporonic acid (7-ACA) are known to be the most important intermediates for the production of antibiotics used in the pharmaceutical industry.

7-ADCA is for example obtained by chemical or

enzymatic cleavage (deacylation) of phenylacetyldesacetoxy cephalosporanic acid yielding 7-amino desacetoxy cephalosporanic acid and phenyl acetic acid.

Phenylacetyldesacetoxy cephalosporanic acid is normally produced by chemical treatment of penicillin G sulfoxide, which is formed from penicillin G. In this production process a large amount of chemicals are required to ensure that the desired reaction take place. This is both expensive and places a heavy burden on waste management. Moreover, the total yield of the process, is not very high.

To overcome some of the drawbacks of the chemical process a fermentative process has been disclosed for the production of 7-ADCA, 7-amino desacetyl cephalosporanic acid (7-ADAC) and 7-ACA, involving fermentative production of *N*-substituted β-lactams, such as adipyl-7-ADCA, adipyl-7-ADAC or adipyl-7-ACA by a recombinant *Penicillium chrysogenum* strain capable of expressing a desacetoxycephalosporanic acid synthetase (DAOCS) also known as "expandase" from a transgene (EP 0 532 341, EP 0 540 210, WO 93/08287, WO 95/04148). The expandase takes care of the expansion of the 5-membered ring of certain N-acylated penicillanic acids, thereby yielding the corresponding N-acylated desacetoxycephalosporanic acids.

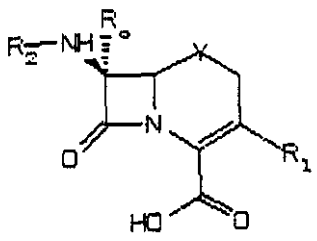
In order to yield the economically most important non-acylated cephalosporins, such as 7-ADCA, 7-ADAC and 7-ACA, the acyl groups are enzymatically removed with a suitable acylase.

Known processes for recovering chemically or enzymatically produced penicillanic and cephalosporanic acids are not effective for the recovery of the *N*-substituted β-lactam intermediates and deacylated amino-β-lactams. The main problem with the recovery of the fermentatively produced cephalosporin compounds mentioned above is the complexity of the broth, or culture filtrate. The broth usually comprises various penicillanic acids, such as alpha-aminoadipyl-6-penicillanic acid, alpha-hydroxyadipyl-6-penicillanic acid, 6-aminopenicillanic acid

(6-APA), various cephalosporanic acids including alpha-aminoadipyl- and hydroxyadipyl-7-ADCA and a lot of proteinaceous material. Known recovery procedures do not give an acceptable quality of the cephalosporanic acid product in terms of purity. In deacylation this leads to problems in terms of reduced enzyme half-life, slower bioconversion rate and more expenses in the recovery after bioconversion and/or unacceptable contaminant levels. Moreover, after deacylation, such impurities prevent or at least hamper the recovery of the desired deacylated cephalosporin compound of the desired specifications.

Summary of the invention

The invention provides for a method for the recovery of a cephalosporanic acid compound of the general formula (I):



(I)

wherein

- R_0 is hydrogen or C_{1-3} alkoxy;
- Y is CH_2, oxygen, sulphur, or an oxidised form of sulphur;
- R_1 is any of the groups selected from the group consisting of
  - hydrogen,
  - hydroxy,
  - halogen,
  - saturated or unsaturated, straight or branched alkyl (1 - 5 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with

hydroxy, halogen, aryl, alkoxy (1 - 3 carbon atoms), or acyl;

- alkoxy (1-3 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy or halogen; or
- cycloalkyl (3 - 8 carbon atoms) optionally substituted with hydroxy, halogen, amino;
- aryl;
- heteroaryl; and

• R_2 is selected from the group consisting of adipyl (1,4-dicarboxybutane), succinyl, glutaryl, adipyl, pimelyl, suberyl, 2-(carboxyethylthio)acetyl, 3-(carboxy-ethylthio)propionyl, higher alkyl saturated and higher alkyl unsaturated dicarboxylic acids,

from a complex mixture comprising in addition to the compound of the general formula 6-aminopenicillanic acid (6-APA) and optionally one or more N-substituted ß-lactam compounds,

comprising the steps of:

(a) acidifying the complex mixture to a pH below 6.5 and maintaining the mixture below said pH at a temperature of between 10°C and 150°C; and/or

(b) contacting the complex mixture with a carbon dioxide source; and

(c) recovering the cephalosporanic acid compound of the formula (I) from the mixture obtained after steps (a) and/or (b).

Preferably in step (a) the temperature is kept between about 50 °C and about 130 °C, preferably between 70 and 120 °C, for between 10 seconds and about 1 week and the pH is kept at or below pH 4.5. According to a preferred method the compound of formula (I) has been produced by fermentation of a micro-organism capable thereof, the complex mixture being a broth, a culture filtrate or any culture liquid derivable from the broth after fermentation.

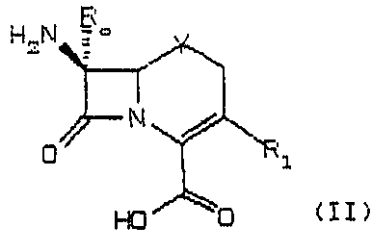
Preferred compounds of the general formula (I) are

selected from the group consisting of adipyl-7-ADCA, adipyl-7-ADAC and adipyl-7-ACA.

According to another aspect of the invention step (c) is performed by subjecting the mixture obtained after steps (a) and/or (b) to chromatography, preferably adsorption chromatography, more preferably Hydrophobic Interaction Chromatography.

According to another aspect of the invention the use of chromatography in a process of recovering a cephalosporin compound according to formula (I) is provided, preferably by adsorption chromatography, more preferably Hydrophobic Interaction Chromatography, still more preferably using Simulated Moving Bed technology.

According to yet another aspect of the invention a method is provided for making a compound of formula (II):



(II)

wherein

- R_0 is hydrogen or C_{1-3} alkoxy;
- Y is CH_2, oxygen, sulphur, or an oxidised form of sulphur;
- R_1 is any of the groups selected from the group consisting of
  - hydrogen,
  - hydroxy,
  - halogen,
  - saturated or unsaturated, straight or branched alkyl (1 - 5 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy, halogen, aryl, alkoxy (1 - 3 carbon atoms), or acyl;

- alkoxy (1-3 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy or halogen; or
- cycloalkyl (3 - 8 carbon atoms) optionally substituted with hydroxy, halogen, amino;
- aryl;
- heteroaryl;

comprising the steps of making a compound according to formula (I) wherein R_0, Y and R_1 are as above and R_2 is selected from the group consisting of adipyl (1,4-dicarboxybutane), succinyl, glutaryl, adipyl, pimelyl, suberyl, 2-(carboxyethylthio)acetyl, 3-(carboxyethylthio)-propionyl, higher alkyl saturated and higher alkyl unsaturated dicarboxylic acids;
deacylating the compound of formula (I) to obtain a conversion solution which comprises a compound according to formula (II).
The conversion solution preferably further comprises the cleaved side chain designated R_2.

According to a preferred embodiment, the process comprises the further step of recovering the compound of formula (II) from the solution by crystallisation, preferably preceded and/or followed (after solubilisation of the crude crystals i.e. by crystallisation) by treatment of the solution with an agent selected such as activated carbon or an adsorber resin. According to another aspect of the invention during or before crystallisation and/or recrystallisation a solvent such as methanol, ethanol, (iso)propanol, isobutanol, n-butanol, or acetone or a combination of any of the mentioned agents is added. Preferred adsorber resins are selected from XAD16 (CAS No. 102419-63-8), XAD1600 (CAS No. 153796-66-8) and HP20 (CAS No. 55353-13-4). Preferred according to the invention is a method wherein the 6-aminopenicillanic acid (6-APA) level is 10 ppm or less with respect to the compound of formula (II). According to another aspect, a process is provided wherein following the deacylation the solution is treated to remove,

at least partially, the cleaved side chain represented by R_2. This step may be performed, or repeated, after crystallisation and solubilisation (i.e. recrystallisation) of the compound of formula (II). Also removal of the cleaved side-chain may be carried out on the mother liquor obtained after crystallisation or recrystallisation.

Thus, a process is provided wherein said treatment to remove, at least partially, the cleaved side chain is followed by solubilisation of the crude crystals and recrystallisation of the compound of formula (II).

Preferably said treatment to remove the cleaved side chain comprises subjecting the conversion solution, or the mother liquor, or both, to membrane filtration at a pH below 5, preferably below 4, more preferably near or below 3. Accordingly, the use is provided of membrane filtration to remove a dicarboxylic acid from a mixture comprising the dicarboxylic acid and a ß-lactam antibiotic. The mixture is preferably a mother liquid obtained after crystallisation of a compound of the formula (II) or the mixture obtained after deacylation of the compound of formula (I). Membrane filtration takes preferably place at a pH of about 5 or less, preferably at pH 4 or less, yet more preferably by nanofiltration at or below pH 3.

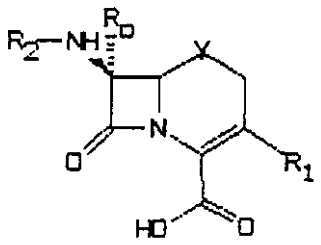
According to another aspect of the invention, a process is provided wherein the side chain R_2 is, at least partially, removed from the conversion mixture by crystallisation and/or recrystallisation.

According to still another aspect of the invention, a process is provided wherein the side chain R_2 is, at least partially, removed from the conversion mixture by acidifying the mixture to a pH lower than 3 and next contacting this mixture with an organic solvent, for instance amyl acetate, butyl actetate, ethyl acetate, methyl isobutyl ketone, cyclohexanone, isobutanol or n-butanol.

Detailed description of the invention

The invention pertains to a method for the recovery of a cephalosporanic acid compound of the general formula

(I):

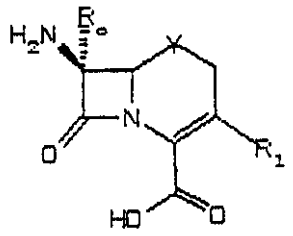


(I)

wherein

- R_0 is hydrogen or C_{1-3} alkoxy;
- Y is CH_2, oxygen, sulphur, or an oxidised form of sulphur;
- R_1 is any of the groups selected from the group consisting of
  - hydrogen,
  - hydroxy,
  - halogen,
  - saturated or unsaturated, straight or branched alkyl (1 - 5 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy, halogen, aryl, alkoxy (1 - 3 carbon atoms), or acyl;
  - alkoxy (1-3 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy or halogen; or
  - cycloalkyl (3 - 8 carbon atoms) optionally substituted with hydroxy, halogen, amino;
  - aryl;
  - heteroaryl; and
- R_2 is selected from the group consisting of adipyl (1,4-dicarboxybutane), succinyl, glutaryl, adipyl, pimelyl, suberyl, 2-(carboxyethylthio)acetyl, 3-(carboxy-ethylthio)propionyl, higher alkyl saturated and higher

alkyl unsaturated dicarboxylic acids,
from a complex mixture comprising in addition to the compound of the general formula 6-aminopenicillanic acid (6-APA) and optionally one or more N-substituted penicillanic acid compounds,
comprising the steps of:
(a) acidifying the complex mixture to a pH below 6.5 and maintaining the mixture below said pH at a temperature of between 10°C and 150°C; and/or
(b) contacting the complex mixture with a carbon dioxide source; and
(c) recovering the cephalosporanic acid compound of the formula from the mixture obtained after steps (a) and/or (b). The invention relates further to a process for the preparation of cephalosporins having the general formula (II):



(II)

wherein

- R_0 is hydrogen or C_{1-3} alkoxy;
- Y is CH_2, oxygen, sulphur, or an oxidised form of sulphur;
- R_1 is any of the groups selected from the group consisting of
  - hydrogen,
  - hydroxy,
  - halogen,
  - saturated or unsaturated, straight or branched alkyl (1 - 5 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with

hydroxy, halogen, aryl, alkoxy (1 - 3 carbon atoms), or acyl;

- alkoxy (1-3 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy or halogen; or
- cycloalkyl (3 - 8 carbon atoms) optionally substituted with hydroxy, halogen, amino;
- aryl;
- heteroaryl.

The compound according to formula (I) may be produced by any series of steps which yield a complex mixture as defined herein, from which the recovery of the compound according to formula (I) is accomplished. For the purposes of the specification and claims, a complex mixture is defined as a mixture comprising a N-substituted cephalosporin compound and substituted or unsubstituted β-lactam compounds.

The compound of formula (II) is obtained by the following series of steps:

(a) recovering, preferably purifying, the compound of formula (I);

(b) deacylating the preferably purified compound of formula (I) to obtain a solution comprising the compound of formula (II) (the conversion solution); and

(c) recovering, preferably purifying the compound of formula (II).

One of the obstacles of producing *N*-substituted cephalosporanic acid fermentatively is the presence of unwanted contaminating β-lactam components, for instance N-substituted 6-amino penicillanic acid. According to one embodiment of the invention, it has been found that these contaminations can be remarkably reduced by incubating the broth, the filtrate of the broth or a liquid derived from the broth using any biomass separation technique, under acidified conditions, preferably accompanied with an

elevated temperature. The broth is acidified down to a pH lower than 6.5, preferable lower than 4.5, using at least one known acid, for instance sulphuric acid, hydrochloric acid or nitric acid or a combination thereof. Operating temperature is in the range of 20 to 150 °C, preferably at 70 to 120 °C. Residence time at these conditions is in the range of a few seconds (at 150 °C), or several days (at 20 °C), preferably 10 seconds to 60 minutes. The pH/temperature treatment is preferably carried out for a period which provides for an N-substituted 6-APA reduction of a factor 100, preferably 1000, more preferably 1,000,000 with respect to the compound of formula (II). This step can be carried out either before or after biomass separation and can be performed batch wise or continuously.

