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(54) PROCESS FOR PURIFYING CEPHALOGLYCIN ANTIBIOTIC AND THE ANTIBIOTIC THUS OBTAINED

(71) We, ELI LILLY AND COMPANY, a Corporation of the State of Indiana, United States of America, of 307, East McCarty Street, Indianapolis, State of Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a process for purifying cephaloglycin antibiotic and to the antibiotic prepared by the process.

More specifically, the present invention provides a process for purifying cephaloglycin antibiotic which comprises (a) dissolving crude cephaloglycin with the aid of acid in a solvent mixture, (b) adding a base to the cephaloglycin solution in a quantity sufficient to precipitate cephaloglycin from the solution, and (c) separating the precipitated cephaloglycin from the base treated solvent, said solvent mixture being acetonitrile, methanol and water with the methanol being an optional part of step (a), said methanol not used in step (a) being added as part of step (b) with the base, and the proportions of the components of the acetonitrile:methanol:water of the solvent mixture being from 3:1:3 to 3:3:1 to 1:3:3 by volume.

7 - (D - α - aminophenylacetamide)cephalosporanic acid and its salt are herein referred to by the generic name, cephaloglycin.

A better solvent and precipitation system and an improved process incorporating that system has been found for converting cephaloglycin from a crude or semi-pure state to a denser, more pure cephaloglycin, substantially free of phenyl-glycine reactant. One variation of our process comprises (1) dissolving cephaloglycin with the aid of an acid in a solvent mixture comprising (a) from 1 to 3 parts by volume of acetonitrile, (b) 1 part to 3 parts by volume of water, and (c) from 1 part to 3 parts by volume of methanol, said solvent mixture containing a total of

from 3 to 9 parts by volume, (2) treating the cephaloglycin-containing solution from step 1 with sufficient base to precipitate cephaloglycin and (3) separating the precipitated cephaloglycin from the base-treated solution mixture.

An alternative procedure which can be used is to dissolve cephaloglycin in a mixture of the acetonitrile and water components with the aid of acid, with or without a portion of the methanol component being present initially in the mixture, and then to add a portion or all of the methanol component with the base in step 2. This alternative procedure helps to slow the crystallization process to obtain denser, more easily filtered cephaloglycin crystals.

Optionally and preferably the separated cephaloglycin crystals obtained from step (3) above are mixed with water or water vapour, to form crystalline cephaloglycin di-hydrate therefrom, either directly, or after washing the separated cephaloglycin with an alkanol such as methanol, and drying. We find that the improved physical properties of the cephaloglycin crystals obtained by the above procedure (steps 1—3) can be preserved by converting this cephaloglycin to the di-hydrate by exposure to high relative humidity.

When cephaloglycin is dissolved in a solvent system which eventually contains acetonitrile, water, and methanol, and base is added to precipitate the cephaloglycin, according to our procedure described above, the crystalline cephaloglycin obtained is denser, more pure, and is more completely precipitated from the solution, thus improving the yields of recovered cephaloglycin from given quantities of dissolved cephaloglycin. Thus, the so-called steps 1 and 2 of the process of this invention can be conducted separately, as first described above, or the two steps can be merged by the addition of the methanol with the base.

The useful range of the acetonitrile:methanol:water solvent mixture used in the pro-

[Price 25p]

cess of this invention for cephaloglycin purification and yield enhancement is within the proportions of 1:3:3 to 3:1:3, to 3:3:1, by volume, of acetonitrile:methanol:water, respectively. Outside these general limits, gels, very fine crystals, or other undesired forms of cephaloglycin are obtained.

The acid used to effect cephaloglycin solution in the solvent mixture can be any strong water miscible acid having a pKa of less than 4. The preferred acids are the economical mineral acids such as hydrochloric, sulfuric, nitric, and orthophosphoric acids. Hydrocarbon-sulfonic acids, especially the C₁ to C₆-alkanesulfonic acids such as methanesulfonic, propanesulfonic, and hexanesulfonic acids as well as the C₆ to C₁₂-aromatic hydrocarbonsulfonic acids such as benzenesulfonic, p-toluenesulfonic, alpha-naphthalenesulfonic acid, and biphenylsulfonic acids, and the halogenated C₆ to C₁₂-aromatic hydrocarbon sulfonic acid and p-bromobenzenesulfonic acids can also be used. Weaker C₁ to C₆-alkanoic carboxylic acids such as acetic or propionic can also be used in that they make up for their weaker acidity by adding their solvent properties to the solvent system.

