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CHLOROCEFADROXIL MONOHYDRATE

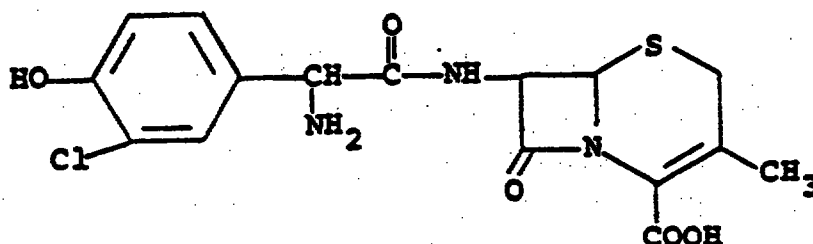
BACKGROUND OF THE INVENTION

1. Field of the Invention

The crystalline cephalosporin monohydrate of the present invention possesses in general the usual attributes of that family of antibacterial agents and is particularly useful in pharmaceutical formulations for treatment of bacterial infections by oral administration.

2. Description of the Prior Art

The cephalosporin compound 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid is disclosed and claimed in U.S. Patent 3,489,751. This compound, generally referred to hereinafter as chlorocefadroxil, has the structural formula



Chlorocefadroxil is active as a broad spectrum antibiotic effective in controlling diseases caused by a wide variety of Gram-positive and Gram-negative microorganisms. It is of particular interest as an oral cephalosporin antibiotic.

U.S. Patent 3,489,751 discloses the preparation of chlorocefadroxil by acylation of 7-aminodesacetoxycephalosporanic acid (7-ADCA) with an amino-protected derivative of D(-)- α -amino- α -(3-chloro-4-hydroxyphenyl)acetic acid. Of the various amino-protecting acylating agents disclosed, the highest yields were obtained with D(-)- α -(3-chloro-4-hydroxyphenyl)- α -(t-butoxycarbonylamino)acetic acid via the so-called t-BOC method. The yields in this process, however, were not as high as are desired

for commercial production and the reagent used in the t-BOC process is very expensive.

U.S. Patent 3,985,741 discloses preparation of chlorocefadroxil by acylation of 7-ADCA with the mixed anhydride of D(-)- α -(3-chloro-4-hydroxyphenyl)glycine when the latter's α -amino group has been blocked with a β -keto compound such as methyl acetoacetate. This process, while having certain definite advantages over the t-BOC procedure, is still not as efficient as is desired for a commercially feasible production process. Also disclosed in this patent is a crystalline dimethylformamide solvate of chlorocefadroxil containing 1.5 moles dimethylformamide per mole of chlorocefadroxil. The dimethylformamide solvate is slurried in boiling methanol until the solvate dissociates and the resulting suspension then cooled to provide a purified form of chlorocefadroxil. There is no indication, however, that the chlorocefadroxil produced according to the method of this patent is in the form of a crystalline hydrate.

U.S. Patent 3,781,282 discloses in a teaching example (Example 7) the preparation of chlorocefadroxil by dissolution of a chlorocefadroxil-DMF solvate in acidified water followed by neutralization with triethylamine. There is no indication from this reference that the chlorocefadroxil product would be in the form of a crystalline monohydrate or indeed that it would even be in a crystalline form.

U.S. Patent 4,160,863 discloses a crystalline monohydrate of 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid (also called cefadroxil) which is prepared in a similar manner to the chlorocefadroxil monohydrate disclosed and claimed in the present application.

In view of the many important advantages of chlorocefadroxil, it would be desirable to have a commercially useful process for preparing this antibiotic in higher yields and with lower production costs than afforded by the prior art processes. Additionally, it would be desirable to provide chlorocefadroxil

in a stable crystalline form such as a crystalline hydrate which would enable the antibiotic to be prepared into suitable pharmaceutical formulations for antibacterial use, e.g. aqueous suspensions.

SUMMARY OF THE INVENTION

The present invention provides a novel crystalline monohydrate of chlorocefadroxil and processes for preparing said monohydrate. Also provided is an improved acylation process for preparing chlorocefadroxil which results in excellent product yields and lower production costs when compared with prior art processes.

DESCRIPTION OF THE DRAWINGS

The accompanying drawing, FIG. 1, illustrates the characteristic infrared absorption spectrum of chlorocefadroxil monohydrate (KBr pellet) as obtained by the procedures described herein.

DETAILED DESCRIPTION OF THE INVENTION

According to one aspect, the present invention provides an improved process for preparing chlorocefadroxil, or a pharmaceutically acceptable salt thereof, which process comprises

- (a) silylating 7-aminodesacetoxycephalosporanic acid in an inert substantially anhydrous aprotic solvent;
- (b) acylating the so-produced silylated 7-aminodesacetoxycephalosporanic acid with D(-)- α -amino- α -(3-chloro-4-hydroxyphenyl)acetyl chloride hydrochloride in an inert substantially anhydrous aprotic solvent in the presence of an acid acceptor;

(c) cleaving any silyl groups of the acylation product by hydrolysis or alcoholysis; and

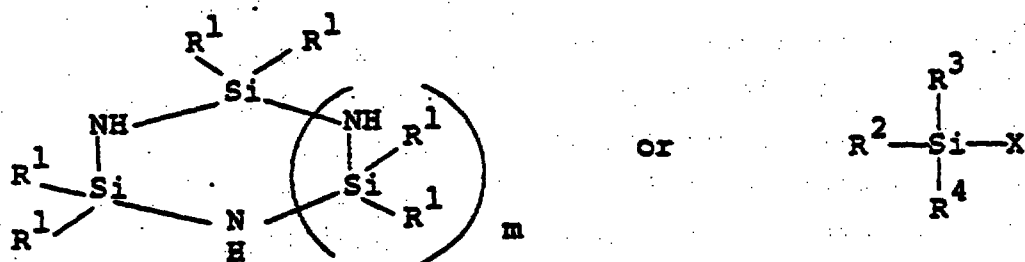
(d) recovering the desired cephalosporanic acid, or a pharmaceutically acceptable salt thereof.

The pharmaceutically acceptable salts referred to above include, for example, (1) non-toxic pharmaceutically acceptable salts of the acidic carboxylic acid group such as the sodium, potassium, calcium, aluminium and ammonium salts and nontoxic substituted ammonium salts with amines such as tri(lower)alkylamines, procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bisdehydroabietyleneethylenediamine, N-(lower)alkylpiperidines such as N-ethylpiperidine and other amines which have been used to form salts of benzyl-penicillin; and (2) nontoxic pharmaceutically acceptable acid addition salts (i.e., salts of the basic nitrogen) such as (a) the mineral acid addition salts such as hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate, sulfonate, phosphate, etc. and (b) the organic acid addition salts such as the maleate, acetate, citrate, tartrate, oxalate, succinate, benzoate, fumarate, malate, mandelate, ascorbate, β -naphthalene sulfonate and p-toluenesulfonate. As used herein the term "(lower)alkyl" is defined as including straight and branched chain saturated hydrocarbon radicals having from 1 to 10 carbons inclusive.

