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(54) PROCESS FOR MAKING CRYSTALLINE ALKALI METAL SALTS OF α -CARBOXYBENZYL-PENICILLIN

(71) We, PFIZER INC., formerly known as Chas. Pfizer & Co. Inc., a Corporation organized under the laws of the State of Delaware, United States of America, of 235 East 42nd Street, New York 17, State of New York, 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:—

This invention relates to a convenient and economic process for producing crystalline, stable forms of α -carboxybenzylpenicillin. More particularly, it relates to the production of crystalline mono-alkali metal and crystalline di-alkali metal salts of α -carboxybenzylpenicillin.

The valuable broad spectrum penicillin, α -carboxybenzylpenicillin, is described in United States Patent 3,142,673 and in British Patent 1,004,670 along with the preparation of various salts of this valuable penicillin, including the di-alkali metal salts. United States Patent 3,142,673 also described the preparation of the mono-alkali metal salts. However, the alkali metal salts, as produced by the procedures of these references, are amorphous rather than crystalline materials.

The relatively high polar nature of α -carboxybenzylpenicillin — coupled with its susceptibility to degradation, particularly at elevated temperatures and especially in alkaline solutions of pH 9 or higher or in acid solutions of pH 4 or lower, to products such as benzylpenicillin, penicilloic acid, and penicillenic acid and the presence of inorganic salts arising from the methods used for its preparation — renders the formation of its crystalline alkali metal salts difficult.

An amorphous salt is, in general, less desirable than is a crystalline form thereof from a preparative, sales, storage, and use standpoint. The physical properties of an amorphous salt,

such as ease of handling, dispersion in solution, rate of solution, color of the salt, and solution thereof, are generally inferior to those of a crystalline form of the same salt. Additionally, amorphous forms of a salt are frequently more hygroscopic than is a crystalline form of said salt.

For pharmaceutical usage, the above-mentioned inferior properties of an amorphous salt, relative to those of a crystalline form of the salt, are especially detrimental. The formation of acceptable dosage forms, such as solutions, suspensions, elixirs, tablets, capsules, and various pharmaceutically elegant preparations required and demanded by the medical and pharmaceutical professions, is rendered difficult or impossible with an amorphous salt.

In the case of α -carboxybenzylpenicillin, the disadvantages of amorphous forms of its mono- and dialkali metal salts are of greater concern since it is an extremely effective broad spectrum penicillin. The preparation of crystalline forms of its alkali salts and, particularly, of its sodium salt is, therefore, of vital interest to the pharmaceutical and medical professions.

It has been found that stable, crystalline forms of the mono-alkali metal and di-alkali metal salts of α -carboxybenzylpenicillin, including its epimers or stereoisomers, can be conveniently prepared from aqueous or organic solvent solutions of α -carboxybenzylpenicillin and salts thereof, and from crude and amorphous forms of α -carboxybenzylpenicillin and salts thereof. The process utilizes readily available equipment and is characterized by ease of manipulation, overall economy, and high quality of the crystalline salts. The stable crystalline products of the present invention are useful in the same dosage forms and amounts for the same purposes as are the amorphous prior art products but do not share the above-mentioned shortcomings of the amorphous products.

According to the present invention there is provided a process for making a crystalline

alkali metal salt of α -carboxybenzylpenicillin which comprises admixing a solution of α -carboxybenzylpenicillin in a solvent selected from ethylacetate, n-butanol, methylethyl ketone or methyl isobutyl ketone said solution containing from 3 per cent to 20 per cent by weight of water, with an alkali metal salt selected from

(a) an alkali metal salt of an organic acid selected from 2-ethyl hexanoic, caproic, oleic, ascorbic, glycolic, propionic, acetic, succinic, cinnamic, caprylic and citric acids;

(b) an alkali metal hydroxide; and

(c) an alkali metal methoxide, wherein the alkali metal is sodium or potassium, and the product is isolated.

By the term " α -carboxybenzylpenicillin and salts thereof" is meant not only the alkali metal salts, e.g. sodium and potassium, but also the alkaline earth salts (calcium, magnesium, barium), the ammonium salt and substituted ammonium salt, e.g., procaine, dibenzylamine, N,N' - dibenzylethylenediamine, 1 - emphenamine, N - ethylpiperidine, diethylamine, triethylamine, and salts with other amines which have been used to form salts with benzylpenicillin. It further includes the mono- and di- salts of α -carboxybenzylpenicillin and its epimers. However, in view of the acidification step in the herein-described process which converts the above-mentioned salts to the acid form of α -carboxybenzylpenicillin, all the salts are equivalent in this process. From a practical standpoint, the crude and/or amorphous disodium salt of α -carboxybenzylpenicillin is the usual starting material for this process.

Alpha - carboxybenzylpenicillin is highly susceptible to degradation, e.g., decarboxylation to benzylpenicillin and hydrolysis to penicilloic and penicillic acids. Decarboxylation is readily brought about by heat. Hydrolysis occurs at both acid and alkaline pH levels and especially at pH values above 9 and below 4. Hydrolytic degradation, of course, increases with an increase in temperature.

