

- 1 -

PREPARATION OF β -LACTAM ANTIBIOTICS IN
THE PRESENCE OF UREA OR AMIDE

5

The present invention relates to an improved process for the preparation of β -lactam antibiotic compounds in the presence of urea or derivatives thereof and/or amide or derivatives thereof.

10

It is commonly accepted that the most economical approach for the isolation of compounds such as β -lactam antibiotics or their intermediates from their solutions comprising the same is crystallization.

15

Various processes have been proposed for the preparation of the semi-synthetic β -lactam antibiotic compounds such as penicillins and cephalosporins. These proposals generally involve the acylation of 6-aminopenicillanic acid (6-APA) or 7-aminocephalosporanic acid (7-ACA) or a protective derivative thereof using, for example, an acyl halide serving to introduce the desired acyl group. For example in European patent application EP 0011513, a process has been described for the preparation of zwitterionic antibiotics, for instance, ampicillin, amoxicillin or cephalixin through acylation of silylated 6-aminopenicillanic acid or 7-aminocephalosporanic acid with a phenylglycyl halide or p-hydroxyphenylglycyl halide in the presence of N-alkylpyrrolidone as an acid acceptor. Similarly United States patent US 4,248,780 for example, describes the preparation of zwitterionic antibiotic ampicillin through acylation of silylated 6-aminopenicillanic acid

25

30

- 2 -

with a phenylglycylchloride hydrochloride by using urea as a base.

Furthermore, a number of patent applications and patents disclose preparation method of α -
5 aminoacylpenicillanic acid derivatives by acylating 6-APA with mixed anhydrides derived from modified Dane salts of D-2-amino-(p-hydroxyphenyl)-acetic acid, such as described in German patent Applications Ser. No. 1,302,847, No. 2,020,133 and No. 2,065,879 and British
10 Patents No. 1,327,270 and No. 1,347,979.

United States patent US 4,358,588 describes an improved process for the preparation of D- α -amino-p-hydroxyphenyl-acetamide-penicillanic acid and cephalosporanic acid derivatives by reacting an
15 appropriate mixed anhydride of a Dane salt with a protected derivative, for example 6-APA or 7-ACA in the presence of a tertiary amine. This type of acylation is referred to as Dane salt coupling reaction.

Thereafter, the β -lactam antibiotic
20 compounds are isolated from an acidic aqueous solution, obtained by acidic hydrolysis of the reaction mixture after the Dane salt coupling reaction, by adding a base and followed by filtration.

A major drawback of the processes for the
25 preparation of β -lactam antibiotic compounds is the crystallization of unwanted by-products, among which the hydrochloric acid salts of the β -lactam antibiotic compounds are the most notorious one. These unwanted crystallization of by-products can occur at any stage of
30 the hydrolysis or crystallization procedure.

The formation of acid salts of the β -lactam

- 3 -

antibiotic compounds is strongly dependent upon temperature, pH and time during the acidic hydrolysis of the Dane salt coupling reaction. Lower temperatures, a lower pH and prolonged time during the hydrolysis of the Dane salt coupling reaction result in the production of an increased amount of acid salt of the corresponding β -lactam antibiotic compound. At higher temperatures, however, the formation of the corresponding acid salt of the β -lactam during hydrolysis of the Dane salt coupling reaction is reduced but on the other hand the cleavage of the β -lactam is enhanced. Also, the presence of seeding crystals corresponding to acid salt of the β -lactam compound, produced during the hydrolysis of the Dane salt coupling reaction, facilitate the formation of the acid salts of the β -lactam antibiotic compounds. Especially the formation of the seeding material during the hydrolysis of Dane coupling reaction is enhanced on an industrial scale process compared to a laboratory scale process. Thus, the crystalline products resulting from the Dane salt coupling reaction often contain unacceptable levels of impurities.