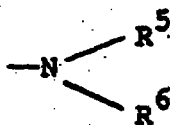
In the above process 7-ADCA is first silylated by reaction with a silylating agent in an inert substantially anhydrous aprotic solvent.

Suitable solvents for the silylation reaction include such inert substantially anhydrous organic solvents as methylene chloride, tetrahydrofuran, chloroform, tetrachloroethane, nitromethane, benzene and diethyl ether. A preferred solvent is methylene chloride.

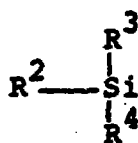
Silylating agents useful in the above process are known in the art [see, for example, U.S. Patents Numbers 3,654,266, 3,575,970, 3,499,909, 3,349,622, 3,595,855, 3,249,622 and U.K. Patent Numbers 1,339,605, 959,853 and 1,008,468]. While any known silylating agent may be employed, it is preferred to use an agent selected from those of the formula



wherein R^2 , R^3 and R^4 are hydrogen, halogen, (lower)alkyl, halo(lower)alkyl, phenyl, benzyl, tolyl or dimethylaminophenyl, at least one of the said R^2 , R^3 and R^4 groups being other than halogen or hydrogen; R^1 is (lower)alkyl; m is an integer of 1 to 2 and X is halogen or



wherein R^5 is hydrogen or (lower)alkyl and R^6 is (lower)alkyl or



wherein R^2 , R^3 and R^4 are as defined above.

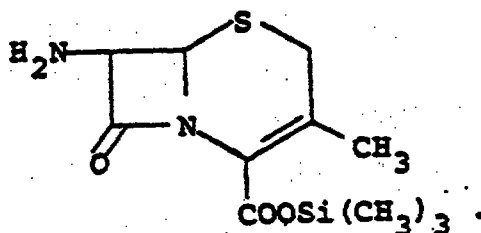
Examples of suitable silylating agents include trimethylchlorosilane, hexamethyldisilazane, triethylchlorosilane, methyltrichlorosilane, dimethyldichlorosilane, triethylbromosilane, tri-n-propylchlorosilane, bromomethyldimethylchlorosilane, tri-n-butylchlorosilane, methyldiethylchlorosilane, dimethylethylchlorosilane, phenyldimethylbromosilane,

benzylmethylethylchlorosilane, phenylethylmethylchlorosilane, triphenylchlorosilane, triphenylfluorosilane, tri-o-tolylchlorosilane, tri-p-dimethylaminophenylchlorosilane, N-ethyltriethylsilylamine, hexaethyldisilazane, triphenylsilylamine, tri-n-propylsilylamine, tetraethyldimethyldisilazane, tetramethyldiethyldisilazane, tetramethyldiphenyldisilazane, hexaphenyldisilazane and hexa-p-tolyldisilazane. Other suitable silylating agents are hexaalkylcyclotrisilazanes or octaalkylcyclotetrasilazanes and silylamides and silylureides such as trialkylsilylacetamide and a bis-trialkylsilylacetamide. The most preferred silylating agents are trimethylchlorosilane and hexamethyldisilazane.

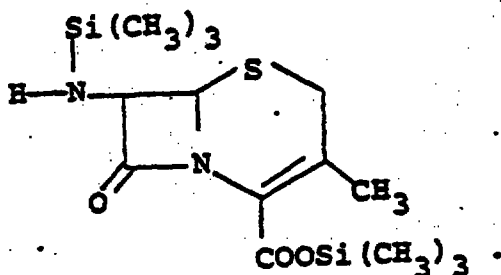
Where a silyl halide, e.g. trimethylchlorosilane, is employed as the silylating agent, the silylation step is carried out in an inert, substantially anhydrous, aprotic solvent in the presence of an acid (hydrogen halide) acceptor, preferably propylene oxide or a nitrogen base such as triethylamine, trimethylamine, dimethylaniline, quinoline, lutidine or pyridine. Preferred acid acceptors are propylene oxide, triethylamine or a mixture of triethylamine and dimethylaniline. Where a silazane, e.g. hexamethyldisilazane, is employed, the silylation step is conveniently effected by heating the silazane and 7-ADCA so that ammonia or amine derivatives formed as by-products of the reaction are distilled off.

In preparing silylated 7-ADCA in the above process, theoretically from one to two molar equivalents of silylating agent can be employed per mole of 7-ADCA to give mono- or disilylated 7-ADCA or mixtures thereof. Thus, when 7-ADCA is reacted with about one equivalent of silylating agent, there is formed monosilylated 7-ADCA. In the case where trimethylchlorosilane or hexamethyldisilazane are used, for example, the product

has the formula



The disilyl derivative of 7-ADCA may be prepared by employing in the silylation step at least two equivalents of silylating agent per mole of 7-ADCA. When the preferred trimethylchlorosilane or hexamethyldisilazane are used, disilylated 7-ADCA is formed having the formula



The silylation step may be conducted over a wide temperature range, e.g. room temperature up to the reflux temperature of the solvent system. Advantageous results are generally obtained at room temperature with the silyl halides (20°-30° C.) and with elevated temperatures, e.g. reflux temperature, in the case of the silazanes which are generally less active.

Either the mono- or disilylated 7-ADCA or a mixture thereof may then be acylated with D(-)-α-amino-α-(3-chloro-4-hydroxyphenyl)acetyl chloride hydrochloride (most preferably in the form of a dioxane solvate) to form in situ a silylated chlorocefadroxil intermediate. Any silyl groups present after acylation are then removed by hydrolysis or alcoholysis and the desired chlorocefadroxil end-product recovered from the reaction medium, e.g. by neutralization to the isoelectric point whereupon the chlorocefadroxil precipitates out of solution.

The solvents employed in acylation of the silylated 7-ADCA are defined above in connection with silylation step (a).

A preferred temperature range for the acylation step is from -20°C . to $+70^{\circ}\text{C}$. The temperature is not critical, however, and temperatures higher or lower than those within the preferred limits may be used. The most preferred acylation temperature is between -10° and $+10^{\circ}\text{C}$.

The acylation procedure is preferably carried out in the presence of an acid acceptor which may be the same as or different from that employed in preparing the silylated 7-ADCA. Best results are obtained if a weaker (i.e. $\text{pK}_{\text{a}} < 7$) tertiary amine base such as dimethylaniline, pyridine or quinoline is used. Preferably, there is also incorporated a mineral acid salt of a weak tertiary amine, e.g. the hydrochloride salt of dimethylaniline, so as to inactivate any excess amine (see, e.g. U.S. Patent No. 3,678,037).