The process of this invention, therefore, achieves a balance as regards the above parameters of temperature and pH. Additionally, other parameters, such as solubility factors and concentrations of reactants, are carefully woven into a unified process which conveniently affords the desired crystalline mono-alkali and di-alkali metal salts. By the term "alkali metal" salts, as used herein, is meant the sodium and potassium salts.

The process, in general, comprises a multiple extraction process which permits formation of concentrated solutions of α -carboxybenzylpenicillin without the use of heat and avoids the need for spray-dry or freeze-dry processes.

The present invention also provides a process for making a crystalline alkali metal salt of α -carboxybenzylpenicillin, wherein an n-

butanol solution of α -carboxybenzylpenicillin containing at least 5 per cent α -carboxybenzylpenicillin by weight and from 3 per cent to 15 per cent by weight of water is treated with either:—

(a) at least a 2:1 molar proportion of sodium 2 - ethyl hexanoate in n - butanol and the crystalline disodium salt is separated therefrom,

(b) from 0.8 to 1.2 molar proportions of sodium 2 - ethyl hexanoate in n-butanol and the disodium salt is separated therefrom.

The invention further provides a process for making a crystalline alkali metal salt of α -carboxybenzylpenicillin, which comprises extracting an aqueous solution of the acid form of α - carboxybenzylpenicillin having a pH of from 2 to 3.5 with a solvent selected from n-butanol and methyl isobutyl ketone, drying the thus-produced extract to a water content of from 3 per cent to 20 per cent water by weight, then admixing said extract with an alkali metal 2 - ethyl hexanoate wherein the alkali metal is selected from sodium and potassium.

Preparation of the crystalline sodium and potassium salts of α - carboxybenzylpenicillin from aqueous solutions of α - carboxybenzylpenicillin and salts thereof, such as are produced by the methods of United States Patent 3,142,673 and British Patent 1,004,670, or by alkaline hydrolysis of an aryl, e.g., phenyl, ester of α -carboxybenzylpenicillin, according to the present invention, comprises:

(a) adjusting the pH of an aqueous alkaline solution of α -carboxybenzylpenicillin to 2 to 3.5,

(b) extracting the acid solution thus produced with a water-immiscible organic solvent selected from ethylacetate, n-butanol, methylethyl ketone and methyl isobutyl ketone,

(c) back-extracting the water-immiscible organic solvent extract with water at a pH of from 6.5 to 8.0,

(d) adjusting the pH of the aqueous extract thus produced to a value of from 2 to 3.5,

(e) extracting the aqueous acid solution thus produced with a water-immiscible organic solvent selected from n-butanol and methyl isobutyl ketone,

(f) drying said water-immiscible organic solvent extract to a water content of from 3 per cent to 20 per cent by weight,

(g) admixing said dried extract with an alkali metal 2 - ethyl hexanoate and

(h) separating the crystalline alkali metal salt.

The back extraction step (b) may be conducted at a pH of from 6.8 to 7.5.

The novel process of this invention can, as mentioned above, be used to prepare crystalline sodium and potassium salts of α - carboxybenzylpenicillin from aqueous solutions of α -carboxybenzylpenicillin and salts thereof, and

from crude and from amphoteric forms of α -carboxybenzylpenicillin and its salts.

It is desirable, for the purpose of this invention, that the concentration of α -carboxybenzylpenicillin in the aqueous alkaline solution is from 0.5 per cent to 10 per cent by weight. It is preferred that the penicillin be present at a concentration of from 2 per cent to 5 per cent by weight in order to achieve optimum extraction of α -carboxybenzylpenicillin in subsequent steps.

The pH of the aqueous solution must be brought to a value of from 2 to 3.5. The favored pH range is from 2 to 3; the preferred range is from 2 to 2.5. The pH adjustment is conveniently accomplished by means of a mineral acid, such as hydrochloric, sulfuric, and hydrobromic acids. The acid solution is then extracted with a water-immiscible organic solvent, such as n-butanol, methyl isobutyl ketone. The preferred solvents for this extraction are n-butanol and methyl isobutyl ketone in view of their favorable partition coefficients for α -carboxybenzylpenicillin. Although ethyl acetate and methyl ethyl ketone may be used in the process of the present invention, because of their greater solubility in water compared to that of the preferred solvents, recovery yields are not so high. This loss in yield is corrected by adding high concentrations of inorganic salts to the aqueous phase prior to extraction with these solvents.

This extraction step can, of course, be conducted as a batch-wise or continuous process. Irrespective of the type of process used, a total volume of solvent equal to about one-half to one-third the volume of the aqueous acid solution has been found to permit satisfactory extraction of the α -carboxybenzylpenicillin. Larger volumes can, of course, be used but necessitate the handling and recovery of unnecessary volumes of solvent. Smaller volumes are not desirable since the recovery of desired product is decreased. The volume of organic solvent is advantageously regulated so as to provide a concentration of α -carboxybenzylpenicillin in the extract of from 5 per cent to 10 per cent.