According to another embodiment of the invention contaminating penicillin components, for instance N-substituted 6-APA, are remarkably reduced by contacting the broth, the filtrate of the broth, the eluate, the conversion solution or the dissolved contaminated cephalosporin according to formula (I), typically at pH 5 to 7, with carbon dioxide. Carbon dioxide can be added to the solution in any suitable way, such as solid or gaseous form or as solution of carbonate ions. The solution is contacted with the CO_2 source at a temperature of 10 to 60 °C, preferably 20 to 40 °C, where said solution is saturated with molecular CO_2 for 4 to 10 hours. After reduction of the penicillin components, purification of the cephalosporins, according to formula 1 can be obtained as mentioned earlier.

The complex mixture as defined herein may have any origin, but is preferably a culture broth or a culture filtrate obtained after fermenting under conditions giving rise to production, a micro-organism capable of producing an 7-N-acylated version of the compound of the general formula (I), wherein the acyl-group may be any acyl-group which supports the ring-expanding enzyme (desacetoxycephalosporin synthetase - DAOCS - or a bifunctional expandase/hydroxylase

occasionally referred to as desacetylcephalosporin synthetase DACS) in the cephalosporin biosynthetic pathway. Bioprocesses for producing 7-N acyl-substituted compounds according to formula (I) *in vivo* are disclosed in WO 93/05158 (adipyl-7-ADCA); WO 93/08287 (adipyl-7-ADAC and adipyl-7-ACA), WO 95/04148 (2-(carboxyethylthio)acetyl-7-ADCA), WO 95/04149 (3-(carboxyethylthio)propionyl-7-ADCA) and higher alkyl saturated or unsaturated dicarboxylic acids. The relevant parts of these PCT-applications are herein incorporated by reference. Preferred acyl groups are dicarboxylic acid groups in general, such as adipyl (1,4-dicarboxybutane), 2-(carboxyethylthio)acetyl, 3-(carboxyethylthio)propionyl, muconic acid and the like. Suitable host organisms include but are not limited to *Penicillium chrysogenum* and *Acremonium chrysogenum*. Suitable sources of expandases, including bifunctional expandase/hydroxylases include but are not limited to *Streptomyces clavuligerus* and *Acremonium chrysogenum*. Methods for transformation, selection of transformed cells and expression regulating elements for filamentous fungi, which may be used to genetically modify host cells, are well known in the art of recombinant DNA technology of ß-lactam producing (filamentous) fungi.

Preferably, the broth is subjected first to biomass separation such as filtration by any suitable means, such as membrane filtration, vacuum filtration, ultrafiltration or a combination thereof, prior to acidification and the optional temperature increase. Any other means of biomass separation is suitable as well.

After the pH-lowering step and the optional temperature step, the recovered compound according to the formula (I) is preferably subjected to further purification to remove, at least partially, unwanted β-lactam components, especially unwanted *N*-substituted cephalosporins and penicillins. The further purification may be carried out by extraction using an organic solvent. In the case of extraction, it is found to be advantageous to wash the

extract, back extract the *N*-substituted cephalosporin from the organic phase to an aqueous phase and stripping the aqueous phase. The extracting organic solvent may be selected from amyl acetate, butyl acetate, ethyl acetate, methyl isobutyl ketone, cyclohexanone, iso-butanol or n-butanol, and the like. A preferred purification step in the process is the usage of chromatography for the purification of *N*-substituted cephalosporin, rather than extraction using organic solvents. The advantage of chromatography is in the absence of solvents, which cause waste problems and problems of containment, as well as improved purity of the final product. Preferred is ion exchange chromatography or adsorption chromatography, more preferably Hydrophobic Interaction Chromatography. The filtrate is subjected to chromatography using an adsorbent. An adsorbent includes activated carbon, *e.g.* Norit CG-1 or Cecarbon GAC 40; or an adsorber resin, such as styrene-divinylbenzene copolymerisates, for example Dianion HP 20 (CAS No. 55353-13-4), Dianion HP 21 (CAS No. 92529-04-9), Dianion SP 207 (CAS No. 98225-81-1) or Dianion SP825, from Mitsubishi Kasei Corporation or Amberlite XAD 1180 (CAS No. 97396-56-0), Amberlite XAD 1600 (CAS No. 153796-66-8) or Amberlite XAD 16 (CAS No. 102419-63-8) from Rohm and Haas or Amberchrom CG 161 (CAS No. 131688-63-6) from TosoHaas; preferably XAD 16 or XAD 1600.

Before adsorbing the *N*-substituted cephalosporin the complex mixture is adjusted to a pH of 1.0 to 5.0, preferably 2.5 to 3.5, by the means of one or more known acids, for instance sulphuric acid, hydrochloric acid or nitric acid or a combination thereof. Operating temperature is the range of 0 to 50 °C, preferably at 5 to 25 °C. Operating pressure is in the range of 0 to 1.0 MPa overpressure.

Unwanted β-lactam components, especially unwanted *N*-substituted cephalosporins, such as alpha-aminoadipylcephalosporanic acids, also adsorb on the adsorbent but are displaced by the wanted *N*-substituted

cephalosporin.

After adsorbing, washing with water is applied to remove unwanted β-lactam components from the void volume between the adsorbent and to desorb weakly bound unwanted β-lactam components from the adsorbent. The water can be acidified down to a pH of 1.0 by the means of one or more known acids, for instance sulphuric acid, hydrochloric acid or nitric acid or a combination thereof. To increase the osmotic pressure, salts may be added to the water. Operating temperature is the range of 0 to 50 °C, preferably at 20 to 40 °C. Operating pressure is in the range of 0 to 1.0 MPa overpressure.

Elution may be carried out with a suitable buffer, such as acetate, phosphate, carbonate, bicarbonate or adipate but also diluted organic solvents (e.g. acetone, isopropanol) or diluted bases (*e.g.* ammonium, caustic) can be used. Operating temperature is the range of 0 to 80 °C, preferably at 10 to 40 °C. Operating pressure is in the range of 0 to 1.0 MPa overpressure.

Regeneration of the adsorbent can be done by any common applied method, such as with dilute bases, dilute acids, or with water miscible solvents (such as acetone, methanol, ethanol or iso-propanol), or a combination thereof. Optionally heating up to 100°C may be performed.

The regeneration liquids can be removed by washing with water. The water can be acidified down to a pH of 1.0 by the means of one or more known acids, for instance sulphuric acid, hydrochloric acid or nitric acid or a combination thereof.

The chromatography step can be performed in several types of equipment, such as in a single column but also the simulated moving bed technology can be applied. For this simulated moving bed technology several types of equipment are available, such as the ADSEP system from U.S. Filter, the ISEP/CSEP-system from Advanced Separation Technology, the 'merry-go-around'-system from *e.g.* Applexion or the

SORBEX-system from Universal Oil Products Company (UOP).

Alternatively, the buffer can be removed from the eluate by means of nanofiltration. The characteristics of the membrane in this membrane filtration show a high retention for the wanted *N*-substituted cephalosporin and a low retention for the buffer.

Optionally a concentration step is applied by any means of suitable concentration such as vacuum evaporation, reversed osmosis, nanofiltration, or narofiltration after chromatography or extraction.

The recovered N-acylated compound is subsequently subjected to deacylation using any suitable method known in the art. A preferred method is enzymatic deacylation using a suitable dicarboxylate acylase. Numerous suitable acylases, wild-type or mutated, are known in the art including but not limited to those from *Bacillus* (EP 0 525 861; EP 0 405 846), *Pseudomonas* (EP 0 482 844; EP 0 525 861; EP 0 475 652; EP 0663 445), *Achromobacter* (EP 0 525 861), *Alcaligenes faecalis* (EP 0 638 649), *Acinetobacter* (EP 0 469 919), *Arthrobacter* (EP 0 283 218), *Escherichia coli* (US 3,945,888), *Kluyvera citrophila*, *Proteus rettgeri* (US 3,915,798) and the like. The dicarboxylate acylase is preferably from *Pseudomonas* SE83 or SY-77. Optionally, the acylase may be a mutated form, as disclosed in WO 91/16435, WO 97/20053, WO 97/40175 to increase or alter the affinity towards the substrate. Another way of deacylating the N-acylated cephalosporin compound according to the invention is by way of contacting the substrate with a micro-organism capable of producing the acylase, as disclosed in US Patent No. 5,677,141.

The acylase may be immobilised (US 3,930,949), either on membranes (EP 0 243 404) or free flowing carriers such as glutaraldehyde based carriers or aza-lacton polymers (EP 0 730 035), using techniques as such well known in the art. Non-immobilised acylase is also contemplated, using membranes to separate the reaction mixture (retentate) from the product (permeate), such as disclosed in US Patent No. 5,521,068. The process may be batch-wise or (semi-

)continuous, this is all well known and not crucial with respect to the invention. The enzymatic deacylation reaction is usually carried out in a stirred tank reactor with or without, preferably inert, sieve plates, to easily separate the immobilised enzyme from the reaction product. The pH is usually regulated during the reaction to compensate for the pH change as a result of the (dicarboxylic) side-chain removal by any type of base such as ammonium, caustic, carbonate, bicarbonate. The pH can be regulated in the reactor and/or in a circulating loop over the reactor. Other parameters may also be regulated, such as temperature, deacylated product or side-chain concentration, and the like, taking account of the effect of such parameters on the reaction rate and/or the equilibrium.

Additional stabilising agents can be added before and/or during deacylation, such as sulphite ($S_2O_5^{2-}$, HSO_3^-, SO_3^{2-}), EDTA, dithiotreitol (DTT).

Usually, the deacylated cephalosporin compound of the general formula (I) is subsequently recovered using any suitable combination of steps. Optionally a concentration step can be applied by any means such as vacuum evaporation, reversed osmosis, nanofiltration, or narofiltration before crystallisation. Optionally a water miscible solvent can be added. Optionally, before crystallisation the solution can be purified by treating with activated carbon or an adsorber resin. Optionally, before crystallisation the side chain can be removed, characterised by acidifying the aqueous phase, extracting the side chain to an extracting organic solvent and separating the phases. The extracting organic solvent may be selected from amyl acetate, butyl acetate, ethyl acetate, methyl isobutyl ketone, cyclohexanone, iso-butanol, n-butanol, and the like.

The product can be crystallised from the resulting aqueous phase in several ways. The most preferred mode of operation is neutralising the aqueous solution and subsequently lowering the pH in 1 to 6 steps down to a pH 3 to 5 using one or more known acids such as H_2SO_4, HCl, HNO_3,

or a combination thereof. This is preferably carried out in continuous mode using an interconnected set of 1 to 6 continuously operated crystallisers in series. Also batch crystallisation, semi-continuous crystallisation or concordance crystallisation can be applied. It is possible to perform the crystallisation directly in the same way as above, without the first neutralisation. According to one embodiment of this invention it has been found that a water miscible solvent, such as methanol, ethanol, iso-propanol, n-butanol, acetone and the like, can be added to improve the quality of the cephalosporin according to formula (II). Optionally, before crystallisation the solution can be treated by activated carbon or by an adsorbent resin in order to improve the quality of the compound according to formula (II).

It has been found that the quality of the cephalosporin according to formula (II) can be further improved by recrystallisation, optionally after treatment with adsorber resins, active coal and/or ethanol and/or acetate. This is characterised by dissolving the cephalosporin according to formula (II) at a pH in the range of 0.5 to 10.0, preferably between 7.5 to 8.5 and crystallisation of the product. The product can be crystallised in several ways. The most preferred mode of operation is lowering of the pH in 1 to 6 steps down to a pH 3 to 5 using one or more known acids, such as H_2SO_4, HCl, HNO_3, or a combination thereof. This can be carried out in continuous mode using an interconnected set of 1 to 6 continuously operated crystallisers in series. Also batch crystallisation, semi-continuous crystallisation or concordance crystallisation can be applied. According to one embodiment of this invention it has been found that a water miscible solvent, such as methanol, ethanol, (iso)propanol, acetone, iso-butanol and n-butanol, can be added to improve the quality of the cephalosporin according to formula (II).

It has been found further, that the quality of the cephalosporin according to formula (II) can be improved by treating the conversion solution and/or the solution of the

dissolved cephalosporin according to formula (II) with an adsorbent. An adsorbent includes activated carbon, *e.g.* Norit Ultra SX; or an adsorber resin, such as styrene-divinylbenzene copolymerisates, for example Dianion HP 20 (CAS No. 55353-13-4), Dianion HP 21 (CAS No. 92529-04-9) or Dianion SP 207 (CAS No. 98225-81-1) from Mitsubishi Kasei Corporation or Amberlite XAD 1180 (CAS No. 97396-56-0), Amberlite XAD 1600 (CAS No. 153796-66-8) or Amberlite XAD 16 (CAS No. 102419-63-8) from Rohm and Haas or Amberchrom CG 161 (CAS No. 131688-63-6) from TosoHaas; preferably XAD 16, XAD 1600 or HP20.

The crystals are isolated by filtration or centrifugation and dried in a conventional continuous or batch dryer. The crystals can be milled by any type of mill, such as ball mill, jet mill and the like.

Optionally a water miscible solvent can be added during the crystallisation. After dissolving, the solution can be treated with activated carbon or an adsorber resin.

This procedure will gave a better overall yield and product quality than the currently known process, mentioned before.

According to another aspect of the invention, a method is provided for removing and recovering adipic acid from the conversion solution or mother liquid (the liquid obtained after crystallisation of the compound according to the formula (II)). It is found, that adipic acid can advantageously separated using membrane filtration at low pH, such as below pH 5, preferably below pH 4, more preferably below at or near pH 3. Preferred according to the invention is an embodiment wherein filtration is carried out by reversed osmosis.

In addition to saving raw materials, the advantage of doing so resides in the purity and/or yield upon crystallisation of the so-treated solution.

The invention is further illustrated by the following non-limiting examples.