It was found surprisingly that β -lactam antibiotic compounds free of acid salts of the corresponding β -lactams can be prepared under addition of urea or derivatives thereof and/or amide or derivatives thereof after the Dane salt coupling reaction at low temperature. Urea or derivatives thereof and/or amide or derivatives thereof can also be added during the purification of crude β -lactam antibiotic compounds during the crystallization phase. Besides the lower content of the hydrochloric acid salt of the β -

lactam produced, these processes also result in a higher yield of the end product. This method has nowhere been described or suggested for β -lactam compounds until now.

5 Summary of the invention

The present invention provides an improved process for the preparation of β -lactam antibiotic compounds in the presence of urea or derivatives thereof and/or amide or derivatives thereof. The β -lactam

10 compounds are crystallized by the addition of a base to an acidic solution of a β -lactam compound obtained by the addition of an acid to a solution or suspension of a β -lactam compound obtained by

(a) an acylation reaction of 6-amino-penicillanic acid,
15 7-amino-cephalosporanic acid, 7-amino-3'-desacetoxycephalosporanic acid or 3-chloro-7-aminodesacetoxydesmethylcephalosporanic acid or derivatives thereof with a mixed-anhydride of the Dane salt of any one of the compounds comprising D-phenyl-
20 glycine, D-p-hydroxyphenyl-glycine, D-2(1,4-cyclohexadien-1-yl)glycine or (2-aminothiazol-4-yl)-(2-methoxy-imino-acetic acid) in one or more organic solvents, or

(b) dissolving or suspending a crude β -lactam compound
25 in a solution comprising water and/or one or more organic solvents, characterised by the addition, to the solution or suspension of a β -lactam compound, of urea or derivatives thereof and/or amide or derivatives thereof.

30 Before starting the crystallization process through the addition of a base, the pH of the solution

- 5 -

or suspension of a β -lactam compound is maintained at the desired pH by the addition of an acid. Normally, the pH is maintained between 0.1 and 3.5 and preferably between 0.3 and 2.5 depending on the type of β -lactam compound to be produced. When the solution of the β -lactam compound is obtained by (a), the acid that is added may assist in the acid hydrolysis of the reaction mixture from the acylation reaction.

The temperature at which the acid is added is preferably between -50°C and 10°C , preferably between -5°C and 5°C and most preferably between -5°C and 0°C . The acid may be any acid, preferably an inorganic acid, for example hydrochloric acid, nitric acid or sulphuric acid. Preferably, the acid is hydrochloric acid.

The temperature during the crystallization may vary. Suitably, the crystallization temperature is between -10°C and 50°C , preferably between -5°C and 35°C . It will be clear for the skilled in the art that the preferred conditions will depend on the β -lactam compound to be prepared.

The β -lactam antibiotic compounds can be isolated in a conventional manner, by adjusting the pH to the isoelectric point of the corresponding β -lactam compounds.

The β -lactam compounds are for instance penicillins and cephalosporins. Examples of penicillins are amoxicillin, ampicillin and epicillin. Examples of cephalosporins are cefaclor, cefadroxil, cefetamet, cefotaxim, cephalixin, cephaloglycin, cephradine and cefroxadine.

Detailed description of the invention

The process for the preparation of crystalline β -lactam antibiotic compounds according to the present invention comprises the addition of a base

5 to an acidic solution of a β -lactam compound obtained by the addition of an acid to a solution or suspension of a β -lactam compound obtained by

(a) an acylation reaction of 6-amino-penicillanic acid (6-APA), 7-amino-cephalosporanic acid (7-ACA), 7-amino-3'-desacetoxycephalosporanic acid (7-ADCA) or 3-chloro-7-aminodesacetoxydesmethylcephalosporanic acid (7-ACCA) or derivatives thereof with a mixed-anhydride of the

10 Dane salt of any one of the compounds comprising D-phenyl-glycine, D-p-hydroxyphenyl-glycine, D-2(1,4-cyclohexadien-1-yl)glycine or (2-aminothiazol-4-yl)-(2-methoxy-imino-acetic acid) in one or more organic solvents, or

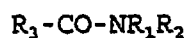
15 (b) dissolving or suspending a crude β -lactam compound in a solution comprising water and/or one or more organic solvents,

20 characterised by the addition, to the solution or suspension of a β -lactam compound, of urea or derivatives thereof and/or amide or derivatives thereof with the formula (A) and (B) respectively,

25



(A)



(B)

wherein R_1 , R_2 , R_3 and R_4 are each independently chosen

30 from the group consisting of hydrogen, lower alkyl and allyl, or

- 7 -

R_1 and R_3 form $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{CO}-$ and $-\text{CH}=\text{CH}-\text{CO}-$ resulting together with $-\text{N}-\text{CO}-\text{N}-$ in a five or six membered cyclic ring, $(\text{C}_1 - \text{C}_6)$ alkanol esters of $(\text{C}_1 - \text{C}_6)$ carboxylic acid.