The water-immiscible organic solvent extract is then back-extracted with water at a pH of from 6.5 to 8.0. The preferred pH range is from pH 7.0 to 7.2. This particular step affords opportunity to concentrate the α -carboxybenzylpenicillin into a small volume of solution and to thus facilitate further processing.

The aqueous extract is next adjusted to pH of from 2 to 3.5 with a mineral acid and preferably to a pH of from 2.2 to 3.0 and the acidified solution then extracted with a water-immiscible organic solvent such as these mentioned above. The preferred water-immiscible organic solvents are n-butanol

and methyl isobutyl ketone, in that order. When using methyl isobutyl ketone as solvent, a pH of about 2.2 is favored; and when using n-butanol, the favored pH is about 3.0. It is desirable, in this extraction step, to regulate the volume of organic solvent so as to provide a concentration of from 10 per cent to 20 per cent by weight of α -carboxybenzylpenicillin in the total volume of extract to facilitate subsequent precipitation of the desired crystalline salt.

The water-immiscible organic solvent extract of α -carboxybenzylpenicillin, of course, contains a certain amount of water. This "wet" extract can be used, as such, in subsequent steps to produce the desired crystalline sodium or potassium salt of α -carboxybenzylpenicillin. However, it has been observed that if too much water is present in the extract, that is, if it is too wet, the crystalline product is difficult to filter, apparently because of a very small crystal size. On the other hand, if the extract is too dry, the salts tend to separate as amorphous rather than crystalline products.

It appears that a certain amount of water is highly desirable, in fact necessary, for optimum separation of the crystalline mono- or di-alkali metal salts. Too much water introduces mechanical problems and is, therefore, not desirable; and too little water tends to prevent or, at least, minimize crystal formation. The crystalline disodium salt, in fact, appears to be a hydrate.

It has been found that a water content of from 3 per cent to 20 per cent by weight in the organic solvent extract is conducive to crystal formation; the preferred range is from 7 per cent to 15 per cent by weight water. This degree of dryness is readily and conveniently achieved by drying the organic solvent extract with anhydrous sodium sulfate, the preferred drying agent in this process. Other drying agents, such as anhydrous calcium sulfate and anhydrous magnesium sulfate, can also be used. When using magnesium sulfate, it is desirable to monitor the drying so as to avoid dropping the water content below the aforementioned 3 per cent level. Additionally, mechanical methods, such as filtration through cotton, centrifugation, and azeotropic drying, can also be used.

The "dried" organic solvent extract is then admixed with the appropriate molar proportion of the proper alkali metal salt of a suitable organic acid to form the desired crystalline mono- or di-alkali metal salt of α -carboxybenzylpenicillin. Suitable salts are the alkali metal hydroxides and methoxides; and the alkali metal salts of organic acids having from two to about eighteen carbon atoms such as those enumerated above and which are somewhat soluble in organic solvents such as acetone, methyl isobutyl ketone, ethyl acetate, n-butanol, isopropanol, and/or in the water pre-

sent in the α -carboxybenzylpenicillin containing extract. The presence of from 3 per cent to 20 per cent by weight water in said extract thus serves a dual purpose when using salts such as alkali metal acetates or hydroxides; i.e., as solvent and as a vital component of the crystallizing solution. The alkali metal salt of organic acids having from two to eight carbon atoms, such as acetic, butyric, caproic, and caprylic acids and the alkali metal methoxides are favored. The preferred organic acid salts are sodium and potassium 2-ethyl hexanoate because of their available and adequate solubility in organic solvents.

The sodium or potassium 2-ethyl hexanoate, or other alkali metal salt, is desirably added in the form of a solution in acetone, methyl isobutyl ketone, ethyl acetate, n-butanol, isopropanol, or other organic solvent in which it is soluble to at least 0.05 per cent by weight. The preferred solvent is acetone. When preparation of a crystalline mono-alkali metal salt, e.g., the monosodium salt, is desired, up to one mole of sodium 2-ethyl hexanoate, or other organic acid sodium salt, is used per mole of α -carboxybenzylpenicillin. A molar proportion much greater than 1:1 at this stage tends to reduce the yield of monosodium salt through formation of the disodium salt. A molar proportion considerably less than 1:1 also results in reduced yields through incomplete reaction. A molar ratio of from 0.8:1 to 1.2:1 is preferred for mono- salt formation.

Formation of the crystalline di-alkali metal salts, e.g., the disodium salt, is achieved by using at least a 2:1 molar proportion of sodium 2-ethyl hexanoate (or equivalent salt) to α -carboxybenzylpenicillin. In actual practice, it is preferred to employ up to a 20 per cent excess of sodium 2-ethyl hexanoate (or equivalent salt) to ensure as complete precipitation of the disodium salt as possible. Similar considerations apply to the di-potassium salt.

The crystalline sodium or potassium salt (mono- or di- salt, depending on the proportion of sodium or potassium 2-ethyl hexanoate or other equivalent salt used) which precipitates is separated, e.g., by filtration, and washed or repulped with an organic solvent such as acetone or isopropanol, then dried. Other solvents, such as n-butanol and methyl isobutyl ketone, can be used in this precipitation step but are not favored since they are difficult to remove from the crystalline product.