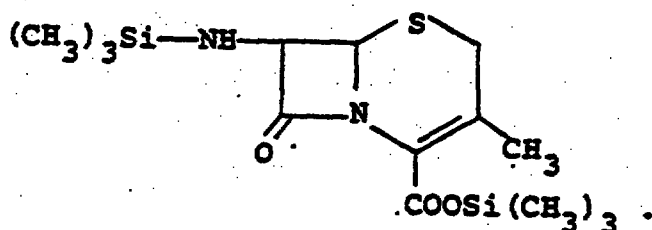
While some reaction will occur regardless of the molar proportion or reactants used, it is preferred in order to obtain maximum yields in the acylation step to use about one mole of acylating agent or a slight molar excess thereof per mole of silylated 7-ADCA.

The silylated chlorocefadroxil acylation product is treated by hydrolysis or alcoholysis to cleave the silyl protecting groups. Thus, the silylated intermediate may be hydrolyzed by addition of water or, more preferably, alcoholized by addition of a suitable alcohol, preferably a $\text{C}_1\text{-C}_4$ alkanol such as methanol, ethanol, n-propanol, isopropanol, n-butanol. A mixture of water and a lower alkanol ($\text{C}_1\text{-C}_4$) may also be employed in the cleavage step.

Chlorocefadroxil may be recovered from the reaction solution by methods customarily employed for the isolation of similar cephalosporins. Thus, the product may be recovered as the neutral molecule by upwardly adjusting the pH of the reaction

mixture until the desired acid precipitates from solution. Preferably a non-aqueous amine base such as triethylamine is used. Chlorocefadroxil in the form of the free acid may be converted to a pharmaceutically acceptable carboxylic acid or acid addition salt by reaction with an appropriate base or acid.

According to a preferred embodiment of the invention, 7-aminodesacetoxycephalosporanic acid is silylated with hexamethyldisilazane in a substantially anhydrous aprotic solvent, preferably methylene chloride, with external heating, preferably at the reflux temperature of the solvent, to form in situ disilylated 7-ADCA of the formula



The disilylated 7-ADCA is then acylated directly in the same solution (preferably at -10° to $+10^{\circ}$ C.) with the D(-)- α -amino- α -(3-chloro-4-hydroxyphenyl)acetyl chloride hydrochloride, preferably in the form of a dioxane solvate, in the presence of an acid acceptor, preferably a tertiary amine base having a $pK_a \leq 7$ such as dimethylaniline, pyridine or quinoline. Following acylation, the silylated chlorocefadroxil acylation product is treated with a C_1 - C_4 alkanol, preferably methanol or n-butanol, to cleave any silyl groups and the product is recovered (after an optional filtration step) by neutralization to the isoelectric point with a tertiary amine base, preferably triethylamine, to effect precipitation.

Use of hexamethyldisilazane as the silylating agent in place of the usual silyl halides such as trimethylchlorosilane eliminates the formation of an acid halide by-product and, consequently, the necessity of employing an acid acceptor in the silylation step. Without the presence in the reaction medium of this acid acceptor, less insoluble salt, e.g. triethylamine-HCl,

is present to interfere with the later recovery steps. By use of hexamethyldisilazane, therefore, higher yields of chlorocefadroxil are achievable than with the conventional trimethylchlorosilane silylation.

In another aspect the present invention provides a novel crystalline monohydrate form of chlorocefadroxil which has been found to be a stable useful form of the cephalosporin antibiotic particularly suitable for pharmaceutical formulations.

The crystalline chlorocefadroxil monohydrate of this invention exhibits essentially the following x-ray powder diffraction properties:

<u>Line</u>	<u>Spacing $d(\text{\AA})$</u>	<u>Relative Intensity</u>
1	8.63	100
2	7.03	23
3	6.68	56
4	6.42	71
5	5.47	39
6	5.25	7
7	4.76	51
8	4.60	29
9	4.48	13
10	4.32	39
11	4.03	82
12	3.96	47
13	3.87	52
14	3.70	32
15	3.53	36
16	3.28	30
17	3.07	23
18	2.98	10

19	2.87	13
20	2.81	21
21	2.72	19
22	2.69	46
23	2.63	15
24	2.53	8
25	2.50	9
26	2.44	13
27	2.31	21
28	2.25	10
29	2.19	9
30	2.14	6
31	2.09	6

The details for this determination of x-ray diffraction properties are as follows:

Flat samples of 2 cm² and of 1 mm of thickness were used with an x-ray powder automatic diffractometer (Phillips PW 1050-70-source CuK α (1.54178Å). Temperature = 22°C.

A very small amount of crystalline sodium fluoride was mixed in with some samples to provide internal calibration. In addition, a sample of pure NaF was run through the complete procedure for the same purpose.

The films were read on a Norelco Debye-Scherrer film reader, recording the positions of the diffraction rings to the nearest 0.05 mm. The data were corrected for film shrinkage and the interplanar spacings (d-spacings) were calculated from the corrected data. A computer program (XRAY, by P. Zugenmaier) was used for all calculations. The accuracy in the resulting d-spacing data was ~1%.

An intensity record of all films was obtained using a Joyce-Loeble Mark IIIC Recording microdensitometer (scan ratio 5:1, 0.1 O.D. wedge). Relative intensities on a scale 1-100 were

assigned to all recognizable diffraction rings using peak intensities corrected for the background reading.

A sample of the crystalline monohydrate product was subjected to infrared analysis and the spectrum of the sample (as KBr disc) is shown in FIG. 1.

A further provision of the present invention is a process for preparing the above-described crystalline chloro- cefadroxil monohydrate, which process comprises

(a) silylating 7-aminodesacetoxycephalosporanic acid in an inert substantially anhydrous aprotic solvent;

(b) acylating the so-produced silylated 7-aminodesacetoxycephalosporanic acid with D(-)- α -amino- α -(3-chloro-4-hydroxyphenyl)acetyl chloride hydrochloride in an inert substantially anhydrous aprotic solvent;

(c) cleaving any silyl groups of the acylation product by hydrolysis or alcoholysis; and

(d) forming the desired monohydrate product by a method selected from

(1) upwardly adjusting the pH of the solution from step (c) in the presence of excess dimethylformamide or acetonitrile to form the dimethylformamide or acetonitrile solvate of 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid; dissolving said solvate in acidified water or a mixture of acidified water and acetonitrile, and upwardly adjusting the pH of said acidified solution to precipitate the desired crystalline monohydrate;

(2) upwardly adjusting the pH of the solution from step (c) in the presence of excess dimethylformamide or acetonitrile to form the dimethylformamide or acetonitrile solvate of 7-[D- α -

amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid and contacting said solvate with water or a partially aqueous medium to precipitate the desired crystalline monohydrate; or

(3) upwardly adjusting the pH of the solution from step (c) to form 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid and contacting said acid with water or a partially aqueous medium to effect crystallization of the desired monohydrate.