Other alkane nitriles such as propionitrile and butyronitrile can be substituted for part or all of the acetonitrile in the solvent system but acetonitrile is preferred. Ethanol and the C₃- to C₆-alkanols can be substituted for part of the methanol, but the use of larger amounts of those alkanols makes the crystallization more difficult to control. Hence, methanol is the preferred alcohol.

The base added in step 2 of the process can be any water-miscible base having a fairly strong pKa, say, above 4. It is preferably added in solution to avoid excessive rise in local pH and is added in quantity sufficient to adjust the pH of the mixture to the pH range in which cephaloglycin precipitates. Generally, maximum precipitation occurs at pH 3.5 to 5.0. The preferred bases are ammonium hydroxide, sodium or potassium hydroxide, C₁ to C₃-trialkylamines such as triethylamine. The base is preferably added in concentrated form with agitation until the rate of cephaloglycin precipitation is satisfactory. A convenient measure is to add the base until it is equivalent to the amount of acid that was added in step 1, but exact stoichiometric equivalency is not required with all solvent systems.

When all or most of the cephaloglycin has precipitated from the solvent mixture, separation therefrom is accomplished by conventional methods, e.g., filtration, centrifugation, washed, if desired, with methanol or acetone, and dried at from 30° to 60° C., preferably under vacuum, for from 1 to 6 hours, or until the weight becomes constant. The cephaloglycin crystals obtained by this process may be dried to contain less than 1 per

cent, by weight, of water, preferably less than 0.5 percent water. The anhydrous cephaloglycin resulting from these procedures have the same anhydrous cephaloglycin X-ray pattern as that obtained by previous procedures and the anhydrous cephaloglycin crystals are substantially free of phenylglycine carried through the manufacturing process and obtained in improved yield, relative to the yields by prior processes.

The anhydrous cephaloglycin product of this process can be used as such for forming aqueous solutions and suspensions useful for antibiotic therapy. It is preferably converted to the cephaloglycin dihydrate crystalline form (approximately 8 to 10 percent water, by weight) for use in making dry solid reconstitutable formulations for antibiotic therapy. The improved bulk flow and density properties of the anhydrous cephaloglycin crystals obtained by the process of this invention can be preserved by exposing the substantially anhydrous cephaloglycin to high relative humidity in a suitable tumbling or mixing apparatus until the cephaloglycin dihydrate is formed. Other hydration methods can be used, however. As an example, the substantially anhydrous cephaloglycin, prepared as described above, can be re-hydrated to a water content of 8 to 10 percent by weight by grinding the anhydrous cephaloglycin crystals for about 0.5 hours in water, filtering the mixture, washing the solid with acetone, and then drying in a vacuum oven to constant weight.

An example of a pharmaceutical formulation containing the re-hydrated cephaloglycin and useful for antibiotic administration is the following.

Formula for Suspension Cephaloglycin to

Provide 250 mg./5 Cubic Centimeters		Weight	
Crystalline cephaloglycin hydrate	5.5 g.		
Sucrose, Baker's grade	50.0 g.		
Saccharin, soluble	0.2 g.		
Sodium cyclamate	1.0 g.		110
Sodium chloride, U.S.P. powder	0.5 g.		
Citric acid powder	0.1 g.		
Sodium citrate powder	0.2 g.		
F, D, and C Yellow No. 6, aluminum lake certified	0.05 g.		115
Imitation orange juice flavor, 59.107/AP Firmenich, Inc.	1.0 g.		
Peppermint flavor E 1395	0.1 g.		
		58.65 g.	

Samples of the above formulations can be stored as a dry powder mixture in sealed containers at 25, 57, and 50° C. for time periods up to 1 month. When samples are taken for standard microbiological assay of cephaloglycin content of the dry mixture, or in preparation of the aqueous suspension for administration, a proper amount of purified

water was added to make the suspension containing about 50 mg. of cephaloglycin per cubic centimeter of suspension.