Experimental

A fermentation broth comprising adipyl-7-ADCA as a complex mixture, comprising *inter alia* 6-APA, adipyl-6-APA and alpha-amino-adipyl-7-cephalosporanic acid as undesired contaminants, is obtained by fermenting a *Penicillium chrysogenum* strain transformed with an expandase (desacetoxycephalosporin C synthetase) from *Streptomyces clavuligerus*, as described in International patent application WO 93/05158, published on March 18, 1993.

The transformed *Penicillium strain* was cultured as described in Example 1 of WO 93/05158, incorporated by reference herein.

After 5 to 7 days of fermentation, the broth was taken for recovery experiments.

This complex mixture can also be simulated by making an aqueous mixture of 6-aminopenicillanic acid, adipyl-6-aminopenicillanic acid, alpha-aminoadipyl-6-amino-penicillanic acid, adipyl-7-aminodesacetoxycephalosporanic acid, and alpha-aminoadipyl-7-cephalosporanic acid.

EXAMPLE 1

pH/HEAT-TREATMENT

This example shows the advantages of a pH-treatment, preferably a combined pH- plus increased temperature treatment, on the removal of unwanted β-lactam components, from complex mixtures.

Broth from a fermentation of *Penicillium chrysogenum* (see experimental), containing a complex mixture of adipyl-7-ADCA and penicillanic acid and cephalosporanic acid contaminants is filtrated. The concentrate is washed with process water until the total volume of the combined filtrates was approx. 2 times the initial broth volume.

The following experiments have been carried out:

A. Part of the filtrate is acidified to pH=3.5; heated up to 70 °C and after 30 minutes cooled to 40 °C;

B. Part of the permeate is acidified to pH=2.7; heated up to 110 °C and after 4 minutes cooled to 25 °C; or

C. Part of the permeate is acidified to pH=3.0 and not further treated.

The pre-treated solutions are then subjected to the following treatments to obtain a compound according to formula (II); 7-ADCA.

adsorption chromatography

The three solutions (A to C) were subjected to filtration over a Seitz K100 filter, whereafter the solution was pumped at a pH of 3.0 over a column filled with 1.6 litre of XAD-1600 resin; next the resin was washed with 4.8 litre water, and eluted with 0.2 M bicarbonate-solution. The first eluate fraction (1.1 litre) is taken out and discarded. The second fraction (3.2 litre) is collected and analysed. The resin is purified by washing with caustic and acetone, and conditioned again with acidified water.

concentrating

The eluate was concentrated at 20 to 30 °C vacuum (5-10mm Hg) till a concentration of 40 grams adipyl-7-ADCA per litre was obtained.

enzymatic deacylation

Subsequently, the adipyl-7-ADCA is treated with acylase as follows. To 1 litre of eluate, 1 gram of sodium metabisulfite, 20 mM EDTA and 100 g immobilised acylase (comprising Pseudomonas SE83 dicarboxylate acylase) was added. At 30 °C, the solution was stirred for two hours. The pH was held at 8.5 with 4 N sodium hydroxide. The immobilised acylase and the liquid were separated with a glass sintered filter.

crystallisation of 7-ADCA

The 7-amino desacetoxy cephalosporanic acid (7-ADCA)

was precipitated by lowering the pH to 3.6, under stirring, at a temperature of 30 °C; in 45 minutes the pH of the solution was lowered to 3.6 with 6 N sulphuric acid. After cooling to 20 °C, the crystals were isolated on a glass sintered filter, washed with water and dried at 35 °C.

resolving 7-ADCA crystals

The 7-ADCA was dissolved with the aid of ammonia. To that end 15 grams of 7-ADCA was suspended in 255 ml water. The 7-ADCA was dissolved with the aid of 4 N ammonium hydroxide at a pH of 7.5-8.5. After filtration over a glass sintered filter, water was added to obtain 300 ml of solution.

treatment with adsorber resin

The solution was treated with adsorber resin. In 45 minutes the solution was pumped over 15 ml of XAD1600. Subsequently, 75 ml of water was pumped over the resin to obtain 375 ml of solution.

recrystallisation

The 7-ADCA was precipitated by lowering the pH to 3.6 under stirring, at a temperature of 30 °C; in 45 minutes the pH was lowered to 3.6 with 6 N sulphuric acid. After cooling to 20 °C, the crystals were isolated on a glass sintered filter, washed with water and dried at 35 °C.

The 7-ADCA so produced shows good results in terms of 6-APA reduction. (6-APA ratio is with respect to 7-ADCA).

Table 1a. Results of experiment 1A, 1B and 1C.

Experiment	6-amino penicillanic acid content (ppm)
1A	<10
1B	<10
1C	950

Clearly, the pH/temperature treatment reduces the level of 6-aminopenicillanic acid contamination of the adipyl-7-ADCA preparation.

The relationship between pH, Temperature and Time of treatment was determined for a fixed reduction of 6-aminopenicillanic acid of 10^{-6} (Table 1b).

Table 1b

6-APA reduction	pH	Temp. (C)	Time (s)	Time (min)	Time (h)
10^{-6}	3	25	35050	584	9.74
10^{-6}	3	50	3057	50.9	0.85
10^{-6}	3	75	378	6.3	0.11
10^{-6}	3	100	62	1.0	0.02
10^{-6}	4	25	148857	2481	41.35
10^{-6}	4	50	12982	216.4	3.61
10^{-6}	4	75	1607	26.8	0.45
10^{-6}	4	100	263	4.4	0.07

EXAMPLE 2

ADSORPTION CHROMATOGRAPHY

This example shows the effect of (a) the degree of loading of the column when adsorption chromatography is used (2A to 2D), (b) the effect of washing the column with different amounts of water prior to elution (2E to 2G), (c) the effect of the pH of the feed on the purification of adipyl-7-ADCA (2H to 2J). The embodiment where adsorption chromatography is carried out in a Simulated Moving Bed mode is given as Experiment 2K.

The broth is pre-treated as described in Example 1A. The adipyl-7-amino-desacetoxy cephalosporanic acid was subsequently purified by adsorption chromatography by pumping the solution over a column filled with 1.6 litre of XAD-1600 resin, washed with different amounts of water (2A to 2D and 2H to 2K: 4.8 litre; 2E to 2F: see Table 2b), and eluted with 0.2 M bicarbonate-solution. The first eluate fraction (1.1 litre) is taken out and discarded. The second fraction (3.2 litre) is collected and analysed. The resin is purified by washing with caustic and acetone, and conditioned again with acidified water. Several changes in process conditions were applied (see table 2).

Reduction is calculated as: $(comp\text{-}i_{feed} / comp\text{-}1_{feed}) / (comp\text{-}i_{eluate} / comp\text{-}1_{eluate})$.

Table 2a. Results of experiment 2

Exp	Feed			Eluate (g)			Reduction (-)		comp 4 (ppm)
	comp 1 (g)	comp 2 (g)	comp 3 (g)	comp 1 (g)	comp 2 (g)	comp 3 (g)	comp 2	comp 3	
2A	34	3.3	9.3	30	3.1	5.9	1	1	
2B	80	5.9	18.5	70	0.5	0.09	10	173	<6
2C	127	10.5	32.8	76	0.3	0.07	24	301	19
2D	255	18.2	59.4	67	0.1	0.03	38	501	30

comp 1: adipyl-7-ADCA

comp 2: alpha-hydroxy adipyl-7-ADCA

comp 3: alpha-amino adipyl-7-ADCA

comp 4: 6-APA content relative to comp 1

These results clearly show the positive effect of overloading the column on the reduction of compounds 2 and 3 in the eluate.

Table 2b. Results of experiment 2

Exp	Feed			Wash	Eluate			Reduction (-)	
	comp 1 (g)	comp 2 (g)	comp 3 (g)	(1)	comp 1 (g)	comp 2 (g)	comp 3 (g)	comp 2	comp 3
2E	85	6.6	14.0	1.6	81	2.1	1.2	3	11
2F	74	6.4	12.6	4.8	71	1.1	0.13	6	94
2G	75	5.8	13.0	7.3	68	0.5	0.1	12	122

comp 1: adipyl-7-ADCA
comp 2: alpha-hydroxyadipyl-7-ADCA
comp 3: alpha-amino adipyl-7-ADCA

The example 2b shows the positive effect of extended washing, prior to elution with sodium bicarbonate, on the reduction of undesired cephalosporin compounds.

Table 2c

Exp	Feed				Eluate			Reduction (-)	
	comp 1 (g)	comp 2 (g)	comp 3 (g)	pH (-)	comp 1 (g)	comp 2 (g)	comp 3 (g)	comp 2	comp 3
2H	79	8.4	22.2	2.5	83	0.6	0.09	15	248
2I	80	5.9	18.5	2.9	70	0.5	0.09	10	183
2J	83	8.2	21.7	3.5	62	0.5	0.14	12	118

comp 1: adipyl-7-ADCA
comp 2: alpha-hydroxy adipyl-7-ADCA
comp 3: alpha-amino adipyl-7-ADCA

The above example shows the effect of the pH at which crystallisation was carried out, on the reduction of unwanted 7-N acylated cephalosporin compounds. H_2SO_4 was used as acid.

Table 2d. Results of experiment 2 (30 litres of resin in an SMB-system was applied)

Exp	Feed				Eluate				Reduction (-)		
	comp 1 (kg)	comp 2 (kg)	comp 3 (kg)	comp 4 (kg)	comp 1 (kg)	comp 2 (kg)	comp 3 (kg)	comp 4 (kg)	comp 2	comp 3	comp 4
2K	1.46	0.08	0.20	0.15	1.38	0.01	0.01	0.02	7	18	7

comp 1: dipyl-7-ADCA
comp 2: alpha-hydroxyadipyl-7-ADCA
comp 3: alpha-aminoadipyl-7-ADCA
comp 4: adipic acid

This example illustrates the use of adsorption chromatography performed according to the so-called Simulated Moving Bed technique, on a kilogram scale. The technique may readily be scaled up further.

The so treated fractions 2A to 2K were treated with acylase to produce 7-ADCA as described in Example 1. Excellent conversion results were obtained, as illustrated in Example 3.

EXAMPLE 3

ENZYMATIC CONVERSION

This example illustrates the results of enzymatic conversion of adipyl-7-ADCA to 7-ADCA. The adipyl-7-ADCA was recovered as disclosed in Example 2K (pH-treatment according to Example 1A, adsorption chromatography was optimised in terms of overloading and washing). The conversion was carried out as described in Example 1, at the pH indicated

in Table 3. Experiment A to E represent different batches.

Table 3

Exp	substrate comp 1 (mmol)	Substrate comp 2 (mmol)	pH (-)	Product stream comp 1 (mmol)	product stream comp 2 (mmol)
A	69.7	2.2	8.5	1.1	68.6
B	144.2	2.4	8.5	5.9	143.3
C	181.4	2.3	8.5	13.5	174.5
D	113.1	1.7	8	5.4	108.7
E	113.4	3.1	9	1.2	112.2

comp 1: adipyl-7-ADCA
comp 2: 7-ADCA

The conversion rate and yields are superior when the adipyl-7-ADCA is pre-treated using the pH/temperature step, as compared to no treatment. The further purification using chromatography brings further improvement in terms of purity (not shown in the Table).

EXAMPLE 4

CRUDE CRYSTALLISATION

The broth is pH/heat-treated (Example 1) and enriched in adipyl-7-ADCA by adsorption chromatography as described in Example 2. Subsequently, conversion was carried out as described in Example 1.

- The conversion solution (the solution obtained after deacylation) was concentrated with reversed osmosis to increase the concentration.

Part of the solution was taken and the 7-ADCA was crystallised by lowering the pH to the pH 3.6, 4 or 5 (see table 4a).

Table 4a Crude crystallisation

Exp	comp 1 in solution	pH (-)	product after isolation, washing and drying (g)	comp 1 in product (%)
A	49.5	3.6	48.9	97.5
B	49.5	4	48.7	97.4
C	49.5	5	48.2	98
comp 1: 7-ADCA				

Crystallisation was satisfactory at all pH tested.

In the following experiment the pH was 3.6. The effect of concentrating the solution is illustrated.

Table 4b Crude crystallisation

Exp	comp 1 in solution (g)	product after isolation, washing and drying (g)	comp 1 in product (%)
D	15.5	14.8	94.3
E	36.1	35.7	95.3
F	49.5	48.9	97.5
comp 1: 7-ADCA			

Clearly, there is an effect of the concentration of 7-ADCA in the conversion solution on purity and yield after crystallisation.

The following example illustrates the effect of different adsorbers on product quality (colour in solution and clarity).

Table 4c Crude crystallisation					
Exp	comp 1 in solution (g)	treatment	comp 1 in product (%)	colour in solution at 425 nm (-)	clarity in HCl (EBC)
G	25	HP20	97.2	0.16	3.6
H	25	HP20 (2 times)	97.8	0.11	2.4
I	25	IRA67	97.2	0.18	6
J	25	IRA67+HP20	97.7	0.1	0.8
K	25	none	96.3	0.39	7.3
comp 1: 7-ADCA					

EXAMPLE 5

TREATMENT OF DISSOLVED 7-AMINO DESACETOXY CEPHALOSPORANIC ACID

This example shows the effect on clarity and colour of 7-ADCA, after treating 7-ADCA solution with different adsorber resins, prior to crystallisation.

A solution comprising 7-ADCA is made as disclosed in Example 2K (the adsorption chromatography column used is a XAD-1600 resin).

Table 5

Exp	comp 1 dissolved (g)	treatment	Colour in solution at 425 nm (-)	Clarity in HCl (EBC)
A	40	--	0.18	4.2
B	40	XAD16	0.05	1.8
C	40	HP20	n.d.	0.5
D	40	XAD1600	0.09	0.5
E	25		n.d.	3.6
F	25	+1% EtOH	0.11	0.6
G	50	+3% EtOH	0.18	0.9
H	50	+2% Coal	0.04	n.d.
I	50		0.17	1.4
J	50	+5% Coal	0.03	0.8

comp 1: 7-amino desacetoxy cephalosporanic acid

EXAMPLE 6

RECOVERY OF ADIPYL-7-ADCA USING EXTRACTION WITH N-BUTANOL

Broth comprising adipyl-7-ADCA is treated as described in Example 1.