The products are conveniently dried at 40° to 65°C. Other methods commonly used in the art are also applicable, as those skilled in the art will recognize, e.g., air-drying, drying under nitrogen, drying *in vacuo*. Higher temperatures, while operative, require close surveillance to avoid decomposition of the product and/or loss of water of hydration. A certain amount of water of hydration is desirable for stability of the crystalline disodium salt.

The crystalline disodium salt thus obtained is somewhat hygroscopic but considerably less so than is the amorphous disodium salt. It appears to be a monohydrate. The crystalline monosodium and monopotassium salts are considerably less soluble in water than are corresponding di-sodium and di-potassium salts.

The temperature at which these various operations are conducted is not especially critical. However, because of the instability of α -carboxybenzylpenicillin in aqueous acid or aqueous alkaline solutions it is advantageous to carry out the entire process, with the exception of the final drying step, at a temperature of from 0°C. to 30°C., the preferred range from 5°C. to 25°C. The only step of the process which can deviate from this range without detriment to the product is the final step of drying the crystalline salts. When using an alkali metal acetate or hydroxide as the neutralizing salt it is desirable to operate at the upper limit of this temperature; e.g., 20°—30°C. in order to achieve solubility of said salt in the α -carboxybenzylpenicillin containing extract.

Adaptation of the above-described sequence [steps (a) to (h)] to the conversion of crude and/or amorphous forms of α -carboxybenzylpenicillin and salts thereof to the crystalline mono- or di-alkali metal salts follows the above sequence in part. It is, of course, obvious that if one begins with an amorphous form of the acid, disodium salt, or other salt such as the potassium salt, one need not begin at step (a) above but may begin at step (c), step (d) or step (e), depending on the form, e.g., acid or salt and purity of the material to be converted.

In addition to the above-described process, various alternative extraction procedures are possible, as those skilled in the art will recognize. One such sequence which affords the crystalline mono- or di-alkali metal salts, and which is also applicable to aqueous reaction mixtures of α -carboxybenzylpenicillin and salts thereof produced by the prior art methods and especially to those produced by the alkaline hydrolysis of esters of α -carboxybenzylpenicillin, such as α -carboxybenzylpenicillin, is a simplified version of the above-described process. This sequence comprises, in general, adjustment of the pH of the aqueous solution to a value of 6 to 7, followed by extraction of the neutral or essentially neutral solution with a water-immiscible organic solvent of the type mentioned in the above sequence and preferably with n-butanol or methyl isobutyl ketone. The aqueous solution is next adjusted to a pH of from 2 to 3.5 and extracted with n-butanol or methyl isobutyl ketone. The organic extract is then partially dried and treated with sodium or potassium 2-ethyl hexanoate or other equivalent salt, as described above. The same conditions and materials described in detail above apply in

this alternative sequence. As those skilled in the art will recognize, the di-alkali metal salts of α -carboxybenzylpenicillin can be obtained by further neutralization of the mono-alkali metal salts. For this purpose, of course, the same, or a different, alkali metal salt used to prepare the mono-alkali metal salt can be employed. It is convenient to use an alkali metal hydroxide or bicarbonate for this additional neutralization. Our copending Application No. 18292/69 (Serial No. 1,261,647) describes and claims a process for the hydrolysis of α -carboxybenzyloxybenzylpenicillin and α -carboxybenzyloxybenzyl penicillins.

The following examples are provided to further illustrate the process of the present invention. They are, however, not to be construed as limiting the invention in any way.

EXAMPLE I

20 Crystalline Disodium α - Carboxybenzylpenicillin from Crude Amorphous α - Carboxybenzylpenicillin

Crude disodium α - carboxybenzylpenicillin (250 g.), prepared according to Example 3 of British Patent 1,004,670 (see Preparation A), is dissolved in water (2.5 liters), methyl isobutyl ketone (600 ml.) added, and the mixture cooled to 5°C. The pH then adjusted to 2.2 by the addition, with stirring of 2N hydrochloric acid (approximately 650 ml.) over a 20-minute period. The mixture is thoroughly mixed and the methyl isobutyl ketone separated. The aqueous acid is extracted further with 2 x 600 ml. of methyl isobutyl ketone.

The extracts are combined, water (1250 ml.) added, and the mixture cooled to 8°C. The pH is adjusted to 7.0 by addition of a saturated aqueous solution of sodium bicarbonate (1600 ml.) and the aqueous phase separated. n-Butanol (650 ml.) is added to the aqueous phase which is cooled to 5°-8°C. and adjusted to pH 3.0 by means of 2N hydrochloric acid (850 ml.). The mixture is thoroughly agitated, the n-butanol phase separated, and the aqueous phase extracted twice more with 400 and 250 ml. volumes of n - butanol. The n - butanol extracts are combined, dried with anhydrous sodium sulfate (600 g.), and filtered first through filter paper and then through a "Milli-pore" filter unit (solvent resistant membrane type, porosity 2.2 μ). ("Milli-pore" is a Registered Trade Mark).