The invention is further illustrated by the following detailed examples.

EXAMPLE 1

A 10 g. portion of cephaloglycin was suspended in 400 ml. of a solvent mixture consisting of acetonitrile:methanol:water in a 1:1:1 proportion by volume. To this suspension 3 ml. of concentrated hydrochloric acid (12N) was added dropwise until the cephaloglycin solution being effected became clear, except for a few lumps which were removed by filtration. To this resulting cephaloglycin solution, concentrated ammonium hydroxide (14 N) was added dropwise until the amount thereof with equivalent to the acid added. The resulting mixture was allowed to stand while cephaloglycin crystals formed. The crystals were separated from the neutralized solvent mixture and dried at 30° C. under vacuum to a water content of less than 0.5 percent by weight.

EXAMPLE 2

In this example, 10 g. of cephaloglycin was suspended in 100 ml. of an acetonitrile:water mixture in 1:1 by volume mixture and then about 2.5 ml. of concentrated hydrochloric acid was added until solution of the cephaloglycin was effected. Then the methanol (50 ml.) was added with the equivalent amount of ammonia used to neutralize the acid and to effect cephaloglycin crystallization. Upon separation of the cephaloglycin crystals from the neutralized solvent system and drying under vacuum at 30° C. for three hours, the crystals were of the anhydrous cephaloglycin crystalline form and were better formed and of larger size than those obtained from the formamide-methanol method.

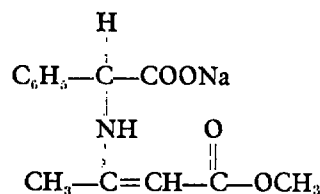
EXAMPLE 3

This example sets forth a typical procedure for preparing cephaloglycin by acylating 7-aminocephthalosporonic acid (7-ACA) with an N-blocked-amino mixed anhydride form of phenylglycine, followed by an example of the re-crystallization and purification procedure of this invention.

Blocking the Amino Group

D - Alpha - phenylglycine, levorotatory, was slurried in benzene in a stainless steel still. A 50 percent solution of sodium hydroxide was added, and the mixture was heated at 50°—70° C. for one hour. Methyl acetorefluxed until all water had been removed by azeotropic distillation. The reaction mixture was diluted with an equal volume of acetone, then transferred to a brine pot

and chilled. The sodium 3 - (D - alpha - carboxybenzylamino) crotonate salt crystals



were filtered, washed with acetone, air dried at 65° C., and ground to a fine powder.

7-ACA Tosylate Salt Preparation

7 - Aminocephalosporonic acid (7-ACA) was slurried in acetone in a glass-lined brine pot and a solution of p-toluene-sulfonic acid (tosylate) monohydrate in acetone was added at 5° C. to -5° C. The mixture was stirred for one hour, then filtered to remove insoluble impurities. The 7-ACA tosylate dihydrate was precipitated from the filtrate by the addition of water, separated by filtration, washed with acetone, and air dried at room temperature.

Mixed Anhydride Formation

The sodium 3 - (D - alpha - carboxybenzylamino)crotonate and a catalytic amount of N,N - dimethylbenzylamine were slurried in acetonitrile in a glass-lined or stainless steel brine pot and the slurry chilled to -10° C. to 0° C. Methyl chloroformate was added rapidly and the mixture was stirred a few minutes to form the methyl mixed anhydride of the N-blocked phenylglycine. Meanwhile, the 7-ACA.tosylate was converted to the soluble triethylamine salt in 50 percent water-50 percent-(acetonitrile mixture of 0° C. to 5° C.

Acylation and Removal of N-Protecting

The 7-ACA.triethylamine salt solution was added rapidly to the mixed anhydride while stirring. Stirring was continued for a few minutes, and then any insoluble material was dissolved with triethylamine. After one-half hour stirring, the solution was adjusted to pH 1.5 with concentrated hydrochloric acid at -5° to +5° C. to effect cleavage of the amino-protecting group from the cephaloglycin molecule. Methanol was added with stirring for an additional 1.5 hour and the pH was raised to 4.5 to precipitate the crude cephaloglycin crystals. The methanol enhances the crystal size of the cephaloglycin for filtration or centrifugation purposes.