After acidification, part of the adipyl-ADCA is purified by adsorption chromatography. The solution is pumped over a column filled with XAD-16 resin, washed with water, and eluted with 0.2 M acetate-solution. The first eluate fraction with low adipyl desacetoxy cephalosporanic acid content is taken out and discarded. The second fraction is collected. The resin is purified by washing with caustic and acetone, and conditioned again with acidified water.

Part of the adipyl-7-ADCA is purified by means of extraction, followed by washing of the extract, back extraction of the *N*-substituted cephalosporin from the organic phase to an aqueous phase and stripping the aqueous phase; the extracting

organic solvent is n-butanol.

The adipyl-7-ADCA is treated with immobilised acylase to produce 7-amino desacetoxy cephalosporanic acid (7-ADCA). Part of the 7-ADCA is isolated by lowering the pH. The adipyl desacetoxy cephalosporanic acid was dissolved with the aid of caustic. The 7-amino desacetoxy cephalosporanic acid was isolated by lowering the pH.

Part of the 7-amino desacetoxy cephalosporanic acid aqueous solution is acidified, and the side chain is extracted to an extracting organic solvent and next the phases are separated; the extracting organic solvent was n-butanol,

Finally the crystal cake was filtrated, washed and dried.

Table 6

Exp	description	comp 1 in product (%)	colour 1 in solution at 425 nm (-)	clarity in HCl (EBC)
A	extraction/extraction /crystallisation	98.3	0.13	2.4
B	chromatography/extraction/ crystallisation	98.9	0.09	1.5
C	chromatography/ crystallisation	97.6	0.12	-
D	chromatography/ crystallisation/ recrystallisation	98.7	0.05	1.1

Comp 1: 7-amino desacetoxy cephalosporanic acid

The results using a single extraction are not shown in the table, but the purity is far worse than when chromatography is used, even without recrystallisation. A combination of chromatography and recrystallisation produces or a combination of chromatography and extraction produces the best results.

EXAMPLE 7

Recovery of adipic acid from 7-ADCA crystallisation mother liquors

7-ADCA crystallisation mother liquors are obtained as described in example 4. Adipic acid is determined using HPLC: Aminex HPX-87H column, 300 mm x 7.8 mm, filled with 9 um cation gel (Biorad), eluted at 65°C with a 0.2M solution of H_2SO_4 in water, detection using an RI Waters 410 refractometer. In the examples given below, optimisation is directed at purity, not at yield.

EXAMPLE 7A

Recovery of adipic acid using acidification

At 20°C, the pH of 7-ADCA crystallisation mother liquor (250 ml, 13.6 g/l adipic acid) was lowered to 0.7 using a 12M solution of H_2SO_4 in water. After 16 h at 0°C, no crystallisation could be detected. The pH was raised to 3.4 using a 6M solution of KOH in water. The resulting crystal was recovered by filtration to give 7.7 g of material which was a mixture of salt and adipic acid which was not further analysed.

EXAMPLE 7B

Recovery of adipic acid using acidification and concentration

At 20°C, the pH of 7-ADCA crystallisation mother liquor (500 ml, 9.4 g/l adipic acid) was lowered to 1.5 using a 12M solution of H_2SO_4 in water and concentrated under reduced pressure at 40°C to give a sticky mixture that was isolated by filtration and dried to give 6.9 g adipic acid with a purity of 53% (yield 78%).

EXAMPLE 7C

RECOVERY OF ADIPIC ACID USING REVERSE OSMOSIS WITH NANOMAX 50 MEMBRANE

At 20°C, the pH of 7-ADCA crystallisation mother liquor (500 ml, 9.4 to 18.2 g/l adipic acid, see table) was adjusted to the value mentioned in the table using either a 6M solution of KOH in water or a 12M solution of H_2SO_4 in water. The resulting solution was subjected to reverse osmosis using a Nanomax 50 membrane from Millipore. With the aid of nitrogen gas, a pressure of 30 bar was applied to give a filtrate and a retentate in which the amount of adipic acid was determined using HPLC. In most cases, a work-up procedure was applied that consisted of concentration under reduced pressure until crystallisation began, followed filtration of the product and drying.

Table 7

pH	Adipic acid (g/l)			Retention	Volume permeate	Yield after work-up	Purity after work-up
	Start	Permeate	Retentate	(%)	(ml)	(g)	(%)
1.5	13.6	11.0	14.8	26	400	4.4	96
2.0	18.2	13.1	20.8	37	260	2.4	99
3.0	9.4	6.7	8.7	23	360	1.9	97
7.2	13.6	5.3	31.9	83	400	no work-up	no work-up

EXAMPLE 7D

RECOVERY OF ADIPIC ACID USING REVERSE OSMOSIS WITH DK U19F MEMBRANE AT PH 2.0

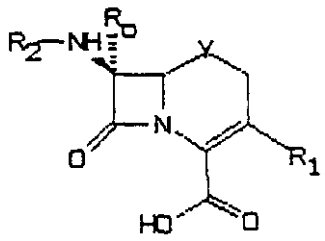
The pH of 7-ADCA crystallisation mother liquor (100 l, containing 25.0 g/l adipic acid) was lowered to 2.0 using 2.9 l of a 12M solution of H_2SO_4 in water. The resulting solution was subjected to reverse osmosis with 57 l water using a DK U19F membrane in a 2.5 m^2 membrane filtration unit P2-B200 from Hydro Air Research. At a pressure of 30 bar an average flux of 8.8 $l/m^2/h$ was reached to give the results summarised in the table.

Table 8

Component	Concentration (g/l)			Retention (%)
	Start	Permeate	Retentate	
Adipic acid	25.0	11.6	17.8	35
7-ADCA	0.72	<0.01	0.72	>99
Adipyl-7-ADCA	1.51	<0.03	1.54	>98

Claims

1. A method for the recovery of an N-substituted cephalosporanic acid compound of the general formula (I):



(I)

wherein

- R_0 is hydrogen or C_{1-3} alkoxy;
- Y is CH_2, oxygen, sulphur, or an oxidised form of sulphur;
- R_1 is any of the groups selected from the group consisting of
  - hydrogen,
  - hydroxy,
  - halogen,
  - saturated or unsaturated, straight or branched alkyl (1 - 5 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy, halogen, aryl, alkoxy (1 - 3 carbon atoms), or acyl;
  - alkoxy (1-3 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy or halogen; or
  - cycloalkyl (3 - 8 carbon atoms) optionally substituted with hydroxy, halogen, amino;
  - aryl;
  - heteroaryl; and
- R_2 is selected from the group consisting of adipyl (1,4-

dicarboxybutane), succinyl, glutaryl, adipyl, pimelyl, suberyl, 2-(carboxyethylthio)acetyl, 3-(carboxy-ethylthio)propionyl, higher alkyl saturated and higher alkyl unsaturated dicarboxylic acids,

from a complex mixture comprising in addition to the compound of the general formula 6-aminopenicillanic acid (6-APA) and optionally one or more N-substituted ß-lactam compounds,

comprising the steps of:

(a) acidifying the complex mixture to a pH below 6.5 and maintaining the mixture below said pH at a temperature of between 10°C and 150°C; and/or

(b) contacting the complex mixture with a carbon dioxide source; and

(c) recovering the cephalosporanic acid compound of the formula (I) from the mixture obtained after steps (a) and/or (b).

2. A method according to claim 1, wherein in step (a) the temperature is kept between about 50 °C and about 130 °C, preferably between 70 and 120 °C, for between 10 seconds and about 1 day and the pH is kept at or below pH 4.5.

3. A method according to claim 1 or 2, wherein the compound has been produced by fermentation of a micro-organism capable thereof and wherein the complex mixture is a broth, a culture filtrate or any culture liquid derivable from the broth after fermentation.

4. A method according to any one of claims 1 to 3, wherein the compound of the general formula is selected from the group consisting of adipyl-7-ADCA, adipyl-7-ADAC and adipyl-7-ACA.

5. A method according to any one of the previous claims, wherein step (c) is performed by subjecting the mixture obtained after steps (a) and/or (b) to chromatography.

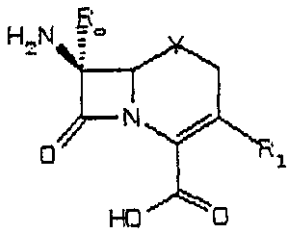
6. A method according to claim 5, wherein chromatography is adsorption chromatography, more preferably Hydrophobic Interaction Chromatography.

7. Use of chromatography in a process of recovering an N-substituted cephalosporin compound according to formula (I) in claim 1.

8. Use according to claim 7, wherein the chromatography is adsorption chromatography, preferably Hydrophobic Interaction Chromatography.

9. Use according to claim 8, wherein the chromatography is performed using Simulating Moving Bed technology.

10. A method for preparing a compound of formula (II):



(II)

wherein

- R_0 is hydrogen or C_{1-3} alkoxy;
- Y is CH_2, oxygen, sulphur, or an oxidised form of sulphur;
- R_1 is any of the groups selected from the group consisting of
  - hydrogen,
  - hydroxy,
  - halogen,
  - saturated or unsaturated, straight or branched alkyl (1 - 5 carbon atoms; optionally replaced by one or

more heteroatoms), optionally substituted with hydroxy, halogen, aryl, alkoxy (1 - 3 carbon atoms), or acyl;

- alkoxy (1-3 carbon atoms; optionally replaced by one or more heteroatoms), optionally substituted with hydroxy or halogen; or
- cycloalkyl (3 - 8 carbon atoms) optionally substituted with hydroxy, halogen, amino;
- aryl;
- heteroaryl,

comprising the steps of making a compound according to formula (I) using a process according to any one of claims 1 to 6;
deacylating the compound of formula (I) to obtain a conversion solution which comprises a compound according to formula (II).

11. A method according to claim 10, wherein the conversion solution further comprises the cleaved side chain designated R_2.

12. The process of claim 10, wherein the deacylation is performed enzymatically using a dicarboxyl acylase.

13. A process according to any one of claims 10 to 12, comprising the further step of recovering the compound of formula (II) from the solution by crystallisation.

14. A process according to claim 13, wherein crystallisation is preceded by treatment of the solution with an agent selected from the group consisting of an adsorber resin, active coal, methanol, ethanol, (iso)propanol, isobutanol, n-butanol, acetone or a combination of any of the mentioned agents.

15. A process according to claim 14, wherein at least an adsorber resin is used selected from XAD16, XAD1600 and

HP20.

16. A method according to claim 10 or 11, wherein the 6-aminopenicillanic acid (6-APA) level is 10 ppm or less with respect to the compound of formula (II).

17. A process according to claim 11 or 12, wherein following the deacylation the solution is treated to remove, at least partially, the cleaved side chain represented by R_2.

18. A process according to claim 17, wherein the treatment to remove, at least partially, the cleaved side chain is carried out on the mother liquor obtained after crystallisation.

19. A process according to claim 18, wherein said treatment to remove, at least partially, the cleaved side chain is followed by solubilisation of the crude crystals and recrystallisation of the compound of formula (II).

20. A process according to claim 18, wherein crystallisation is preceded by treatment of the solution with an agent selected from the group consisting of an adsorber resin, active coal, methanol, ethanol, (iso)propanol, isobutanol, n-butanol and acetone, or a combination of any of these mentioned agents.

21. A process according to any one of claims 17 to 20, wherein said treatment comprises subjecting the conversion solution, or the mother liquid, to membrane filtration at a pH below 5, preferably below 4, more preferably near or below 3.

22. Use of membrane filtration to remove a dicarboxylic acid from a mixture comprising the dicarboxylic acid and a ß-lactam antibiotic.

23. Use according to claim 22, wherein the mixture is a mother liquid obtained after crystallisation of a compound of the formula (II).

24. The use according to claim 22 or 23, wherein the filtration takes place at a pH of about 5 or less, preferably at pH 4 or less.