To the filtrate, there is then added sodium 2-ethyl hexanoate (2.1 equivalents in 950 ml. acetone) over a 1.5-hour period. Acetone is added as needed to maintain an easily stirrable suspension (total volume added is 2300 ml.). Stirring is continued for one hour at room temperature following addition of the sodium 2-ethyl hexanoate, after which the product is removed by filtration. The filter cake is reslurried in acetone at room temperature for 45 minutes, then filtered, and the reslurry step again repeated. The crystalline disodium salt is dried overnight under a stream of nitro-

gen. Further drying is accomplished by heating the product to about 65°C. in a drying oven. Overall yield=178.5 g., 71 per cent.

The use of 1.05 equivalents of sodium 2-ethyl hexanoate in the above sequence in place of 2.1 equivalents produces the crystalline monosodium salt.

EXAMPLE II

Crystalline Disodium α - Carboxybenzylpenicillin from Crude α - Carboxybenzylpenicillin

A sample of crude α - carboxybenzylpenicillin (7.5 g.) is dissolved in water (75 ml.) to provide a 10 per cent solution of the crude acid and cooled to 10°C. The pH is adjusted to 2.2 by means of 2N sulfuric acid, the solution extracted with methyl isobutyl ketone (3 x 20 ml.), and the combined extract dried with anhydrous calcium sulfate. The dried extract is filtered, cooled to 8°C., and treated dropwise with 18 ml. of an acetone solution of sodium 2 - ethyl hexanoate (containing 0.2075 mg. of sodium salt per ml.). Acetone is added as needed to permit efficient stirring. After one hour at 8°-10°C., the crystalline disodium salt is removed by filtration, washed with acetone, and dried at 40°C.

Repetition of this procedure but using anhydrous magnesium sulfate, anhydrous sodium sulfate, centrifugation, filtration through cotton, or azeotropic drying in place of anhydrous calcium sulfate also produces the crystalline disodium salt.

EXAMPLE III

Crystalline Disodium α - Carboxybenzylpenicillin from an Ester of α - Carboxybenzylpenicillin

A. Phenylmalonic Acid Monophenyl Ester

Phosphorous oxychloride (421 g.) and phenol (576 g.) are added to phenylmalonic acid (1000 g.) in a 3-neck, 12-liter flask. The contents of the flask are mixed well and then heated on a steam bath for 4.5 hours under an atmosphere of nitrogen. The temperature of the mixture is not allowed to rise above 87°C. Under these conditions, the mixture forms a clear melt within about 15 minutes, and evolution of hydrogen chloride is almost complete in 4 hours. Benzene (3.8 liters) is added to the reaction vessel and the mixture refluxed for 45 minutes. The hot benzene solution is decanted from the gummy phosphoric acid and phenol into ice/water, the pH of the aqueous phase adjusted to 6.5 by addition of 8N sodium hydroxide and finally to pH 7.8 by addition of 10 per cent sodium carbonate solution. The mixture is mixed well and the aqueous extract collected. The benzene layer is washed with a small volume of water which is then combined with the first aqueous extract, and the combined aqueous solution adjusted to pH 2.2 by addition of concentrated hydrochloric acid. The heavy precipitate produced is extracted into chloroform (total volume, 5 liters),

- the extract washed once with water, dried (anhydrous sodium sulfate), and evaporated to a solid which crystallizes when triturated with hexane. The product is filtered, washed with a small volume of 5% chloroform in hexane, and air-dried. Yield=430 g., m.p. 103°—110°C. (33%).

B. Phenylmalonyl Chloride Monophenyl Ester

- Thionyl chloride (92.5 g.) is added to a solution of phenylmalonic acid monophenyl ester in methylene chloride (1000 ml.) and the solution refluxed gently for 4 hours. Toluene (50 ml.) is added and the mixture evaporated under reduced pressure to provide the desired product as a syrupy amber liquid.

C. α - Carbophenoxybenzylpenicillin N - Ethylpiperidine Salt

- A solution of 6 - aminopenicillanic acid (86.4 g.) in water (850 ml.) is adjusted to pH 6.7 by addition of 8N sodium hydroxide, then cooled to 10°C. Phenylmalonyl chloride monophenyl ester is added in one portion and the mixture stirred vigorously for 40 minutes while the pH is maintained at pH 6.5—7.0 by addition of 2N sodium hydroxide. The cooling bath is removed from the reaction vessel 10 minutes after addition of the acid chloride is complete. The reaction mixture is acidified (pH 2.1) by addition of 2N hydrochloric acid and extracted with ethyl acetate. The combined ethyl acetate extracts are shaken with a 10% solution of potassium bicarbonate, the aqueous solution (pH 7.0—8.0) separated, and washed once with a small volume of ethyl acetate which is discarded. Fresh ethyl acetate is added to the aqueous solution followed by sufficient potassium chloride to salt the product out of the aqueous phase. The aqueous layer is discarded and fresh water added to the ethyl acetate solution. The potassium salt of the penicillin ester dissolves readily in the fresh water, and the ethyl acetate layer is discarded. The aqueous solution containing the desired product is acidified (pH 2.2) with 2N hydrochloric acid, extracted with ethyl acetate, and the extract dried (Na_2SO_4) and evaporated to provide 117 g. of crude α -carboxybenzylpenicillin phenyl ester. This material is redissolved in ethyl acetate (1000 ml.) and 35.3 ml. of N-ethylpiperidine added. The amine salt which crystallizes is collected by filtration and air-dried (97.0 g., 42.7%); m.p. 145°—147°C.