In preparing crystalline chlorocefadroxil monohydrate according to the above process, the silylation, acylation and silyl group cleavage steps are carried out as described previously in connection with the improved acylation procedure for preparing chlorocefadroxil.

The desired crystalline monohydrate may then be prepared according to any one of several alternative routes.

In one method, the solution of chlorocefadroxil following the solvolysis step is neutralized with a basic substance, e.g. a tertiary amine base such as triethylamine, in the presence of excess dimethylformamide or acetonitrile until the dimethylformamide or acetonitrile solvate of chlorocefadroxil precipitates from solution. The solvate may then be collected and washed (preferably not dried) to give a crystalline material. Chlorocefadroxil dimethylformamide or acetonitrile solvate may be converted to the desired chlorocefadroxil monohydrate by dissolving the solvate in acidified water or a mixture of acidified water and acetonitrile and then neutralizing the acidified solution to precipitate the monohydrate product.

Dissolution of the chlorocefadroxil dimethylformamide or acetonitrile solvate occurs at a pH of around 2-2.4 which can be achieved by addition of a mineral acid, e.g. HCl, to a mixture of the solvate in either water or an acetonitrile-water mixture. Solid impurities may be removed at this stage of the process by

filtration of the acidified solution after treatment with activated carbon and/or filter aid.

The acidified solution is then neutralized, preferably with agitation and with warming to 35-60° C., by addition of a suitable base, e.g. an aliphatic tertiary amine such as triethylamine, to raise the solution pH to the point where chlorocefadroxil monohydrate crystallizes from solution.

Acetonitrile is preferably added to the solution as an antisolvent (precipitating agent) during neutralization to achieve maximum recovery of the desired product. Yields are also improved by seeding the solution with seed crystals of the desired monohydrate prior to and/or during the final neutralization step.

An alternative method for preparing the crystalline chlorocefadroxil monohydrate in the above process involves preparing chlorocefadroxil dimethylformamide or acetonitrile solvate as described above and contacting said solvate with water or a partially aqueous medium until the desired monohydrate crystallizes from the solvent system.

The chlorocefadroxil dimethylformamide or acetonitrile solvate is dissolved in water or a mixture of water and an organic solvent such as acetonitrile, acetone, a C₁-C₅ alkanol (e.g. methanol, ethanol, n-propanol, isopropanol, n-butanol, amyl alcohol), or a mixture thereof. The use of partially aqueous organic solvent systems is preferred since the organic solvents take up many of the impurities and result in a purer end-product.

When mixtures of water and organic solvents are employed, the ratios of the solvent components may be varied over a wide range without serious adverse effects. The preferred solvent ratios have been determined for several partially aqueous solvent systems and are as follows:

water:acetone (1:3) (v/v)

water:isopropanol (1:3) (v/v)
water:acetonitrile (1:3) (v/v)
water:n-butanol (1:1) (v/v).

With the water-acetonitrile system, it is preferred to add n-butanol (preferably after solubilization of the solvate) to ensure that the solvent system remains as a single homogeneous phase during crystallization. Preferably, sufficient n-butanol is added to this crystallization system so as to achieve a final solvent ratio of water-acetonitrile:n-butanol (1:2:1) (v/v).

The concentration of solvate in the aqueous or partially aqueous crystallization medium is not critical. Best yields have been obtained, however, when concentrations of between about 400 and 800 grams/liter of solution are employed. The solvate is preferably added to the solvent system in increments and with stirring over a period of time which is dependent on the quantity of solvate used, i.e. from a few minutes up to several hours.

Crystallization may be carried out over a wide temperature range, i.e. from room temperature up to the boiling point of the solvent system. Good results are obtained in a temperature range of from 35°-60° C., most preferably 40°-45° C.

Yields of monohydrate are improved by seeding the solution of dimethylformamide or acetonitrile solvate with seed crystals of chlorocefadroxil monohydrate.

Yet another method of preparing the desired monohydrate in the above process comprises (1) preparing the silylated chlorocefadroxil and cleaving the silyl protecting groups by hydrolysis or alcoholysis as described above, (2) neutralizing the solution from the cleavage step to the isoelectric point of chlorocefadroxil (~pH 5.7-5.8) with a suitable base, preferably an aliphatic tertiary amine such as triethylamine, to precipitate impure or primary grade chlorocefadroxil, and (3) contacting said impure chlorocefadroxil with water or a mixture of water with a suitable organic solvent, preferably acetonitrile, acetone, a C₁-C₅ alkanol (e.g. methanol, ethanol, n-propanol, isopropanol,

n-butanol, amyl alcohol) or mixture thereof, until chlorocefadroxil monohydrate crystallizes from solution.

Neutralization of the chlorocefadroxil solution to form impure or primary grade chlorocefadroxil (amorphous) can be conveniently carried out at room temperature by gradual addition of the base to the stirred solution. The impure chlorocefadroxil may then be crystallized in the same manner as described above for the chlorocefadroxil dimethylformamide or acetonitrile solvate. As in the case of the dimethylformamide or acetonitrile solvate crystallization procedure, the most preferred solvent system is water:acetonitrile:n-butanol (1:2:1) (v/v).

A most preferred embodiment of the present invention is the process of preparing crystalline chlorocefadroxil monohydrate from either chlorocefadroxil dimethylformamide or acetonitrile solvate or impure (primary grade) chlorocefadroxil by the steps of

(a) dissolving the dimethylformamide or acetonitrile solvate of 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid in acidified water or a mixture of acidified water and acetonitrile; and upwardly adjusting the pH of said acidified solution until the desired monohydrate crystallizes from solution; or

(b) contacting 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid or the dimethylformamide or acetonitrile solvate thereof with water or a partially aqueous medium until the desired monohydrate crystallizes from solution.

The dimethylformamide or acetonitrile solvate and chlorocefadroxil starting materials used in the above process may be prepared by the processes described in the present application or by other known processes, e.g. the processes disclosed in U.S. Patent Nos. 3,489,751 and 3,985,741.

Preferred conditions for forming chlorocefadroxil monohydrate in the above process are as described above in connection with the previously disclosed overall reaction scheme, i.e. the combined silylation, acylation and monohydrate production steps.