- 5 Preferably, R_1 , R_2 , R_3 and R_4 in formula (A) are each chosen from hydrogen or methyl, and more preferably R_1 , R_2 , R_3 and R_4 are each hydrogen.

 The amount of urea or derivatives thereof and/or amide or derivatives thereof present during the crystallization process may vary, and preferably is
10 between 0.001 mol/mol β -lactam and 7.5 mol/mol β -lactam, more preferably between 0.1 mol/mol β -lactam and 5.25 mol/mol β -lactam.

- By derivatives of 6-APA, 7-ACA, 7-ADCA and
15 7-ACCA have been meant salts and esters thereof.

 Preferably, a small amount of a $(\text{C}_1 - \text{C}_6)$ alkanol, specifically methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, or a ketone, specifically acetone, methylethylketone,
20 methylisobutylketone or an ester, specifically methyl acetate, ethyl acetate, isopropyl acetate, butyl acetate or an ester of $(\text{C}_1 - \text{C}_6)$ carboxylates with a $(\text{C}_1 - \text{C}_6)$ alkanol or a mixture thereof is added as a co-solvent to the solution or suspension of a β -lactam compound.

- 25 The Dane salt coupling reaction is carried out in a dry water-insoluble organic solvent, for instance dichloromethane. When the solution or suspension of a β -lactam compound is obtained by (a), the co-solvent is preferably added after the Dane salt
30 coupling reaction.

 The concentration of the co-solvent is

between 0.1% and 60%, preferably between 25% and 60% depending on the type of the β -lactam compound to be prepared.

The pH of the acidic solution is maintained
5 between 0.1 and 3.5, preferably between 0.3 and 2.5, by using an acid. The temperature of the solution during the addition of the acid may vary, and is usually between -50°C and 10°C, preferably between -5°C and 5°C and more preferably between -5°C and 0°C. At higher
10 temperatures, the cleavage of the sensitive β -lactam ring of the antibiotic compound is enhanced.

The acid is an inorganic acid, for example hydrochloric acid, sulphuric acid, nitric acid, phosphoric acid or an organic acid, for example formic
15 acid and suitable acetic acid or derivatives thereof. Preferably, hydrochloric acid is used.

The β -lactam antibiotic compound is isolated in conventional manner, by adjusting the pH between 2.0 and 7.0, preferably to the isoelectric point of the said
20 β -lactam antibiotic compound by addition of a base.

Furthermore, seeding material is optionally added to the crystallization solution before the addition of the base.

The base is an inorganic base, preferably
25 sodium hydroxide, potassium hydroxide, ammonium hydroxide or an organic base, preferably an amine.

Accordingly, after filtration and drying crystalline products substantially free of impurities such as the acid salt of the same, β -lactam compounds
30 are obtained whereas known procedures without application of urea or derivatives thereof and/or amide

- 9 -

or derivatives thereof give higher levels of impurity, especially when the crystallization is performed at a lower temperature.

The process of the present invention is particularly suitable for the preparation of β -lactam antibiotics in the form of a zwitterionic amino acid. Examples of said antibiotics are amoxicillin, ampicillin, epicillin, cefaclor, cefadroxil, cefetamet, cefotaxim, cephalixin, cephaloglycin, cephradine and cefroxadine.

According to a further aspect of the present invention the β -lactam compound is advantageously crystallized by simultaneous addition of the β -lactam solution and a base to a crystallization vessel as described in non-published European patent application No. 97203484.7. This crystallization process comprises simultaneous addition of the acidic solution of a β -lactam compound containing urea or derivatives thereof and/or amide or derivatives thereof and a titrant, namely the base, to a crystallization vessel.