D. Hydrolysis of α - Carbophenoxybenzylpenicillin N - Ethylpiperidine Salt

- Boric acid (61.8 g.) and potassium chloride (74.56 g.) are dissolved in water and sufficient water added to the solution to bring the volume to 2 liters. A sufficient amount of 0.5N sodium hydroxide is added to adjust the solution to pH 9.0. α - Carbophenoxybenzylpeni-

cillin phenyl ester N - ethylpiperidine salt is dissolved in this buffer solution and the mixture stirred at room temperature (25°C.) for 2.5 hours. The reaction mixture becomes more acidic as hydrolysis occurs until, at the cessation of reaction, the mixture is pH 8.45. The mixture becomes turbid during the last 15 minutes of the reaction due to separation of by-product phenol from the weakly basic solution.

E. Isolation of Crystalline Disodium Salt

The reaction is cooled to 0°—10°C. and methyl isobutyl ketone (400 ml.) added, followed by sufficient 2N hydrochloric acid (ca. 250 ml.) to acidify the mixture to pH 2.2. The mixture is allowed to separate, the methyl isobutyl ketone extract collected, and the aqueous liquor extracted with two more volumes (each 300 ml.) of methyl isobutyl ketone. Water (200 ml.) is added to the combined extracts, the mixture cooled to 0°—10°C., and a solution of sodium bicarbonate (8 g. in 20 ml. H_2O) added dropwise over a 5-minute period. To the mixture, now pH 5.8, a small volume of saturated sodium bicarbonate solution is cautiously added to bring the pH to 7.0. The aqueous extract of α -carboxybenzylpenicillin sodium salt is collected and the methyl isobutyl ketone liquor extracted with two separate volumes (150 ml.) of water. n-Butanol (100 ml.) is added to the combined aqueous extracts and the mixture stirred and chilled to 0°—10°C. Sufficient 2N hydrochloric acid is added to bring the mixture to pH 3.0, the mixture allowed to separate, and the butanol layer collected. The acidic aqueous liquor is extracted twice with 75 ml. volumes of butanol, the combined butanol solutions (250 ml.) dried over anhydrous sodium sulfate (300 g.) for one hour, then filtered into a 1000 ml. 3-neck flask equipped with a mechanical stirrer and an addition funnel. An acetone solution of sodium 2-ethyl hexanoate (169 ml. containing 207.5 mg. of salt per ml.) is added dropwise over a 20 to 30-minute period. When approximately one-half of the solution has been added, the mixture is seeded with crystalline disodium salt. The mixture is stirred for 30 minutes following addition of the sodium ethyl hexanoate, then acetone (225 ml.) added, and the mixture stirred for an additional 15 minutes. The crystalline disodium salt is collected by filtration on a sintered glass funnel under an atmosphere of dry nitrogen, and the filter cake washed with acetone (500 ml.). The cake is slurried in fresh acetone (1000 ml.), the slurry stirred at room temperature for 30 minutes, filtered as described above, and allowed to dry on the filter in a stream of dry nitrogen for 16 hours. The product is a white, free-flowing crystalline powder, slightly hygroscopic and readily soluble in water.

Yield = 25.6 g. (60%), m.p. 198°—201°C. dec.

- 5 Repetition of the above procedure but using an n-butanol solution of sodium 2-ethyl hexanoate in place of an acetone solution produces substantially the same results.

EXAMPLE IV

- 10 An aqueous hydrolysis mixture prepared according to the procedure of Example III—D is adjusted to pH 7 by addition of 2N hydrochloric acid, then extracted with n-butanol (3×200 ml.). The n-butanol extract is discarded. n-Butanol (100 ml.) is added to the
15 aqueous phase, the mixture stirred, cooled to 5°—10°C., and brought to pH 2.2 by means of 2N hydrochloric acid. The n-butanol phase is allowed to separate, collected, and the aqueous layer extracted twice again with n-butanol (75 ml. each). The combined n-butanol extracts
20 are dried over anhydrous sodium sulfate, filtered, and treated at 10°C. with sodium 2-ethyl hexanoate (169 ml. of acetone solution containing 207.5 mg. of sodium salt per ml.) over a 30-minute period. The mixture is
25 stirred for one-half hour, 250 ml. acetone added, and stirring continued for an additional

15 minutes. The crystalline disodium salt is collected and dried under nitrogen.

EXAMPLE V

Repetition of the procedure of Example III—E but using 1.00 equivalent of sodium 2-ethyl hexanoate in place of 2.10 equivalents produces the crystalline monosodium salt.