By employing the preferred reaction conditions described above, the present invention makes possible the production of primary grade chlorocefadroxil in excellent yields and subsequent conversion of said chlorocefadroxil or its dimethylformamide or acetonitrile solvate to chlorocefadroxil monohydrate in activity yields of up to 85%.

The crystalline monohydrate prepared according to any of the above processes can be recovered by conventional methods, e.g. filtration, and then washed, dried and prepared into pharmaceutical formulations for use in antibiotic therapy in combating various bacterial diseases. Examples of such formations (e.g. capsules or tablets), doses and modes of administration of chlorocefadroxil monohydrate and its pharmaceutical compositions are as described in U.S. Patent Nos. 3,489,751 and 3,985,741 for the amorphous form of chlorocefadroxil.

The invention thus includes a pharmaceutical composition, most preferably a pharmaceutical composition adapted for oral administration, comprising crystalline chlorocefadroxil monohydrate with a suitable inert pharmaceutically acceptable carrier or diluent.

The invention further includes medication for use in a method of treating humans or other animal species (e.g. mammals) for diseases caused by Gram-positive or Gram-negative bacteria, which medication comprises an effective dose of crystalline chlorocefadroxil monohydrate as defined herein or a pharmaceutical composition as hereinbefore defined.

The following examples are given by way of illustration of the present invention. All temperature are in degrees

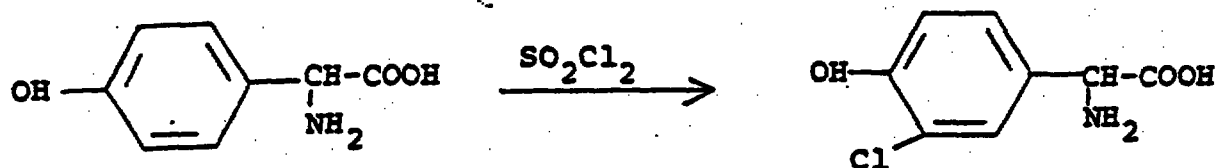
Centigrade. 7-Aminodesacetoxycephalosporanic acid is abbreviated as 7-ADCA, triethylamine as TEA, dimethylaniline as DMA and dimethylformamide as DMF.

Preparation of Starting Materials

Preparation 1

Preparation of D(-)Chloro-3-hydroxy-4-phenylglycine

6



REAGENTS

- D(-)p-hydroxyphenylglycine	10 kg (60 moles)
- Acetic acid	228 liters
- Hydrochloric acid (gas)	6.25 kg
- Sulfuryl chloride	8.1 kg (60 moles)
- Methylene chloride	100 liters
- Soda solution 400 g/liter	= 3 liters
- Acetone	= 15 liters

PROCEDURE

A suspension of 10 kg of D(-)p-hydroxyphenylglycine in 200 liters of acetic acid was heated to 60°C. The hydrochlorate was formed by bubbling 6.25 kg of hydrochloric acid (over approximately 60 minutes); a solution of 8.1 kg of sulfuryl chloride in 28 liters of acetic acid was then added to the solution obtained, maintained at 65-70°C, over 90 minutes. The solution was de-gassed under low pressure, cooled to 20°C and stirred overnight.

The solid was collected and washed with 2 resuspensions in methylene chloride. It was dried in a vacuum at 40°C.

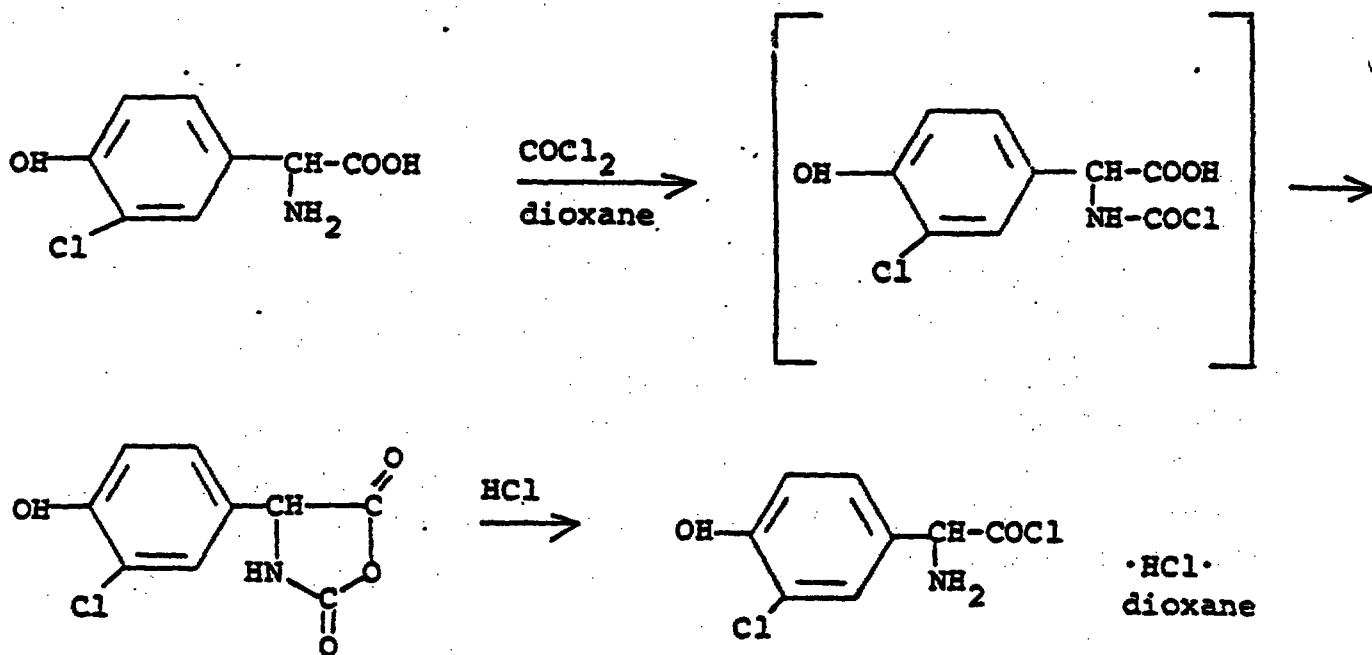
Weight of the chloro-3-hydroxy-4-phenylglycine hydrochlorate obtained = 13 kg.

The hydrochlorate was suspended in 130 liters of water and the mixture brought to 40°C while stirring for 30 minutes. It was cooled to 20°C and the pH adjusted to 1.4 by addition of a 400 g/liter solution of soda (approximately 3 liters). It was stirred for 2 hours at +5°C, filtered and the solid washed 3 times with distilled water (absence of ionizable chlorine in the mother liquors), then once with approximately 15 liters of acetone, and dried at 60°C. It was blended on a Frewitt apparatus and dried to H₂O (KF) ≤ 0.1%.