The process of crystallization of antibiotic compounds, according to the present invention, has a major advantage over the existing methods because better concordance results are possible. The concordance value related to the difference between titration with a strong acid and titration with a strong base has been defined according to the United States Pharmacopoeia, twenty-first revision, dated January 1, 1985. A small concordance value stands for a constant quality product. Ampicillin trihydrate, for instance isolated from crystallization of a solution containing ampicillin

- 10 -

hydrochloride has a concordance value of less than 1 mol %, while crystallization through a prior art procedure of a number of experiments has given a concordance value between 4 and 8 mol %. The β -lactam antibiotic compounds with a small concordance value, i.e. comprising lower amounts of acid salt of the corresponding β -lactam antibiotics, are suitable for pharmaceutical formulation purposes.

Furthermore, the bad effect of storage and temperature (higher than room temperature) on the stability of the β -lactam antibiotic compound is enhanced due to the presence of very small or negligible amounts of the corresponding acid salt of β -lactam antibiotic compound. These acid salts of the β -lactam antibiotic are usually hygroscopic in nature. Thus, the product obtained by the process according to the invention is new.

The invention will now be described with reference to the following Example, which are not to be constructed as being limiting on the invention, and are provided purely for illustrative purposes.

Example

STEP A: Preparation of methoxycarbonyl D- α -(1-carbomethoxypropen-2-yl)amino-p-hydroxyphenyl-acetate. 85 ml of N,N-dimethylacetamide, 116.4 g of potassium D- α -[1-(carbomethoxypropen-2-yl)-amino]-p-hydroxyphenylacetate and 0.3 ml pyridine were added to 267 ml of dichloromethane and the resulting suspension was cooled to -40°C. Subsequently 46.3 g of pivaloylchloride was added to this solution. While

- 11 -

maintaining a temperature of -30°C the mixture was stirred for 90 minutes and then cooled to -50°C .

STEP B: 63.8 g of triethylamine was added to a suspension of 80.0 g of 6-aminopenicillanic acid in 533
5 ml of dichloromethane at 20°C and the mixture was stirred for 90 minutes. The mixture should contain enough water for obtaining a clear solution. At 5°C , 78 g of 2-ethylhexanoic acid was added and the solution was further cooled to -50°C .

10 STEP C: Preparation of amoxicilline trihydrate.

The reaction mixture obtained in STEP A was added in about 3 minutes into the solution obtained in STEP B with vigorous stirring. In 3 hours the temperature was gradually increased from -40°C / -35°C
15 to -10°C / -5°C . Subsequently, a solution of urea (100 g in 800 ml of demineralised water) was added.

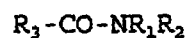
While maintaining the mixture at between -5°C and 0°C concentrated hydrochloric acid was added and hydrolysis was continued for 30 minutes at a pH
20 between 1.4 and 1.5. The organic phase was separated and the product was crystallised from the aqueous layer by addition of approximately 45 ml of 8 N sodium hydroxide solution until pH between 4.8 and 5.0 at a temperature of between 2°C and 5°C . The suspension was kept over
25 night under these conditions, filtered, washed with 100 ml of chilled, demineralised water and 300 ml of chilled acetone. The cake was dried at 35°C till a constant weight was reached to obtain about 143 g of amoxicillin trihydrate with a purity of 86.1% as amoxicillin
30 anhydrous (HPLC).