In like manner, the crystalline mono- and dipotassium salts are prepared by the use of 1.00 and 2.20 equivalents of potassium 2-ethyl hexanoate in place of sodium 2-ethyl hexanoate.

EXAMPLE VI

The procedure of Example I is repeated but using a pH adjustment to 3.3 in the initial acidification step. Substantially the same results are obtained.

EXAMPLE VII

Crystalline Monosodium α - Carboxybenzylpenicillin

Butanol solutions of α - carboxybenzylpenicillin, prepared according to the procedure of Example III—A through III—E, are dried over anhydrous sodium sulfate or magnesium sulfate as noted below then treated with an organic solvent solution of a sodium salt to produce the monosodium salt.

Drying Agent	% H ₂ O in BuOH(1)	Sodium Salt	Solvent	Mole Equiv. (2)	T °C.	% Yield
(a) Na ₂ SO ₄	10.3	2-ethyl hexanoate	ethyl acetate	0.90	0-5	66.5
(b) Na ₂ SO ₄	10.9	2-ethyl hexanoate	ethyl acetate	0.95	15-25	96.5
(c) Na ₂ SO ₄	10.9	2-ethyl hexanoate	n-butanol	0.95	15-25	95.5
(d) Na ₂ SO ₄	10.0	acetate . 3H ₂ O	— ⁽³⁾	0.97	15-25	83.2
(e) Na ₂ SO ₄	15.6	acetate . 3H ₂ O	—	1.1	15-25	82.0
(f) Na ₂ SO ₄	15.5	acetate . H ₂ O	—	1.0	15-25	66.7
(g) MgSO ₄	2.9	acetate . 3H ₂ O	—	1.0	15-25	79.0
(h) MgSO ₄	2.9	acetate . 3H ₂ O	—	1.2	15-25	86.0

(1) % water by weight in n-butanol after drying

(2) molar equivalents of sodium salt used

(3) sufficient water is present in the n-butanol solution to completely dissolve the sodium acetate salt

EXAMPLE VIII

Disodium α - Carboxybenzylpenicillin.

Monosodium α - carboxybenzylpenicillin (0.01 mole) prepared as in Example VII is added to an aqueous solution of sodium hydroxide (0.01 mole in 25 ml. water). The disodium salt is recovered by freeze-drying.

Similarly the di-potassium salt is prepared from the mono-potassium salt of α -carboxybenzylpenicillin and potassium hydroxide.

The above-mentioned alkali metal hydroxides can, of course, be replaced by the corresponding alkali metal bicarbonates or carbonates with substantially the same results.

EXAMPLE IX

Crystalline Mono-alkali Metal Salts of α -Carboxybenzylpenicillin

The procedure of Example VII—(c) is repeated but using the following alkali metal salts in place of sodium 2-ethyl hexanoate:

sodium caproate	potassium citrate
sodium oleate	potassium propionate
sodium ascorbate	potassium oleate
sodium glycolate	potassium caproate
sodium propionate	potassium ascorbate
sodium succinate	potassium glycolate
sodium cinnamate	potassium succinate
sodium caprylate	potassium cinnamate

In each case the crystalline monosodium or potassium salt is produced.

EXAMPLE X

Crystalline Di-alkali Metal Salts of α -Carboxybenzylpenicillin

The procedure of Example IX is repeated but using 2.20 equivalents of the alkali metal salts enumerated therein per equivalent of α -carboxybenzylpenicillin to produce the di-alkali metal salts.

EXAMPLE XI

Following the procedure of Example VII—(e) but using sodium and potassium hydroxide in place of sodium acetate trihydrate produces the corresponding mono-alkali metal salts of α -carboxybenzylpenicillin.

In like manner but using 2.0 equivalents of alkali metal hydroxide per equivalent of α -carboxybenzylpenicillin, the di-alkali metal salts are produced.

EXAMPLE XII

The procedure of Example VII—(h) is followed but substituting sodium and potassium methoxide in methyl isobutyl ketone for sodium acetate trihydrate. In each instance, the mono-alkali metal salt is produced.

The use of 2.0 equivalents of alkali metal methoxide produces the corresponding di-alkali metal salt of α -carboxybenzylpenicillin.

PREPARATION A

Crude Disodium α -Carboxybenzylpenicillin (British Patent 1,004,670—Example 3)

A mixture of phenylmalonic acid (25 g., 0.140 mole) in dry ether (500 ml.), thionyl chloride (17.35 g., 0.145 mole), and a trace

of dimethylformamide is refluxed for 3 hours, then evaporated under reduced pressure at room temperature (finally by co-distillation with dry benzene to remove the last trace of thionyl chloride).