Weight of the chloro-3-hydroxy-4-phenylglycine obtained = 8.1 kg (67% yield).

Preparation 2

Preparation of D(-)Chloro-3-hydroxy-4-phenylglycine chloride hydrochloride



REAGENTS

- D(-)chloro-3-hydroxy-4-phenylglycine 50 g (0.248 mole)
- Dioxane 410 ml (+ washing)
- Phosgene 60 g (0.60 mole)
- Hydrochloric acid (gas) $\approx$ 190 g (5.2 moles)
- Methylene chloride q.s. for washing

PROCEDURE

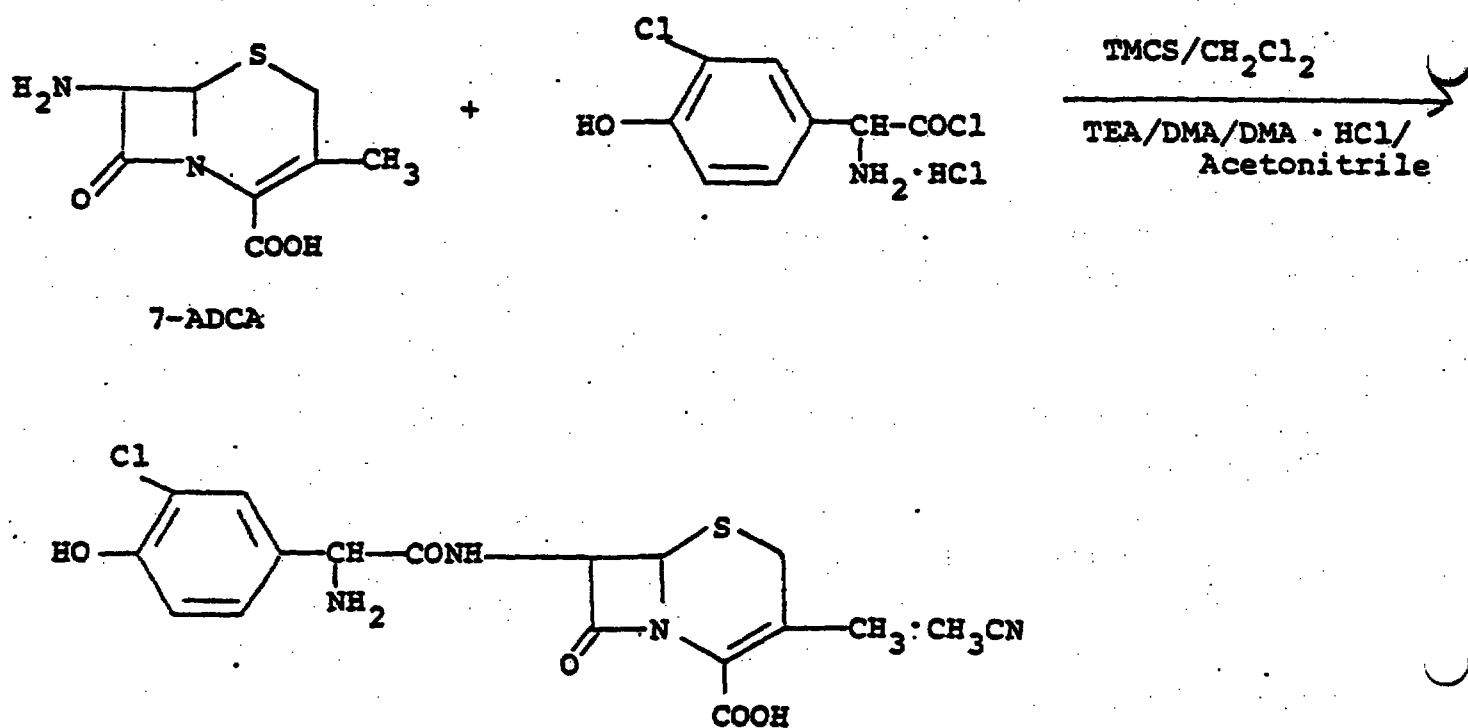
410 ml of anhydrous dioxane (dried on a molecular sieve, KF <0.05%), then 50 g of anhydrous D(-)chloro-3-hydroxy-4-phenylglycine (dried 5 mm Hg/80°, constant weight KF <0.1% and sieved on a 200 mesh sieve) were placed in a 1 liter reactor. 60.0 g of phosgene was passed through in 20 minutes while stirring, [the initial temperature of 20° increases to 35° at the onset of passage of the phosgene while there is transformation of the solid (stirring more difficult), after which the temperature tends to decrease]. When the necessary amount of phosgene had been added, the mixture was heated to 70°C. When the temperature attained 60-65°C, the crystallized mass passed into solution. It was heated for 10 minutes at 70°C after which time the heating was stopped and the solution was concentrated to a volume of 250 ml without external heating (elimination of the excess phosgene); (the temperature is close to 25°C at the end of concentration). The solution was cooled at 8-10°C and hydrochloric acid was passed through as rapidly as possible to maintain the temperature at 28-30°C (the amount of hydrochloric acid added corresponds to a very large excess; the reaction is exothermic until approximately the stoichiometric quantity, i.e., 2 moles, has been passed; the temperature then tends to decrease again, and a temperature of 20-25°C is then maintained). When approximately half of the hydrochloric acid had been passed, the solution was seeded and stirred overnight at 20°C. The chloride hydrochlorate was filtered the next day, preventing contact with atmospheric air. It was washed once with anhydrous dioxane and twice with anhydrous methylene chloride. It was dried in a vacuum at

ambient temperature to yield 73 g ($\approx 85\%$) of title product as a mono-dioxane solvate.

Example 1

Chlorocefadroxil Monohydrate

A. Chlorocefadroxil Acetonitrile Solvate



REAGENTS

7- ADCA	7.2 Kg (33.6 Mol)
- Methylene chloride (K.F.<0.1%)	180.0 l.
- Trimethylchlorosilane (TMCS)	9.0 l. (71.0 Mol)
- N,N-Dimethylaniline (DMA)	4.35 l. (34.3 Mol)
- Triethylamine (TEA)	23.4 l.
- N,N-Dimethylaniline hydrochloride (methylene chloride solution 376 g/l)	4.24 l. (10.1 Mol)
- Chloro-3-hydroxy-4-phenylglycine chloride hydrochloride dioxane solvate (purity 53.8%)	17.0 Kg (34.3 Mol)
- Methanol	3.4 l.
- Acetonitrile	107.0 l.
- City water	51.0 l.