25. The use according to claim 22 to 24, wherein said filtration is by narofiltration at or below pH 3.

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/EP 99/02247

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D501/12 C12P35/00

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 C07D 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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 599 854 A (SHELL DEVELOPMENT COMPANY) 23 March 1948 see page 1, line 74 - page 2, line 65; claims	1-25
A	GB 810 196 A (NATIONAL RESEARCH DEVELOPMENT CORPORATION) 11 March 1959 see page 9 - page 10; claims	1-25
A	WO 96 23797 A (CHEMFERM VOF ;BOESTEN WILHELMUS H J (NL)) 8 August 1996 see claims	1-25
A	EP 0 532 341 A (MERCK & CO INC) 17 March 1993 cited in the application see claims	1-25

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"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
18 June 1999	01/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chouly, J

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 48036 A (FASEL HERMAN PIETER ;VROOM ERIK DE (NL); KRIJGSMAN JOHN (NL); BRAA) 29 October 1998 see abstract, claims especially claims 7,8	1-25
P,A	EP 0 832 892 A (CHEMFERM VOF) 1 April 1998 see claims	1-25
P,A	US 5 801 241 A (AUDIA VICKI H ET AL) 1 September 1998 see claims	1-25

INTERNATIONAL SEARCH REPORT

Inf... ...ation on patent family members

Int... Application No
PCT/EP 99/02247

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C07D501/12
C12P 35/00

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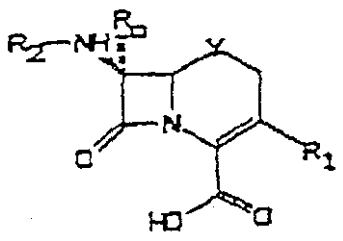
代理人 郭建新

权利要求书4页 说明书26页 附图页数0页

[54]发明名称 发酵生产头孢菌素的新方法

[57]摘要

一种从除通式(Ⅰ)的化合物外还含有6-氨基青霉烷酸(6-APA)和任选一种或多种N-取代青霉烷酸化合物的复杂混合物回收通式(Ⅰ)的N-取代头孢霉烷酸化合物的方法,其中,R_2选自:已二酰(1,4-二羧基丁烷)、丁二酰、戊二酰、已二酰、庚二酰、辛二酰、2-(羧基乙硫基)乙酰、3-(羧基乙硫基)丙酰、高级烷基饱和的和高级烷基不饱和的二羧酸,该方法包括如下步骤:(a)酸化所述复杂混合物至低于6.5的pH并将低于所述pH的混合物保持在10℃和150℃之间的温度;和/或(b)将所述复杂混合物与二氧化碳源接触;以及(c)从步骤(a)和/或(b)之后获得的混合物回收式(Ⅰ)的头孢霉烷酸化合物。



(Ⅰ)

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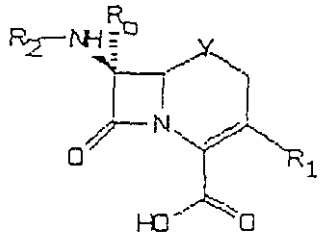
权 利 要 求 书

1. 一种从除通式（I）化合物外还含有 6-氨基青霉烷酸(6-APA)和任选一种或多种 N-取代 β-内酰胺化合物的复杂混合物中回收通式(I)的 N-取代头孢霉烷酸化合物的方法：



(I)

其中，

- R_0 是氢或 C_{1-3} 烷氧基；
- Y 是 CH_2、氧、硫或硫的氧化形式；
- R_1 是选自下组的任一个基：
  - 氢，
  - 羟基，
  - 卤素，
  - 饱和的或不饱和的、直链的或支化的烷基(1~5 个碳原子；任选被一个或多个杂原子替代)，任选被羟基、卤素、芳基、烷氧基(1~3 个碳原子)或酰基取代；
  - 烷氧基(1~3 个碳原子；任选被一个或多个杂原子替代)，任选被羟基或卤素取代；或者
  - 任选被羟基、卤素、氨基取代的环烷基(3~8 个碳原子)；
  - 芳基；
  - 杂芳基；以及
- R_2 选自：己二酰(1,4-二羧基丁烷)、丁二酰、戊二酰、己二酰、庚二酰、辛二酰、2-(羧基乙硫基)乙酰、3-(羧基乙硫基)丙酰、高级烷基饱和的和高级烷基不饱和的二羧酸，

该方法包括如下步骤：

(a)酸化所述复杂混合物至低于6.5的pH并将低于所述pH的混合物保持在10°C和150°C之间的温度；和/或

(b)将所述复杂混合物与二氧化碳源接触；以及

(c)从步骤(a)和/或(b)之后获得的混合物回收式(I)的头孢霉烷酸化合物。

2.权利要求1的方法，其中，在步骤(a)中将温度保持在约50°C和约130°C之间、优选在70和120°C之间达10秒~约1天，而pH则被保持在pH4.5或低于pH4.5。

3.权利要求1或2的方法，其中，所述化合物已通过能生产该化合物的微生物的发酵生产了，并且其中，所述复杂混合物是发酵液、培养物滤液或可得自发酵后的发酵液的任何培养液。

4.权利要求1~3任一项的方法，其中，所述通式化合物选自：己二酰-7-ADCA、己二酰-7-ADAC和己二酰-7-ACA。

5.前述权利要求任一项的方法，其中，这样进行步骤(c)：将步骤(a)和/或(b)之后获得的混合物进行色谱法处理。

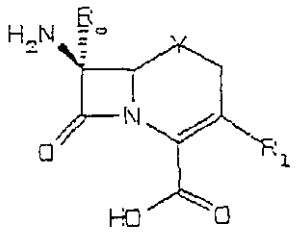
6.权利要求5的方法，其中，所述色谱法是吸附色谱法，更优选是疏水相互作用色谱法。

7.色谱法在回收权利要求1中式(I)的N-取代头孢菌素化合物的过程中的应用。

8.权利要求7的应用，其中，所述色谱法是吸附色谱法，优选是疏水相互作用色谱法。

9.权利要求8的应用，其中，所述色谱法是应用模拟移动床技术进行的。

10.一种制备式(II)化合物的方法：



(II)

其中，

- R_0是氢或C_{1-3}烷氧基；
- Y是CH_2、氧、硫或硫的氧化形式；
- R_1是选自下组的任一个基：
  - 氢，
  - 羟基，
  - 卤素，
  - 饱和的或不饱和的、直链的或支化的烷基(1~5个碳原子；任选被一个或多个杂原子替代)，任选被羟基、卤素、芳基、烷氧基(1~3个碳原子)或酰基取代；
  - 烷氧基(1~3个碳原子；任选被一个或多个杂原子替代)，任选被羟基或卤素取代；或者
  - 任选被羟基、卤素、氨基取代的环烷基(3~8个碳原子)；
  - 芳基；
  - 杂芳基，

该方法包括如下步骤：应用权利要求1~6任一项的方法制备式(I)的化合物；

使式(I)的化合物脱酰而获得包含式(II)化合物的转化液。

11. 权利要求10的方法，其中，所述转化液进一步包含以R_2表示的分裂侧链。

12. 权利要求10的方法，其中，所述脱酰是应用二羧基酰基转移酶酶促进行的。

13. 权利要求10~12任一项的方法，它包括该进一步的步骤：通过结晶从所述溶液回收式(II)的化合物。

14. 权利要求13的方法，其中，在所述结晶以前用选自下列的作用剂处理溶液：吸附树脂、活性炭、甲醇、乙醇、(异)丙醇、异丁醇、正丁醇、丙酮或者任意上述作用剂的组合物。

15. 权利要求14的方法，其中，应用了至少一种选自下列的吸附树脂：XAD16、XAD1600和HP20。

16. 权利要求 10 或 11 的方法，其中，6-氨基青霉烷酸(6-APA)含量相对于式(II)化合物为 10 ppm 或更少。

17. 权利要求 11 或 12 的方法，其中，在脱酰之后处理溶液而至少部分地除去由 R_2 表示的分裂侧链。

18. 权利要求 17 的方法，其中，至少部分地除去分裂侧链的处理是对结晶后获得的母液进行的。

19. 权利要求 18 的方法，其中，所述至少部分地除去分裂侧链的处理之后接着进行粗晶体的加溶和式(II)化合物的重结晶。

20. 权利要求 18 的方法，其中，在结晶之前用选自下列的作用剂处理溶液：吸附树脂、活性炭、甲醇、乙醇、(异)丙醇、异丁醇、正丁醇和丙酮或者上述这些作用剂任意的组合。

21. 权利要求 17~20 任一项的方法，其中，所述处理包括：将转化液或母液在低于 5 的 pH、优选低于 4 的 pH、更优选接近或低于 3 的 pH 下进行膜式过滤。

22. 膜式过滤在从包含二羧酸和β-内酰胺抗生素的混合物中除去二羧酸方面的应用。

23. 权利要求 22 的应用，其中，所述混合物是结晶式(II)的化合物后获得的母液。

24. 权利要求 22 或 23 的应用，其中，所述过滤是在大约 5 或更低的 pH、优选在 pH 4 或更低 pH 下进行的。

25. 权利要求 22~24 的应用，其中，所述过滤是在 pH 3 或低于 pH 3 时通过 narofiltration 进行的。

说　明　书

发酵生产头孢菌素的新方法

本发明涉及一种制备头孢菌素类和头孢菌素衍生物的方法。更具体地说，本发明涉及从头孢菌素类和其它β-内酰胺化合物的复杂混合物回收头孢菌素类及其衍生物。本发明还涉及从β-内酰胺化合物和侧链(例如可通过酶促侧链除去而获得的那些)的混合物回收脱酰头孢菌素类。

制备头孢菌素类的半合成途径大多始于发酵产物(例如青霉素 G、青霉素 V 和头孢菌素 C)，例如按下列文献中公开的方法将它们转化成相应的β-内酰胺环：K.Matsumoto，生物加工技术(Bioprocess.Techn.)，16，(1993)，67-88，J.G.Shewale & H.Sivaraman，生物化学进展(Process Biochemistry)，1989 年 8 月，146-154，T.A.Savidge，工业抗生素的生物技术(Biotechnology of Industrial Antibiotics)(编辑 E.J.Vandamme)Marcel Dekker，New York，1984，或 J.G.Shewale 等，国际生物化学进展(Process Biochemistry International)，1990 年 6 月，97-103。接着通过偶合到合适的侧链上而将获得的β-内酰胺环转化成所需的抗生素，正如尤其在 EP 0 339 751、JP-A-53005185 和 CH-A-640 240 中描述的那样。通过将侧链和β-内酰胺环进行不同的组合，可获得各种青霉素和头孢菌素抗生素。

已知，7-氨基去乙酸基头孢霉烷酸(7-amino desacetoxy cephalosporanic acid)(7-ADCA)和 7-氨基头孢噻嗪酸(7-aminocephalosporonic acid)(7-ACA)是生产制药工业中应用的抗生素的最重要中间体。

7-ADCA 是例如通过化学法或酶法分裂(脱酰作用)苯乙酰去乙酸基头孢霉烷酸(生成 7-氨基去乙酸基头孢霉烷酸和苯乙酸)而获得的。

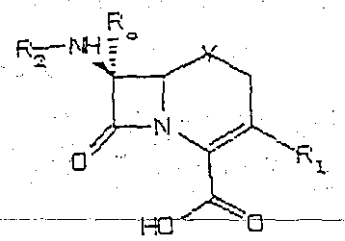
苯乙酰去乙酸基头孢霉烷酸一般是通过化学法处理青霉素 G 亚砜(它是从青霉素 G 生成的)而生产的。在该生产方法中，需要大量化学试剂以保障所需反应的进行。这既昂贵又对废物处理构成沉重负担。此外，该方法的总产率不是很高。

为了克服化学法的某些缺点，已公开了生产 7-ADCA、7-氨基去乙酰基头孢霉烷酸(7-ADAC)和 7-ACA 的发酵法，该方法涉及：通过能从转基因表达去乙酸基头孢霉烷酸合成酶(DAOCS，也被称为“扩展酶”)的重组产黄青霉(Penicillium chrysogenum)菌株发酵生产 N-取代β-内酰胺(例如己二酰-7-ADCA、己二酰-7-ADAC 或己二酰-7-ACA)(EP 0 532 341、EP 0 540 210、WO 93/08287、WO 95/04148)。扩展酶负责某些 N-酰化青霉烷酸的 5 元环扩展，于是生成相应的 N-酰化去乙酸基头孢霉烷酸。

为了生产出经济上最重要的未酰化头孢菌素类(例如 7-ADCA、7-ADAC 和 7-ACA)，用合适的酰基转移酶酶促除去酰基。

回收化学法或酶法生产的青霉烷酸和头孢霉烷酸的已知方法对 N-取代β-内酰胺中间体和脱酰氨基-β-内酰胺的回收来说不是有效的。回收发酵法生产的上述头孢菌素化合物的主要问题是发酵液或培养物滤液的复杂性。发酵液通常包含各种青霉烷酸[例如α-氨基己二酰-6-青霉烷酸、α-羟基己二酰-6-青霉烷酸、6-氨基青霉烷酸(6-APA)]，各种头孢霉烷酸(包括α-氨基己二酰-和羟基己二酰-7-ADCA)和大量蛋白质类物质。已知的回收方法不能给出纯度上可接受品级的头孢霉烷酸产品。在脱酰作用中，这导致如下问题：酶半寿期的缩短、生物转化率的降低和生物转化后的回收费用更高和/或不可接受的污染物含量。此外，脱酰后，这类杂质妨碍或至少阻碍所要求技术规格的所需脱酰头孢菌素化合物的回收。

本发明提供了一种从除通式（I）化合物外还含有 6-氨基青霉烷酸(6-APA)和任选一种或多种 N-取代β-内酰胺化合物的复杂混合物中回收通式(I)的头孢霉烷酸化合物的方法：



(I)

其中，

- R_0是氢或C_{1-3}烷氧基；
- Y是CH_2、氧、硫或硫的氧化形式；
- R_1是选自下组的任一个基：
  - 氢，
  - 羟基，
  - 卤素，
  - 饱和的或不饱和的、直链的或支化的烷基(1~5个碳原子；任选被一个或多个杂原子替代)，任选被羟基、卤素、芳基、烷氧基(1~3个碳原子)或酰基取代；
  - 烷氧基(1~3个碳原子；任选被一个或多个杂原子替代)，任选被羟基或卤素取代；或者
  - 任选被羟基、卤素、氨基取代的环烷基(3~8个碳原子)；
  - 芳基；
  - 杂芳基；以及
- R_2选自：己二酰(1,4-二羧基丁烷)、丁二酰、戊二酰、己二酰、庚二酰(pimelyl)、辛二酰(suberyl)、2-(羧基乙硫基)乙酰、3-(羧基乙硫基)丙酰、高级烷基饱和的和高级烷基不饱和的二羧酸，

该方法包括如下步骤：

(a)酸化所述复杂混合物至低于6.5的pH并将低于所述pH的混合物保持在10°C和150°C之间的温度；和/或

(b)将所述复杂混合物与二氧化碳源接触；以及

(c)从步骤(a)和/或(b)之后获得的混合物回收式(I)的头孢霉烷酸化

合物。

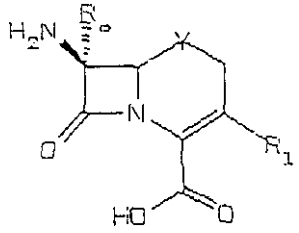
优选地，在步骤(a)中将温度保持在约 50℃和约 130℃之间(优选在 70 和 120℃之间)达 10 秒~约 1 周，而 pH 则被保持在 **pH4.5** 或低于 pH4.5。按一种优选的方法，已通过有能力的微生物的发酵生产了式(I)的化合物，所述复杂混合物是发酵液、培养物滤液或可得自发酵后发酵液的任何培养液。