C L A I M S

1. A process to prepare a crystalline β -lactam compound by the addition of a base to an acidic solution of a β -lactam compound obtained by the addition of an acid to a solution or suspension of a β -lactam compound obtained by
- 5 (a) an acylation reaction of 6-amino-penicillanic acid, 7-amino-cephalosporanic acid, 7-amino-3'-desacetoxycephalosporanic acid or 3-chloro-7-aminodesacetoxydesmethylcephalosporanic acid or derivatives thereof with a mixed-anhydride of the Dane salt of any one of the compound comprising D-phenyl-glycine, D-p-hydroxyphenyl-glycine, D-2(1,4-cyclohexadien-1-yl)glycine or (2-aminothiazol-4-yl)-(2-methoxy-imino-acetic acid)
- 10 in one or more organic solvents, or
- (b) dissolving or suspending a crude β -lactam compound in a solution comprising water and/or one or more organic solvents, characterised by the addition, to the solution or suspension of a β -lactam compound, of urea or derivatives thereof and/or amide or derivatives thereof with the formula (A) and (B) respectively,
- 15
- 20
- 25



(A)



(B)



- wherein R_1 , R_2 , R_3 and R_4 are each independently chosen from the group consisting of hydrogen, lower alkyl and allyl, or
- 30

- 13 -

R_1 and R_3 form $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{CO}-$ and $-\text{CH}=\text{CH}-\text{CO}-$ resulting together with $-\text{N}-\text{CO}-\text{N}-$ in a five or six membered cyclic ring, $(\text{C}_1 - \text{C}_6)$ alkanol esters of $(\text{C}_1 - \text{C}_6)$ carboxylic acid.

- 5 2. A process according to claim 1 wherein in the formula (A), R^1 , R^2 , R^3 and R^4 are each chosen from hydrogen or methyl.
3. A process according to any one of the claims 1-2, wherein when using the solution of a β -lactam
- 10 compound obtained by (a), the temperature at which the acid is added is maintained between -50°C and 10°C .
4. A process according to any one of the claims 1-3, wherein the amount of urea or derivatives thereof
- 15 and/or amide or derivatives thereof is between $0.001 \text{ mol/mol } \beta\text{-lactam}$ and $7.5 \text{ mol/mol } \beta\text{-lactam}$, preferably between $0.1 \text{ mol/mol } \beta\text{-lactam}$ and $5.25 \text{ mol/mol } \beta\text{-lactam}$.
5. A process according to any one of the claims 1-4,
- 20 wherein the solution of the β -lactam antibiotic compound comprises one or more co-solvents chosen from the group of $(\text{C}_1 - \text{C}_6)$ alkanol or ketone or ester of a $(\text{C}_1 - \text{C}_6)$ carboxylate with a $(\text{C}_1 - \text{C}_6)$ alkanol.
- 25 6. A process according to claim 5, wherein when using the solution of a β -lactam compound obtained by (a), the co-solvent or solvents are added after the coupling reaction.
7. A process according to claim 5 or 6, wherein the
- 30 concentration of $(\text{C}_1 - \text{C}_6)$ alkanol or ketone or ester is between 0.1% and 60%.



- 14 -

8. A process according to any one of the claims 1-7,
wherein the pH of the acidic solution before the
addition of a base, has been maintained between
0.1 and 3.5, preferably between 0.3 and 2.5, by
5 using an acid.
9. A process according to claim 8, wherein the acid
is hydrochloric acid.
10. A process according to any one of the claims 1-9,
wherein during crystallization, the pH is
10 maintained between 2.0 and 7.0, preferably close
to the isoelectric point of said β -lactam compound
by addition of a base.
11. A process according to any one of the claims 1-10
wherein the base is an inorganic base, preferably
15 sodium hydroxide, potassium hydroxide or ammonium
hydroxide.
12. A process according to any one of the any one of
the claims 1-11 wherein seeding crystals are added
before the addition of the base.
- 20 13. A process according to any one of the claims 1-12
wherein the temperature during the crystallization
is maintained between -10°C and 50°C , preferably
between -5°C and 35°C .
14. A process according to any one of the claims 1-13
25 wherein the β -lactam compounds are penicillins and
cephalosporins, preferably amoxicillin,
ampicillin, epicillin, cefaclor, cefadroxil,
cefetamet, cefotaxim, cephalaxine, cephaloglycin,
cephradine and cefroxadine.



- 15 -

15. A process according to any one of the preceding claims, wherein the acidic solution of a β -lactam compound and a base are simultaneously added to a crystallization vessel.
- 5 16. A β -lactam compound obtainable by a method according to any of the preceding claims.