PROCEDURE

A 500 l glass-lined reactor was charged with 150 l of anhydrous methylene chloride (KF <0.1%) and 7.2 Kg of 7-ADCA. To the stirred suspension, 9.0 l of TMCS and 4.35 l of DMA were added followed by an addition of 9.4 l of TEA over a 15 minute period while keeping the temperature at 20-25°C. The mixture was stirred one hour at 20°C and cooled at -10°C. There was then added 4.24 l of a 376 g/l methylene chloride solution of DMA·HCl followed by 17 Kg of D(-)chloro-3-hydroxy-4-phenylglycine chloride hydrochloride dioxane solvate (purity 53.8%) in ten portions over a one hour period (the temperature is kept at between -12°C and -8°C). The mixture was stirred 2 hours at -10°C and 3.4 l of methanol was added over a 10 minute period followed by 48 l of tap water (with efficient stirring). The mixture was stirred 15 minutes (temperature 0°C-5°C) and the pH was adjusted to 2.3 by addition of TEA (9.0 l). The aqueous solution was separated and washed twice with 15 l of methylene chloride. There was then added 80 l of acetonitrile and the pH was adjusted to 5.0 by addition of TEA (5.0 l). The solution was seeded and stirred overnight at +10°C. The solid was collected, washed with 15 l of

acetonitrile-water (8:2) and 15 l of acetonitrile and then dried at 40°C to give 11.0 Kg (74% yield from 7-ADCA) of title product.

B. Purification of Chlorocefadroxil Acetonitrile Solvate

REAGENTS:

Chlorocefadroxil acetonitrile solvate (crude)	21.0 Kg
Tap water	115 l
33% HCl	5.2 l
Charcoal	1.5 Kg
Acetonitrile	250 l
Triethylamine (TEA)	8.7 l
Celite	q.s.

PROCEDURE:

Crude chlorocefadroxil acetonitrile solvate (21 Kg) was stirred in 100 l of tap water and the pH was adjusted to 0.8-0.9 by addition of 5.2 l of 33% HCl. To the solution, 1.5 Kg of charcoal was added and the mixture was stirred 30 minutes and filtered through a CELITE pad. The solution and washing water (10 l) was charged into a 250 l glass-lined reactor, 200 l of acetonitrile was added and the pH was adjusted to 2.5 by addition of TEA (3.5 l). The solution was seeded, heated to 40-45°C and the pH adjusted to 5.0 by addition of TEA (5.2 l). The mixture was stirred one hour at 40°C, cooled to 10°C and allowed to stand overnight with stirring at 10°C. The solid was collected, washed with 30 l of acetonitrile-water (1:2) and then 30 l of acetonitrile, and dried at 40°C to give 16.6 Kg (79%) of purified title product.

C. Chlorocefadroxil Monohydrate

REAGENTS:

Chlorocefadroxil acetonitrile solvate (purified)	6.85 Kg
Water	73.0 l
33% HCl	1.7 l
Charcoal	0.7 Kg
Triethylamine (TEA)	2.8 l
CELITE	q.s.

PROCEDURE:

Purified chlorocefadroxil acetonitrile solvate (6.85 Kg) was stirred in 50 l of water and the pH adjusted to 0.8-0.9 by addition of 1.7 l of 33% HCl. Charcoal (0.7 Kg) was added and the mixture was stirred 30 minutes and filtered through a CELITE pad. The solution and washing water (5 l) were transferred into a 100 l glass-lined reactor and heated to 40°C. The pH was adjusted to 1.6-1.8 by addition of TEA (1.24 l) and the solution seeded with chlorocefadroxil monohydrate. The mixture was stirred one half hour at 40°C and the pH adjusted to 4.0 by addition of TEA (1.56 l). The suspension was slowly cooled to 20°C and then stirred two hours at +5°C. The solid was collected, washed three times with 6 l of water and dried at 40°C to give 5.35 Kg (86%) of title product.

Example 2

Chlorocefadroxil Monohydrate (illustrates
preparation without seeding)

Purified chlorocefadroxil acetonitrile solvate (352 g) was suspended in 2.8 l of water and then 36% HCl added to provide a pH of 0.9 (all the material goes into solution). The solution was stirred one-half hour with 36.0 g of charcoal and the mixture filtered through a CELITE pad. The resulting solution was heated to 40°C and the pH adjusted to 1.8 by addition of triethylamine. At this point crystals of chlorocefadroxil monohydrate began to form without seeding. The mixture was stirred one-half hour at 40°C. The pH of the mixture was then adjusted to 4.0 with triethylamine and the suspension stirred an additional two hours at 5°C. The crystalline chlorocefadroxil monohydrate was collected, washed three times with water and dried at 45°C to give 295.5 g of title product. H_2O (KF)=3.74%. The IR spectrum was substantially as shown in FIG. 1 and was identical to that obtained for the sample prepared according to Example 1.



CLAIMS

1. Crystalline 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)-acetamido]-3-methyl-3-cephem-4-carboxylic acid monohydrate exhibiting substantially the following x-ray diffraction properties:

<u>Line</u>	<u>Spacing d($\text{\AA}$)</u>	<u>Relative Intensity</u>
1	8.63	100
2	7.03	23
3	6.68	56
4	6.42	71
5	5.47	39
6	5.25	7
7	4.76	51
8	4.60	29
9	4.48	13
10	4.32	39
11	4.03	82
12	3.96	47
13	3.87	52
14	3.70	32
15	3.53	36
16	3.28	30
17	3.07	23
18	2.98	10
19	2.87	13
20	2.81	21
21	2.72	19
22	2.69	46
23	2.63	15
24	2.53	8
25	2.50	9
26	2.44	13
27	2.31	21



28	2.25	10
29	2.19	9
30	2.14	6
31	2.09	6

2. A process for the preparation of crystalline 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid monohydrate exhibiting substantially the following x-ray diffraction properties:

<u>Line</u>	<u>Spacing d(Å)</u>	<u>Relative Intensity</u>
1	8.63	100
2	7.03	23
3	6.68	56
4	6.42	71
5	5.47	39
6	5.25	7
7	4.76	51
8	4.60	29
9	4.48	13
10	4.32	39
11	4.03	82
12	3.96	47
13	3.87	52
14	3.70	32
15	3.53	36
16	3.28	30
17	3.07	23
18	2.98	10
19	2.87	13
20	2.81	21
21	2.72	19
22	2.69	46
23	2.63	15
24	2.53	8
25	2.50	9
26	2.44	13
27	2.31	21



28	2.25	10
29	2.19	9
30	2.14	6
31	2.09	6

which comprises

(a) silylating 7-aminodesacetoxycephalosporanic acid in an inert substantially anhydrous aprotic solvent;