通式(I)的优选化合物选自：己二酰-7-ADCA、己二酰-7-ADAC 和己二酰-7-ACA。

按本发明的另一方面，这样进行步骤(c)：将步骤(a)和/或(b)之后获得的混合物进行色谱法(优选为吸附色谱法、更优选为疏水相互作用色谱法)处理。

按本发明的又一方面，提供了色谱法在回收式(I)的头孢菌素化合物过程中的应用，优选通过吸附色谱法、更优选为疏水相互作用色谱法，进一步优选应用模拟移动床技术(Simulated Moving Bed technology)。

按本发明的又一方面，提供了一种制备式(II)的化合物的方法：



(II)

其中，

- R_0 是氢或 C_{1-3} 烷氧基；
- Y 是 CH_2、氧、硫或硫的氧化形式；
- R_1 是选自下组的任一个基：
  - 氢，
  - 羟基，

- 卤素，
- 饱和的或不饱和的、直链的或支化的烷基(1~5 个碳原子；任选被一个或多个杂原子替代)，任选被羟基、卤素、芳基、烷氧基(1~3 个碳原子)或酰基取代；
- 烷氧基(1~3 个碳原子；任选被一个或多个杂原子替代)，任选被羟基或卤素取代；或者
- 任选被羟基、卤素、氨基取代的环烷基(3~8 个碳原子)；
- 芳基；
- 杂芳基；

该方法包括如下步骤：制备式(I)的化合物(其中，R_0、Y 和 R_1 如上述定义，并且 R_2 选自：己二酰(1,4-二羧基丁烷)、丁二酰、戊二酰、己二酰、庚二酰、辛二酰、2-(羧基乙硫基)乙酰、3-(羧基乙硫基)丙酰、高级烷基饱和的和高级烷基不饱和的二羧酸)；

使式(I)的化合物脱酰而获得包含式(II)化合物的转化液。

该转化液优选还包含分裂的侧链(表示为 R_2)。

按一个优选的实施方案，所述方法还包括通过结晶从溶液回收式(II)化合物这一步骤，优选在此之前和/或之后(在加溶粗晶体即通过结晶之后)用选定的作用剂(例如活性炭或吸附树脂)处理溶液。按本发明的另一方面，在结晶和/或重结晶期间或之前添加一种溶剂，例如甲醇、乙醇、(异)丙醇、异丁醇、正丁醇或丙酮或者上述试剂任意的组合物。优选的吸附树脂选自：XAD16(CAS No.102419-63-8)、XAD1600(CAS No.153796-66-8)和 HP20(CAS No.55353-13-4)。按本发明优选的是这一方法：其中，6-氨基青霉烷酸(6-APA)含量相对于式(II)化合物为 10ppm 或更少。按另一方面，提供了一种方法，其中，在脱酰作用后，处理溶液而至少部分地除去以 R_2 表示的分裂的侧链。可在式(II)化合物的结晶和加溶(即重结晶)之后进行该步骤或重复该步骤。还可对结晶或重结晶之后获得的母液进行分裂的侧链的除去。

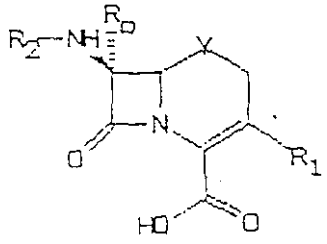
所以，提供了一种方法，其中，在所述处理而至少部分地除去分裂的侧链之后，接着加溶粗晶体和重结晶式(II)的化合物。

优选地，所述处理而除去分裂的侧链包括：在低于 5、优选低于 4、更优选接近或低于 3 的 pH 下，将所述转化液或母液或者这二者进行膜式过滤。因此，提供了膜式过滤在从包含二羧酸和β-内酰胺抗生素的混合物除去二羧酸方面的应用。所述混合物优选是结晶式(II)的化合物之后获得的母液或是式(I)的化合物脱酰后获得的混合物。膜式过滤优选在约为 5 或更小的 pH 下、优选在 pH 4 或更小下进行，更优选在 pH 3 或更低时通过超微过滤(nanofiltration)进行。

按本发明的另一方面，提供了一种方法，其中，通过结晶和/或重结晶从转化混合物至少部分地除去了侧链 R_2。

按本发明的又一方面，提供了一种方法，其中，侧链 R_2 是这样从转化混合物至少部分地被除去的：将混合物酸化至低于 3 的 pH，接着将该混合物与有机溶剂(例如乙酸戊酯、乙酸丁酯、乙酸乙酯、甲基异丁基酮、环己酮、异丁醇或正丁醇)接触。

本发明涉及一种从除通式（I）化合物外还含有 6-氨基青霉烷酸(6-APA)和任选一种或多种 N-取代青霉烷酸化合物的复杂混合物中回收通式(I)的头孢霉烷酸化合物的方法：



(I)

其中，

- R_0 是氢或 C_{1-3} 烷氧基；
- Y 是 CH_2、氧、硫或硫的氧化形式；
- R_1 是选自下组的任一个基：
  - 氢，

- 羟基，
- 卤素，
- 饱和的或不饱和的、直链的或支化的烷基(1~5 个碳原子；任选被一个或多个杂原子替代)，任选被羟基、卤素、芳基、烷氧基(1~3 个碳原子)或酰基取代；
- 烷氧基(1~3 个碳原子；任选被一个或多个杂原子替代)，任选被羟基或卤素取代；或者
- 任选被羟基、卤素、氨基取代的环烷基(3~8 个碳原子)；
- 芳基；
- 杂芳基；以及

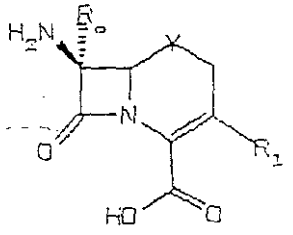
- R_2选自：己二酰(1,4-二羧基丁烷)、丁二酰、戊二酰、己二酰、庚二酰、辛二酰、2-(羧基乙硫基)乙酰、3-(羧基乙硫基)丙酰、高级烷基饱和的和高级烷基不饱和的二羧酸，

该方法包括如下步骤：

(a)酸化所述复杂混合物至低于6.5的pH并将低于所述pH的混合物保持在10°C和150°C之间的温度；和/或

(b)将所述复杂混合物与二氧化碳源接触；以及

(c)从步骤(a)和/或(b)之后获得的混合物回收式(I)的头孢霉烷酸化合物。本发明进一步涉及一种制备具有通式(II)的头孢菌素的方法：



(II)

其中，

- R_0是氢或C_{1-3}烷氧基；
- Y是CH_2、氧、硫或硫的氧化形式；

- R_1是选自下组的任一个基:
  - 氢,
  - 羟基,
  - 卤素,
  - 饱和的或不饱和的、直链的或支化的烷基(1~5 个碳原子; 任选被一个或多个杂原子替代), 任选被羟基、卤素、芳基、烷氧基(1~3 个碳原子)或酰基取代;
  - 烷氧基(1~3 个碳原子; 任选被一个或多个杂原子替代), 任选被羟基或卤素取代; 或者
  - 任选被羟基、卤素、氨基取代的环烷基(3~8 个碳原子);
  - 芳基;
  - 杂芳基。

式(I)的化合物可通过能生成本文定义的复杂混合物的任何系列步骤生产, 从该复杂混合物实现式(I)化合物的回收。对于说明书和权利要求书来说, "复杂混合物"定义为包含 N-取代头孢菌素化合物和取代的或未取代的β-内酰胺化合物的混合物。

式(II)的化合物是通过如下系列步骤获得的:

(a)回收、优选纯化式(I)的化合物;

(b)将优选纯化的式(I)化合物脱酰而获得包含式(II)化合物的溶液(转化液); 以及

(c)回收、优选纯化式(II)的化合物。

发酵法生产 N-取代头孢霉烷酸的障碍之一是存在不希望有的污染性β-内酰胺组分(例如 N-取代 6-氨基青霉烷酸)。按本发明的一个实施方案, 已发现这些污染物可这样被显著减少: 在酸化条件下(优选伴随高温), 将发酵液、发酵液的滤液或通过应用任何生物量分离技术从发酵液获得的液体保温。应用至少一种已知的酸(例如硫酸、盐酸或硝酸或其组合物)将发酵液酸化到低于 6.5 的 pH(优选低于 4.5)。操作温度在 20~150°C 的范围内, 优选在 70~120°C。在这些条件下的保留时间

在数秒(150°C 下)或数天(20°C 下)的范围内，优选为 10 秒~60 分钟。优选进行一段时间的 pH/温度处理而提供 N-取代 6-APA 相对于式(II)化合物的降低倍数为 100、优选为 1000、更优选为 1,000,000。可在生物量分离之前或之后进行该步骤，而且可分批地或连续地进行。

按本发明的另一实施方案，污染性青霉素组分(例如 N-取代 6-APA)是通过将发酵液、发酵液的滤液、洗脱液、转化液或溶解的式(I)的污染性头孢菌素(通常在 pH5~7 时)与二氧化碳接触而显著地减少的。二氧化碳可被以任意合适的形式(例如固体或气体形式或作为碳酸根离子的溶液)加到溶液中。将溶液与 CO_2 源在 10~60°C(优选 20~40°C)的温度下接触，其中，所述溶液被分子 CO_2 饱和 4~10 小时。在减少青霉素组分后，可达到如前述那样的式 1 头孢菌素的纯化。

本文定义的复杂混合物可具有任意来源，但优选是在导致生产的条件下、通过能生产通式(I)化合物的 7-N-酰化形式的微生物发酵后获得的培养液或培养物滤液，其中所述酰基可以是在头孢菌素生物合成途径中支持环扩展酶(去乙酸基头孢菌素合成酶-DAOCS，或双官能扩展酶/羟化酶(有时被称为去乙酰基头孢菌素合成酶 DACS))的任意酰基。体内生产式(I)的 7-N-酰基取代的化合物的生物过程被公开于：WO 93/05158(己二酰-7-ADCA)；WO 93/08287(己二酰-7-ADAC 和己二酰 -7-ACA)，WO 95/04148(2-(羧基乙硫基)乙酰-7-ADCA)，WO 95/04149(3-(羧基乙硫基)丙酰-7-ADCA)和高级烷基饱和的或不饱和的二羧酸。这些 PCT 申请的相关部分被并入本文作参考。优选的酰基一般是二羧酸类，例如己二酰(1,4-二羧基丁烷)、2-(羧基乙硫基)乙酰、3-(羧基乙硫基)丙酰、粘康酸等。合适的宿主生物包括但不限于产黄青霉和产黄支顶孢(Acremonium chrysogenum)。合适的扩展酶(包括双官能扩展酶/羟化酶)源包括但不限于带小棒链霉菌(Streptomyces clavuligerus)和产黄支顶孢。转化的方法、转化细胞的选择和表示丝状真菌的调节因子(它们可被用于基因修饰宿主细胞)都是β-内酰胺生产(丝状)真菌的重组 DNA 技术领域中熟知的。

优选地，在酸化和任选升高温度之前，首先将发酵液进行生物量

分离，例如通过任意合适的方法过滤，例如膜式过滤、真空过滤、超滤或其组合。生物量分离的任何其它方法也是合适的。

在 pH-降低步骤和任选的温度步骤之后，回收的式(I)化合物优选经历进一步纯化从而至少部分地除去不希望有的β-内酰胺组分，尤其是不希望有的 N-取代头孢菌素和青霉素。所述进一步纯化可通过应用有机溶剂萃取而进行。就萃取来说，发现了有利的是：洗涤萃取液，从有机相将 N-取代头孢菌素反萃取到水相，再反萃取水相。萃取有机溶剂可选自乙酸戊酯、乙酸丁酯、乙酸乙酯、甲基异丁基酮、环己酮、异丁醇或正丁醇等。在本方法中，优选的纯化步骤是应用色谱法纯化 N-取代头孢菌素而不是应用有机溶剂萃取。色谱法的优点是不存在溶剂(溶剂引起废物问题和污染问题)，以及终产物纯度的改善。优选的是离子交换色谱法或吸附色谱法，更优选是疏水相互作用色谱法。应用吸附剂将滤液进行色谱处理。吸附剂包括：活性炭，例如 Norit CG-1 或 Cecarbon GAC 40；或者吸附树脂，例如苯乙烯-二乙烯基苯共聚物，诸如得自 Mitsubishi Kasei Corporation 的 Dianion HP 20(CAS No. 55353-13-4)、Dianion HP 21(CAS No. 92529-04-9)、Dianion SP 207(CAS No. 98225-81-1)或 Dianion SP 825 或者得自 Rohm 和 Haas 的 Amberlite XAD 1180(CAS No. 97396-56-0)、Amberlite XAD 1600(CAS No. 153796-66-8)或 Amberlite XAD 16(CAS No. 102419-63-8)或者得自 TosoHaas 的 Amberchrom CG 161(CAS No. 131688-63-6)；优选为 XAD 16 或 XAD 1600。

在吸附 N-取代头孢菌素之前，通过一种或多种已知的酸(例如硫酸、盐酸或硝酸或其组合物)将所述复杂混合物调节至 1.0~5.0(优选 2.5~3.5)的 pH。操作温度在 0~50°C(优选在 5~25 °C)的范围内。操作压力在 0~1.0 MPa 超压的范围内。

不希望有的β-内酰胺组分，尤其不希望有的 N-取代头孢菌素(例如α-氨基己二酰头孢霉烷酸)也吸附在吸附剂上，但被需要的 N-取代头孢菌素置换。

吸附后，采取用水洗涤以除去吸附剂之间空隙容积中不希望有的

β-内酰胺组分，并从吸附剂解吸弱结合的、不希望有的β-内酰胺组分。可通过一种或多种已知的酸(例如硫酸、盐酸或硝酸或其组合)酸化所述水至 1.0 的 pH。要增大渗透压，可以往水中添加盐。操作温度在 0~50°C(优选在 20~40°C)的范围内。操作压力在 0~1.0 MPa 超压的范围内。