Re-crystallization and Purification

The crude cephaloglycin was then redissolved in a 1:1:1 (volume) mixture of water, acetonitrile, and methanol, using hydrochloric acid to complete solution, and then reprecipitated from the solution by adding tri-

ethylamine to precipitate the cephaloglycin. After filtering the cephaloglycin and washing with methanol, it was dried *in vacuo* at 30° C. to a constant weight. It was a substantially anhydrous cephaloglycin containing less than 0.5 percent water, by weight. Increased phenylglycine removal was effected by use of a 2:1:1 (volume) mixture of water, acetonitrile, and methanol, respectively, but the average yield of purified cephaloglycin crystals upon precipitation therefrom tended to be somewhat lower than with the above proportioned solvent mixture.

Rehydration

The substantially anhydrous cephaloglycin was mixed with deionized water in a vacuum drier equipped with an internal agitator of a dough mixer. The excess water was removed by vacuum drying and the cephaloglycin dihydrate was milled as required.

WHAT WE CLAIM IS:—

1. A process for purifying cephaloglycin antibiotic which comprises (a) dissolving crude cephaloglycin with the aid of acid in a solvent mixture, (b) adding a base to the cephaloglycin solution in a quantity sufficient to precipitate cephaloglycin from the solution, and (c) separating the precipitated cephaloglycin from the base treated solvent, said solvent mixture being acetonitrile, methanol and water with the methanol being an optional part of step (a), said methanol not used in step (a) being added as part of step (b) with the base, and the proportions of the components of the acetonitrile:methanol:water of the solvent mixture being from 3:1:3 to 3:3:1 to 1:3:3 by volume.

2. The process of claim 1 which also includes mixing the precipitated cephaloglycin from step (c) with water to form a crystalline cephaloglycin dihydrate.

3. The process of claim 1 or 2 wherein in step (b) a C_1 to C_{18} -trialkylamine is added as the base.

4. The process of claim 1, 2 or 3 wherein in step (a) the solvent mixture comprises acetonitrile, methanol, and water in substantially equal parts by volume and hydrochloric acid is used to effect solution of the cephaloglycin in the solvent mixture.

5. The process of claim 1, 2 or 3 wherein in step (a) the solvent mixture comprises acetonitrile, methanol and water in the approximate volume proportions of 1 volume of acetonitrile, 1 volume of methanol, and 1 to 2 volumes of water, and hydrochloric acid is added in an amount sufficient to effect solution of cephaloglycin in the solvent mixture.

6. The process of any of claims 1 to 5 wherein in step (a) the cephaloglycin is dissolved with the aid of acid in a mixture of the acetonitrile and water components, and the methanol component is added with the base in step (b).

8. A modification of the process claimed in any one of the preceding claims wherein another alkanenitrile is used in place of part or all of the acetonitrile.

9. A modification of the process claimed in any one of the preceding claims wherein a C_2 — C_6 alkanol is used in place of part of the methanol.

10. A process for purifying cephaloglycin antibiotic according to claim 1 substantially as hereinbefore described with particular reference to the examples.

11. Cephaloglycin when prepared in accordance with the process of any of claims 1 to 10.

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Reference has been directed in pursuance of Section 9, Subsection (1) of the Patents Act, 1949, to Patent No. 985,747.

ethylamine to precipitate the cephaloglycin. After filtering the cephaloglycin and washing with methanol, it was dried *in vacuo* at 30° C. to a constant weight. It was a substantially anhydrous cephaloglycin containing less than 0.5 percent water, by weight. Increased phenylglycine removal was effected by use of a 2:1:1 (volume) mixture of water, acetonitrile, and methanol, respectively, but the average yield of purified cephaloglycin crystals upon precipitation therefrom tended to be somewhat lower than with the above proportioned solvent mixture.

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2. The process of claim 1 which also includes mixing the precipitated cephaloglycin from step (c) with water to form a crystalline cephaloglycin dihydrate.

3. The process of claim 1 or 2 wherein in step (b) a C₁ to C₈-trialkylamine is added as the base.

4. The process of claim 1, 2 or 3 wherein in step (a) the solvent mixture comprises acetonitrile, methanol, and water in substantially equal parts by volume and hydrochloric acid is used to effect solution of the cephaloglycin in the solvent mixture.

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