(b) acylating the so-produced silylated 7-amino-desacetoxycephalosporanic acid with D(-)- α -amino- α -(3-chloro-4-hydroxyphenyl)acetyl chloride hydrochloride in an inert substantially anhydrous aprotic solvent in the presence of an acid acceptor;

(c) cleaving any silyl groups of the acylation product by hydrolysis or alcoholysis; and

(d) forming the desired monohydrate product by a method selected from

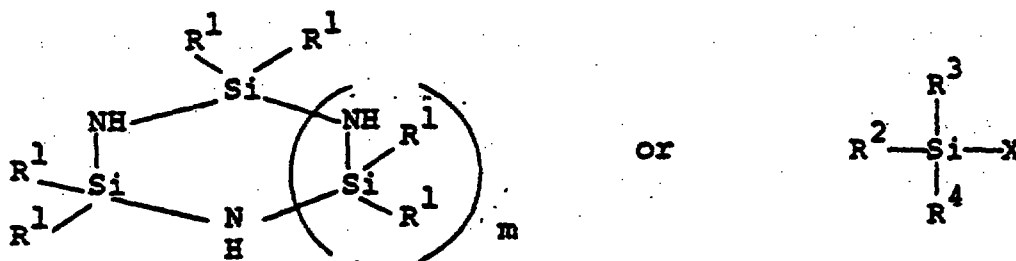
(1) upwardly adjusting the pH of the solution from step (c) in the presence of excess dimethylformamide or acetonitrile to form the dimethylformamide or acetonitrile solvate of 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid; dissolving said solvate in acidified water or a mixture of acidified water and acetonitrile, and upwardly adjusting the pH of said acidified solution to precipitate the desired crystalline monohydrate;

(2) upwardly adjusting the pH of the solution from step (c) in the presence of excess dimethylformamide or acetonitrile to form the dimethylformamide or acetonitrile solvate of 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid and contacting said solvate with water or a partially aqueous medium to precipitate the desired crystalline monohydrate; or

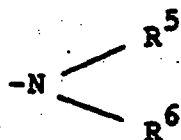


(3) upwardly adjusting the pH of the solution from step (c) to form 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)-acetamido]-3-methyl-3-cephem-4 carboxylic acid and contacting said acid with water or a partially aqueous medium to effect crystallization of the desired monohydrate.

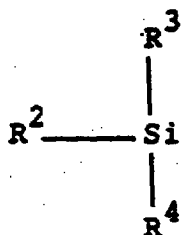
3. A process as claimed in Claim 2 wherein the silylation step (a) is accomplished by reacting 7-aminodesacetoxycephalosporanic acid with a silylating agent selected from those of the formula



wherein R², R³ and R⁴ are hydrogen, halogen, (lower)alkyl, halo(lower)alkyl, phenyl, benzyl, tolyl or dimethylaminophenyl, at least one of the said R², R³ and R⁴ groups being other than halogen or hydrogen; R¹ is (lower)alkyl; m is an integer of 1 to 2 and X is halogen or



wherein R⁵ is hydrogen or (lower)alkyl and R⁶ is (lower)alkyl or



wherein R², R³ and R⁴ are as defined above.

4. A process as claimed in Claim 3 wherein the silylating agent in step (a) is trimethylchlorosilane or hexamethyldisilazane.



5. A process as claimed in Claim 2, 3 or 4 wherein disilylated 7-amino-desacetoxycephalosporanic acid is produced in step (a) by using at least two equivalents of silylating agent per mole of 7-amino-desacetoxycephalosporanic acid.
6. A process as claimed in Claim 2 or 5 wherein step (a) is carried out by silylating 7-aminodesacetoxycephalosporanic acid with trimethylchlorosilane in a substantially anhydrous aprotic solvent in the presence of an acid acceptor.
7. A process as claimed in Claim 6 wherein the silylation step is carried out in a substantially anhydrous methylene chloride solvent system in the presence of an acid acceptor comprising triethylamine or a mixture of triethylamine and dimethylaniline at a temperature of 20-30° C.
8. A process as claimed in Claim 2 or 5 wherein step (a) is carried out by silylating 7-aminodesacetoxycephalosporanic acid with hexamethyldisilazane in a substantially anhydrous aprotic solvent with external heating.
9. A process as claimed in Claim 8 wherein the silylation step is carried out in a substantially anhydrous methylene chloride solvent at reflux temperature.
10. A process as claimed in any of claims 2 to 9 wherein acylation step (b) is carried out in a substantially anhydrous methylene chloride solvent system at a temperature in the range of from -10°C. to +10° C. in the presence of acid acceptor selected from a tertiary amine base having a $pK_a \leq 7$.
11. A process as claimed in Claim 10 wherein the acid acceptor is dimethylaniline.
12. A process as claimed in any of claims 2 to 11 wherein in step (c) silyl groups are cleaved by treatment with water or a C_1 - C_4 alkanol, or a mixture thereof.



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13. A process as claimed in any of claims 2 to 11 wherein in step (c) silyl groups are cleaved by treatment with a C₁-C₄ alkanol.

14. A process as claimed in any of claims 2 to 13 wherein step (d) comprises

(1) upwardly adjusting the pH of the solution from step (c) with triethylamine in the presence of excess dimethylformamide or acetonitrile until the dimethylformamide or acetonitrile solvate of 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid precipitates from solution;

(2) dissolving said solvate in acidified water; and

(3) upwardly adjusting the pH of said solution by addition of triethylamine to precipitate the desired crystalline monohydrate.

15. A process as claimed in Claim 14 wherein the final pH adjustment step to produce the desired crystalline monohydrate is conducted at a temperature of _____ 35-60° C.

16. A process as claimed in claim 14 or 15 wherein acetonitrile is added as an antisolvent during the final pH adjustment step.

17. A process as claimed in claim 14, 15 or 16 wherein seed crystals of the desired 7-[D- α -amino- α -(3-chloro-4-hydroxyphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic acid monohydrate are added prior to or during the final pH adjustment step.

18. A pharmaceutical composition comprising a compound according to Claim 1 together with an inert pharmaceutically acceptable carrier or diluent.



19. Medication comprising an effective anti-bacterial dose of the compound according to claim 1 or a pharmaceutical composition according to claim 18, for use in a method of treating mammals for diseases caused by Gram-positive or Gram-negative bacteria.

20. A process as claimed in claim 2 wherein a procedure substantially as described in Example 1 or 2 is followed.

21. The monohydrate of chlorocefadroxil or a pharmaceutically acceptable salt thereof prepared by a process as claimed in any of claims 2 to 17 or claim 20.

22. A pharmaceutical composition comprising the monohydrate of claim 21 together with a pharmaceutically acceptable carrier or diluent.