可用合适的缓冲剂(例如乙酸盐、磷酸盐、碳酸盐、碳酸氢盐或己二酸盐)进行洗脱，但还可应用稀有机溶剂(例如丙酮、异丙醇)或稀碱(例如铵、苛性碱)。操作温度在 0~80°C(优选在 10~40°C)的范围内。操作压力在 0~1.0 MPa 超压的范围内。

可通过任何常用方法进行吸附剂的再生，例如应用稀碱、稀酸，或者应用水混溶性溶剂(例如丙酮、甲醇、乙醇或异丙醇)或其组合。可以实施任选加热到 100°C。

再生液体可通过用水洗涤除去。可通过一种或多种已知的酸(例如硫酸、盐酸或硝酸或其组合)酸化所述水至 1.0 的 pH。

可在数种装置中进行色谱处理步骤，例如以单一的柱，但还可应用模拟移动床技术。就该模拟移动床技术来说，有数种装置是可获得的，例如 U.S.Filter 的 ADSEP 系统、Advanced Separation Technology 的 ISEP/CSEP 系统、例如 Applexion 的 'merry-go-around' 系统或 Universal Oil Products Company(UOP)的 SORBEX 系统。

还可通过超微过滤除去洗脱液中的缓冲剂。该膜式过滤中膜的特征表现为对需要的 N-取代头孢菌素的高保留和对缓冲剂的低保留。

任选通过任何合适的浓缩方法应用一个浓缩步骤，例如真空蒸发、反渗透、超微过滤或者色谱处理或萃取后的 narofiltration。

接着应用本领域已知的任意合适方法将回收的 N-酰化化合物脱酰。一个优选的方法是应用合适的二羧化酰基转移酶进行酶促脱酰作用。有很多合适的酰基转移酶(野生型或突变型)是本领域已知的，它们包括但不限于得自下列微生物的那些：芽孢杆菌属(Bacillus)(EP 0 525 861; EP 0 405 846)，假单胞菌属(Pseudomonas)(EP 0 482 844; EP 0 525 861; EP 0 475 652; EP 0 663 445)，无色杆菌属

(Achromobacter)(EP 0 525 861)，粪产碱菌(Alcaligenes faecalis)(EP 0 638 649)，不动杆菌属(Acinetobacter)(EP 0 469 919)，节杆菌属(Arthrobacter)(EP 0 283 218)，大肠埃希氏菌(Escherichia coli)(US 3,945,888)，嗜柠檬酸克吕沃尔氏菌(Kluyvera citrophila)，雷氏普罗威登斯菌(Proteus rettgeri)(US 3,915,798)等。二羧化酰基转移酶优选得自假单胞菌属SE83或SY-77。该酰基转移酶任选可以是突变形式(如 WO 91/16435、WO 97/20053、WO 97/40175 中公开的那些)从而增大或改变对底物的亲和性。使本发明的N-酰化头孢菌素化合物脱酰的另一方法是通过将底物与能生产酰基转移酶的微生物接触(如美国专利 No. 5,677,141 中公开的那样)。

可应用本领域熟知的技术将酰基转移酶固定(US 3,930,949)在膜(EP 0 243 404)或自由流动的载体(例如基于戊二醛的载体)或氮杂内酯聚合物(EP 0 730 035)上。非固定化酰基转移酶也是预期的，应用膜分离反应混合物(滞留物)与产物(渗透液)，例如美国专利No..5,521,068 中公开的那样。该方法可以是分批的或(半)连续的，这是完全熟知的并且对本发明来说不是关键的。酶促脱酰反应通常在搅拌釜式反应器(具有或没有优选为惰性的筛板，从而容易分离固定化酶与反应产物)中进行。在反应过程中通常调节 pH 以补偿由于(二羧基)侧链被任意类别的碱(例如铵、苛性碱、碳酸盐、碳酸氢盐)除去引起的 pH 变化。可在反应器中和/或在反应器上方的循环回路(circulating loop)中调节 pH。还可调节其它参数(例如温度、脱酰产物或侧链浓度等)，考虑到这些参数对反应速率和/或平衡的影响。

可在脱酰之前和/或脱酰过程中添加另外的稳定剂，例如亚硫酸根($S_2O_5^{2-}$、HSO_3^-、SO_3^{2-})、EDTA、二硫苏糖醇(dithiotreitol)(DTT)。

通常，接着应用任意合适的步骤组合回收通式(I)的脱酰头孢菌素化合物。任选可采取浓缩步骤，通过例如真空蒸发、反渗透、超微过滤或者结晶前的 narofiltration。任选可添加水混溶性溶剂。任选地，在结晶前可通过用活性炭或吸附树脂处理而纯化溶液。任选地，在结晶前可除去侧链，其特征在于，酸化水相，将侧链萃取到萃取有机溶

剂并分离这两相。萃取有机溶剂可选自乙酸戊酯、乙酸丁酯、乙酸乙酯、甲基异丁基酮、环己酮、异丁醇、正丁醇等。

可用数种方法从形成的水相结晶产品。最优选的操作方法是：中和水溶液，接着应用一种或多种已知的酸(例如 H_2SO_4、HCl、HNO_3 或其组合)分 1~6 步降低 pH 至 pH 3~5。这优选应用 1~6 个互连的一组连续操作的结晶器(按顺序)以连续方式进行。也可采取分批结晶、半连续结晶或协调结晶。可以按与上述相同的方法直接进行结晶而不需首先中和。按本发明的一个实施方案，发现了可添加水混溶性溶剂(例如甲醇、乙醇、异丙醇、正丁醇、丙酮等)而改善式(II)的头孢菌素的质量。任选地，在结晶前可通过用活性炭或用吸附树脂处理而改善式(II)化合物的质量。

已发现了，任选在用吸附树脂、活性炭和/或乙醇和/或乙酸酯处理后，可通过重结晶进一步改善式(II)的头孢菌素的质量。该处理的特征在于，在 0.5~10.0 范围内(优选在 7.5~8.5 之间)的 pH 下溶解式(II)的头孢菌素，再使产物结晶。可以按数种方式使产物结晶。最优选的操作方法是：应用一种或多种已知的酸(例如 H_2SO_4、HCl、HNO_3 或其组合)分 1~6 步降低 pH 至 pH 3~5。这可应用 1~6 个互连的一组连续操作的结晶器(按顺序)以连续方式进行。也可采取分批结晶、半连续结晶或协调结晶。按本发明的一个实施方案，发现了可添加水混溶性溶剂(例如甲醇、乙醇、(异)丙醇、丙酮、异丁醇和正丁醇)而改善式(II)的头孢菌素的质量。

还发现了，可通过用吸附剂处理转化液和/或溶解的式(II)头孢菌素的溶液而改善式(II)的头孢菌素的质量。吸附剂包括：活性炭，例如 Norit Ultra SX；或者吸附树脂，例如苯乙烯-二乙烯基苯共聚物，诸如得自 Mitsubishi Kasei Corporation 的 Dianion HP 20(CAS No. 55353-13-4)、Dianion HP 21(CAS No. 92529-04-9)或 Dianion SP 207(CAS No. 98225-81-1)或者得自 Rohm and Haas 的 Amberlite XAD 1180(CAS No. 97396-56-0)、Amberlite XAD 1600(CAS No. 153796-66-8)或 Amberlite XAD 16(CAS No. 102419-63-8)或者得自 TosoHaas 的

Amberchrom CG 161(CAS No. 131688-63-6)；优选为 XAD 16，XAD 1600 或 HP20。

晶体是通过过滤或离心分离的，再在常规连续式干燥机或分批干燥机中干燥。可通过任意类别的磨(例如球磨、射流磨等)研磨晶体。

任选可在结晶过程中添加水混溶性溶剂。溶解后，可用活性炭或吸附树脂处理溶液。

该操作将比前述目前已知的方法给出更好的总产率和产品质量。

按本发明的另一方面，提供了一种从转化液或母液(结晶式(II)的化合物后获得的液体)除去和回收己二酸的方法。发现了，应用膜式过滤在低 pH(例如低于 pH 5、优选低于 pH 4、更优选低于 pH3，处于 pH3 或接近 pH 3)时可以有利地分离己二酸。按本发明优选的是这一实施方案：其中，通过反渗透进行过滤。

除了节省原料外，这样做的优点还在于这样处理的溶液结晶时的纯度和/或产率。

通过如下非限制性的实施例阐述了本发明。

实验

如 1993 年 3 月 18 日公开的国际专利申请 WO 93/05158 中描述的那样，通过对用得自带小棒链霉菌的扩展酶(去乙酸基头孢菌素 C 合成酶)转化的产黄青霉菌株发酵而获得了一种作为复杂混合物的发酵液，它包含己二酰-7-ADCA，尤其包含作为不希望有的污染物的 6-APA、己二酰-6-APA 和α-氨基-己二酰-7-头孢霉烷酸。

按 WO 93/05158(并入本文作参考)的实施例 1 中描述的那样培养所述转化的青霉属菌株。

发酵 5~7 天后，将发酵液用于回收实验。

还可通过配制下列化合物的水混合物而模拟该复杂混合物：6-氨基青霉烷酸、己二酰-6-氨基青霉烷酸、α-氨基己二酰-6-氨基青霉烷酸、己二酰-7-氨基去乙酸基头孢霉烷酸和α-氨基己二酰-7-头孢霉烷酸。

实施例 1

pH/热-处理

该实施例显示了 pH 处理(优选将 pH 处理与升温处理结合)对于从复杂混合物除去不希望有的β-内酰胺组分的优点。

将得自产黄青霉发酵的发酵液(见实验部分)过滤，所述发酵液包含己二酰-7-ADCA 和青霉烷酸和头孢霉烷酸这些污染物的复杂混合物。应用生产用水洗涤该浓缩液直至合并的滤液总体积约为初始发酵液体积的 2 倍。进行了下列实验:

A. 将部分滤液酸化到 pH=3.5；加热到 70℃，30 分钟后冷却到 40℃;

B. 将部分渗透液酸化到 pH=2.7;加热到 110℃,4 分钟后冷却到 25℃;

或者

C. 将部分渗透液酸化到 pH=3.0 并且未进一步处理。

将该预处理后的溶液进行如下处理而获得式(II)的化合物；7-ADCA。

吸附色谱法

在 Seitz K100 滤器上过滤三份溶液(A~C)，然后将 pH 为 3.0 的溶液泵送到填充了 1.6 升 XAD-1600 树脂的柱上;接着用 4.8 升水洗涤树脂，用 0.2 M 碳酸氢盐溶液洗脱。取出第一次洗出液级分(1.1 升)而弃去。收集第二次级分(3.2 升)并分析。通过用苛性钠和丙酮洗涤而纯化树脂，再次用酸化的水调节。

浓缩

在 20~30℃ 真空(5~10mm Hg)下浓缩洗出液直至获得每升 40 克己二酰-7-ADCA 的浓度。

酶促脱酰

接着，用酰基转移酶按下述方法处理该己二酰-7-ADCA。往 1 升洗出液中添加 1 克焦亚硫酸钠、20 mM EDTA 和 100 g 固定化酰基转移酶(包括假单胞菌属 SE83 二羧化酰基转移酶)。在 30°C 下将溶液搅拌两小时。用 4 N 氢氧化钠将 pH 保持在 8.5。应用玻璃烧结的滤器分离所述固定化酰基转移酶和液体。

7-ADCA 的结晶

在搅拌下、在 30°C 的温度下降低 pH 至 3.6 而使 7-氨基去乙酸基头孢霉烷酸(7-ADCA)沉淀；在 45 分钟内，用 6 N 硫酸使溶液的 pH 降低至 3.6。冷却到 20°C 后，在玻璃烧结的滤器上分离晶体，用水洗涤，在 35°C 下干燥。

溶解 7-ADCA 晶体

借助于氨溶解 7-ADCA。为此，将 15 克 7-ADCA 悬浮于 255 ml 水中。借助于 4 N 氢氧化铵在 7.5~8.5 的 pH 下溶解 7-ADCA。在玻璃烧结的滤器上过滤后，添加水而得 300 ml 溶液。

用吸附树脂处理

用吸附树脂处理了该溶液。在 45 分钟内，将该溶液泵送到 15 ml 的 XAD1600 上。接着，将 75 ml 水泵送到该树脂上而获得 375 ml 溶液。

重结晶

在搅拌下、在 30°C 的温度下降低 pH 至 3.6 而使 7-ADCA 沉淀；在 45 分钟内，用 6 N 硫酸使 pH 降低至 3.6。冷却到 20°C 后，在玻璃烧结的滤器上分离晶体，用水洗涤，在 35°C 下干燥。

从 6-APA 减少来看，这样生产的 7-ADCA 表现出良好的结果。(6-APA 比值是相对于 7-ADCA 求算的)。

表 1a. 实验 1A、1B 和 1C 的结果

实验	6-氨基青霉烷酸含量(ppm)
1A	<10
1B	<10
1C	950

显然，所述 pH/温度处理减小了污染己二酰-7-ADCA 制剂的 6-氨基青霉烷酸的含量。

就恒定减少 10^{-6} 的 6-氨基青霉烷酸而测定了处理的 pH、温度和时间之间的相互关系(表 1b)。

表 1b

6-APA 减少	pH	温度 (C)	时间 (s)	时间 (min)	时间 (h)
10^{-6}	3	25	35050	584	9.74
10^{-6}	3	50	3057	50.9	0.85
10^{-6}	3	75	378	6.3	0.11
10^{-6}	3	100	62	1.0	0.02
10^{-6}	4	25	148857	2481	41.35
10^{-6}	4	50	12982	216.4	3.61
10^{-6}	4	75	1607	26.8	0.45
10^{-6}	4	100	263	4.4	0.07

实施例 2

吸附色谱法

该实施例显示了：(a)当应用吸附色谱法时，柱的载荷度(2A~2D)、(b)在洗脱前用不同量的水洗涤柱 (2E~2G)、(c)物料的 pH 对己二酰

-7-ADCA 的纯化的效果(2H~2J)。以模拟移动床方式进行吸附色谱处理的该实施方案作为"实验 2K"给出。

按实施例 1A 中描述的那样预处理发酵液。接着，通过吸附色谱法纯化己二酰-7-氨基－去乙酸基头孢霉烷酸：将溶液泵送到装填了 1.6 升 XAD-1600 树脂的柱上，用不同量的水洗涤(2A~2D 和 2H~2K：4.8 升；2E~2F：见表 2b)，用 0.2 M 碳酸氢盐溶液洗脱。取出第一次洗出液级分(1.1 升)后弃去。收集第二次级分(3.2 升)并分析。通过用苛性钠和丙酮洗涤而纯化树脂，再次用酸化的水调节。应用了数个处理条件的变量(见表 2)。

减少量按这样计算：(化合物-i $_{物料}$/化合物-1 $_{物料}$)/(化合物-i $_{洗出液}$/化合物-1 $_{洗出液}$)。

表 2a. 实验 2 的结果

实验	物料			洗出液(g)			减少(-)		化合物 4 (ppm)
	化合物 1 (g)	化合物 2 (g)	化合物 3 (g)	化合物 1 (g)	化合物 2 (g)	化合物 3 (g)	化合物 2	化合物 3	
2A	34	3.3	9.3	30	3.1	5.9	1	1	
2B	80	5.9	18.5	70	0.5	0.09	10	173	<6
2C	127	10.5	32.8	76	0.3	0.07	24	301	19
2D	255	18.2	59.4	67	0.1	0.03	38	501	30

化合物 1：己二酰-7-ADCA

化合物 2：α-羟基己二酰-7-ADCA

化合物 3：α-氨基己二酰-7-ADCA

化合物 4：6-APA 相对于化合物 1 的含量

这些结果清楚地表明将柱过载对洗出液中化合物 2 和 3 的减少的正性效果。

表 2b. 实验 2 的结果

实验	物料			洗涤	洗出液			减少 (-)	
	化合物 1 (g)	化合物 2 (g)	化合物 3 (g)	(1)	化合物 1 (g)	化合物 2 (g)	化合物 3 (g)	化合物 2	化合物 3
2E	85	6.6	14.0	1.6	81	2.1	1.2	3	11
2F	74	6.4	12.6	4.8	71	1.1	0.13	6	94
2G	75	5.8	13.0	7.3	68	0.5	0.1	12	122

化合物 1: 己二酰-7-ADCA

化合物 2: α-羟基己二酰-7-ADCA

化合物 3: α-氨基己二酰-7-ADCA

实施例 2b 表明了在用碳酸氢钠洗脱前持续洗涤对减少不希望有的头孢菌素化合物的正性效果。

表 2c

实验	物料				洗出液			减少 (-)	
	化合物 1 (g)	化合物 2 (g)	化合物 3 (g)	pH (-)	化合物 1 (g)	化合物 2 (g)	化合物 3 (g)	化合物 2	化合物 3
2H	79	8.4	22.2	2.5	83	0.6	0.09	15	248
2I	80	5.9	18.5	2.9	70	0.5	0.09	10	183
2J	83	8.2	21.7	3.5	62	0.5	0.14	12	118

化合物 1: 己二酰-7-ADCA

化合物 2: α-羟基己二酰-7-ADCA

化合物 3: α-氨基己二酰-7-ADCA

如上实施例表明了在进行结晶时pH对减少不希望有的7-N酰化头

孢菌素化合物的效果。H_2SO_4被用作酸。

表 2d. 实验 2 的结果(在 SMB 系统中应用了 30 升树脂)

实验	物料				洗出液				减少(-)		
	化合物 1 (kg)	化合物 2 (kg)	化合物 3 (kg)	化合物 4 (kg)	化合物 1 (kg)	化合物 2 (kg)	化合物 3 (kg)	化合物 4 (kg)	化合物 2	化合物 3	化合物 4
2K	1.46	0.08	0.20	0.15	1.38	0.01	0.01	0.02	7	18	7

化合物 1: 己二酰-7-ADCA

化合物 2: α-羟基己二酰-7-ADCA

化合物 3: α-氨基己二酰-7-ADCA

化合物 4: 己二酸

该实施例阐释了按所谓的模拟移动床技术在千克规模上进行的吸附色谱法的应用。该技术可以轻易地进一步放大。

如实施例 1 中描述的那样，用酰基转移酶处理这样处理过的级分 2A~2K 而生产 7-ADCA。获得了优异的转化结果，如实施例 3 中所述。

实施例 3

酶促转化

该实施例阐述了酶促转化己二酰-7-ADCA 至 7-ADCA 的结果。己二酰-7-ADCA 是按实施例 2K 中公开的那样回收的(按实施例 1A 进行 pH-处理，通过过载和洗涤将吸附色谱处理最佳化)。按实施例 1 中描述的那样、在表 3 中所示 pH 下进行转化。实施例 A~E 代表不同批次。

表 3					
实验	底物 化合物 1 (mmol)	底物 化合物 2 (mmol)	pH (-)	产品物流 化合物 1 (mmol)	产品物流 化合物 2 (mmol)
A	69.7	2.2	8.5	1.1	68.6
B	144.2	2.4	8.5	5.9	143.3
C	181.4	2.3	8.5	13.5	174.5
D	113.1	1.7	8	5.4	108.7
E	113.4	3.1	9	1.2	112.2
化合物 1: 己二酰-7-ADCA 化合物 2: 7-ADCA					

当应用 pH/温度步骤预处理己二酰-7-ADCA 时，与未处理相比，转化率和产率都更优。应用色谱法进一步纯化导致纯度的进一步改善(表中未示出)。

实施例 4

粗结晶

将发酵液进行 pH/热-处理(实施例 1)，再通过如实施例 2 中描述的吸附色谱法富集己二酰-7-ADCA。接着，如实施例 1 中描述的那样进行转化。

用反渗透法浓缩转化液(脱酰后获得的溶液)而增大浓度。

取出一部分溶液，通过降低 pH 至 pH 3.6、4 或 5 使 7-ADCA 结晶(见表 4a)。

表 4a. 粗结晶

实验	溶液中的 化合物 1	pH (-)	分离、洗涤和干燥后的产品(g)	产品中的化合物 1 (%)
A	49.5	3.6	48.9	97.5
B	49.5	4	48.7	97.4
C	49.5	5	48.2	98
化合物 1: 7-ADCA				

在全部测试的 pH 下的结晶都令人满意。在如下实验中，pH 为 3.6。阐释了浓缩溶液的效果。

表 4b. 粗结晶

实验	溶液中的化合物 1 (g)	分离、洗涤和干燥后的产品(g)	产品中的化合物 1 (%)
D	15.5	14.8	94.3
E	36.1	35.7	95.3
F	49.5	48.9	97.5

化合物 1: 7-ADCA

显然，转化液中 7-ADCA 的浓度对结晶后的纯度和产率都有影响。

如下实施例阐释了不同吸附剂对产品质量(溶液中的颜色和透明度)的影响。

表 4c. 粗结晶

实验	溶液中的化合物 1 (g)	处理	产品中的化合物 1 (%)	在 425nm 处溶液中的颜色 (-)	在 HCl 中的透明度 (EBC)
G	25	HP20	97.2	0.16	3.6
H	25	HP20 (2 倍)	97.8	0.11	2.4
I	25	IRA67	97.2	0.18	6
J	25	IRA67+HP20	97.7	0.1	0.8
K	25	无	96.3	0.39	7.3

化合物 1: 7-ADCA

实施例 5

溶解的 7-氨基去乙酸基头孢霉烷酸的处理

该实施例显示了在结晶前用不同的吸附树脂处理 7-ADCA 溶液后

对 7-ADCA 的透明度和颜色的影响。

按实施例 2K 中公开的方法制备了含 7-ADCA 的溶液(应用的吸附色谱柱是 XAD-1600 树脂)。

表 5

实验	溶解的化合物 1 (g)	处理	在 425nm 处溶液中的颜色 (-)	在 HCl 中的透明度 (EBC)
A	40	--	0.18	4.2
B	40	XAD16	0.05	1.8
C	40	HP20	未测出	0.5
D	40	XAD1600	0.09	0.5
E	25		未测出	3.6
F	25	+1% EtOH	0.11	0.6
G	50	+3% EtOH	0.18	0.9
H	50	+2% 炭	0.04	未测出
I	50		0.17	1.4
J	50	+5% 炭	0.03	0.8

化合物 1: 7-氨基去乙酸基头孢霉烷酸

实施例 6

用正丁醇萃取回收己二酰-7-ADCA

按实施例 1 中描述的那样处理包含己二酰-7-ADCA 的发酵液。

酸化后，通过吸附色谱法纯化一部分己二酰-ADCA。将该溶液泵送到填充了 XAD-16 树脂的柱上，用水洗涤，用 0.2 M 乙酸盐溶液洗脱。取出含低含量的己二酰去乙酸基头孢霉烷酸的第一次洗出液级分后弃去。收集第二次级分。通过用苛性钠和丙酮洗涤而纯化树脂，再次用酸化的水调节。

这样纯化一部分己二酰-7-ADCA：萃取，接着洗涤萃取液，从有机相将N-取代头孢菌素反萃取到水相，再反萃取水相；萃取有机溶剂是正丁醇。

用固定化酰基转移酶处理己二酰-7-ADCA而生产7-氨基去乙酸基头孢霉烷酸(7-ADCA)。通过降低pH而分离一部分7-ADCA。借助于苛性钠溶解己二酰去乙酸基头孢霉烷酸。通过降低pH而分离7-氨基去乙酸基头孢霉烷酸。

酸化一部分7-氨基去乙酸基头孢霉烷酸水溶液，将所述侧链萃取到萃取有机溶剂中，再进行相分离；萃取有机溶剂是正丁醇。

最后，过滤晶体块，洗涤后干燥。

表6

实验	描述	产品中的化合物 1 (%)	在425nm处溶液中的颜色 (-)	在HCl中的透明度 (EBC)
A	萃取/萃取/结晶	98.3	0.13	2.4
B	色谱处理/萃取/结晶	98.9	0.09	1.5
C	色谱处理/结晶	97.6	0.12	-
D	色谱处理/结晶/重结晶	98.7	0.05	1.1

化合物1：7-氨基去乙酸基头孢霉烷酸

表中未给出采用单独萃取的结果，但纯度比采用色谱处理时（即使未重结晶）差得多。将色谱处理与重结晶组合或者将色谱处理与萃取组合产生最好的结果。

实施例7

从7-ADCA结晶母液回收己二酸

7-ADCA 结晶母液是按实施例 4 中描述的那样获得的。应用 HPLC 测定己二酸：Aminex HPX-87H 柱，300 mm×7.8 mm，填充了 9 um 阳离子凝胶(Biorad)，在65℃下用0.2 M H_2SO_4水溶液洗脱，应用 RI Waters 410 折光计测定。在下文给出的实施例中，最优化旨在纯度，而不是产率。

实施例 7A

采用酸化回收己二酸

在 20℃ 下，用 12 M H_2SO_4 水溶液将 7-ADCA 结晶母液(250 ml，13.6g/l 己二酸)的 pH 降到 0.7。在 0℃ 下 16 h 后，未能检测出结晶。应用 6M KOH 水溶液使 pH 升高到 3.4。通过过滤回收形成的晶体而给出 7.7g 物质(它是盐和己二酸的混合物，未对它进一步分析)。

实施例 7B

采取酸化和浓缩回收己二酸

在20℃下，用12 M H_2SO_4水溶液将7-ADCA结晶母液(500 ml，9.4g/l 己二酸)的 pH 降到 1.5，接着在 40℃ 减压下浓缩而给出一种粘性混合物，通过过滤分离该混合物，干燥后给出 6.9g 己二酸，纯度为 53%(产率为 78%)。

实施例 7C

应用 Nanomax 50 膜的反渗透作用回收己二酸

在 20℃ 下，用 6 M KOH 水溶液或 12 M H_2SO_4水溶液将 7-ADCA 结晶母液(500 ml，9.4~18.2g/l 己二酸，见表)的 pH 调节到表中提到的值。应用得自 Millipore 的 Nanomax 50 膜将形成的溶液进行反渗透。借助于氮气，施加 30 巴的压力而得到滤液和滞留物，其中，采用 HPLC 测定己二酸的量。在大多数情况下，应用一种处理程序，它包括：在

减压下浓缩直至结晶，接着过滤产物并干燥。

	表 7						
pH	己二酸(g/l)			保留	渗透液体积	处理后的产量	处理后的纯度
	起始	渗透液	滞留物	(%)	(ml)	(g)	(%)
1.5	13.6	11.0	14.8	26	400	4.4	96
2.0	18.2	13.1	20.8	37	260	2.4	99
3.0	9.4	6.7	8.7	23	360	1.9	97
7.2	13.6	5.3	31.9	83	400	未处理	未处理

实施例 7D

应用 DK U19F 膜的反渗透作用在 pH 2.0 下回收己二酸

用 2.9 l 的 12 M H_2SO_4 水溶液将 7-ADCA 结晶母液(100 l, 含 25.0g/l 己二酸)的 pH 降到 2.0。用 57 l 水、应用 DK U19F 膜在 2.5 m^2 得自 Hydro Air Research 的膜式过滤装置 P2-B200 中将形成的溶液进行反渗透。在 30 巴的压力下, 达到 8.8 l/m^2/h 的平均流量, 给出表中归纳的结果。

表 8				
组分	浓度(g/l)			保留
	起始	渗透液	滞留物	(%)
己二酸	25.0	11.6	17.8	35
7-ADCA	0.72	<0.01	0.72	>99
己二酰-7-ADCA	1.51	<0.03	1.54	>98