The syrupy residue of mono acid chloride is dissolved in dry ether (300 ml.) and added to a stirred, ice-cold mixture of 6-aminopenicillanic acid (30 g.), water (275 ml.), ether (150 ml.), and N sodium hydroxide solution (135 ml.). The mixture is stirred for 30 minutes, the aqueous phase adjusted to pH 2 by addition of dilute hydrochloric acid, and the layers separated. The ether solution is washed with ice-cold water, saturated with ether (8×300 ml.), and then extracted with water to which sufficient dilute sodium hydroxide has been added to bring the aqueous phase to pH 7. Evaporation of the aqueous solution at low temperature and pressure, followed by drying the residual white powder in a vacuum desiccator, gives the crude disodium salt of α -carboxybenzylpenicillin (33.3 g.).

WHAT WE CLAIM IS:—

1. A process for making a crystalline alkali metal salt of α -carboxybenzylpenicillin which comprises admixing a solution of α -carboxybenzylpenicillin in a solvent selected from ethylacetate, n - butanol, methylethyl ketone or methyl isobutyl ketone, said solution containing from 3 per cent to 20 per cent by weight of water, with an alkali metal salt selected from

(a) an alkali metal salt of an organic acid selected from 2 - ethyl hexanoic, caproic, oleic, ascorbic, glycolic, propionic, acetic, succinic, cinnamic, caprylic and citric acids;
(b) an alkali metal hydroxide; and
(c) an alkali metal methoxide, wherein the alkali metal is of sodium or potassium, and the product is isolated.

2. The process according to claim 1, wherein the solvent is selected from n - butanol or methyl isobutyl ketone.

3. The process according to claim 1, wherein at least a 2:1 molar ratio of an alkali metal salt of group (a) to α -carboxybenzylpenicillin is used and the crystalline dialkali metal salt separated therefrom.

4. The process according to claim 1 or 2, wherein a molar ratio of an alkali metal salt of the group (a) to α -carboxybenzylpenicillin of about 1:1 is used and the crystalline mono-alkali metal salt separated therefrom.

5. The process according to claim 3, wherein the alkali metal salt is selected from an alkali metal salt of acetic acid and an alkali metal salt of 2 - ethyl hexanoic acid.

6. The process according to claim 4, wherein the alkali metal salt of 2-ethyl hexanoic acid is used in a solvent selected from acetone, ethyl acetate, n-butanol and isopropanol.

7. The process according to claim 5, wherein an n-butanol solution of α -carboxybenzylpenicillin containing at least 5 per cent α -carboxy-

EXAMPLE VIII

Disodium α - Carboxybenzylpenicillin.
 Monosodium α - carboxybenzylpenicillin
 (0.01 mole) prepared as in Example VII is
 5 added to an aqueous solution of sodium
 hydroxide (0.01 mole in 25 ml. water). The
 disodium salt is recovered by freeze-drying.

Similarly, the di-potassium salt is prepared
 10 from the mono-potassium salt of α -carboxy-
 benzylpenicillin and potassium hydroxide.

The above-mentioned alkali metal hydrox-
 ides can, of course, be replaced by the corres-
 ponding alkali metal bicarbonates or carbon-
 15 ates with substantially the same results.

EXAMPLE IX

Crystalline Mono-alkali Metal Salts of
 α -Carboxybenzylpenicillin

The procedure of Example VII—(c) is re-
 20 peated but using the following alkali metal
 salts in place of sodium 2-ethyl hexanoate:

sodium caproate	potassium citrate
sodium oleate	potassium propionate
sodium ascorbate	potassium oleate
25 sodium glycolate	potassium caproate
sodium propionate	potassium ascorbate
sodium succinate	potassium glycolate
sodium cinnamate	potassium succinate
sodium caprylate	potassium cinnamate

In each case the crystalline monosodium or
 30 potassium salt is produced.

EXAMPLE X

Crystalline Di-alkali Metal Salts of
 α -Carboxybenzylpenicillin

The procedure of Example IX is repeated
 35 but using 2.20 equivalents of the alkali metal
 salts enumerated therein per equivalent of α -
 carboxybenzylpenicillin to produce the di-alkali
 metal salts.

EXAMPLE XI

Following the procedure of Example
 40 VII—(c) but using sodium and potassium
 hydroxide in place of sodium acetate trihydrate
 produces the corresponding mono-alkali metal
 salts of α -carboxybenzylpenicillin.

In like manner but using 2.0 equivalents of
 45 alkali metal hydroxide per equivalent of α -
 carboxybenzylpenicillin, the di-alkali metal
 salts are produced.

EXAMPLE XII

The procedure of Example VII—(h) is fol-
 50 lowed but substituting sodium and potassium
 methoxide in methyl isobutyl ketone for
 sodium acetate trihydrate. In each instance, the
 mono-alkali metal salt is produced.

The use of 2.0 equivalents of alkali metal
 55 methoxide produces the corresponding di-alkali
 metal salt of α -carboxybenzylpenicillin.

PREPARATION A

Crude Disodium α -Carboxybenzylpenicillin
 60 (British Patent 1,004,670—Example 3)

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of dimethylformamide is refluxed for 3 hours,
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 65 room temperature (finally by co-distillation
 with dry benzene to remove the last trace of
 thionyl chloride).

The syrupy residue of mono acid chloride is
 70 dissolved in dry ether (300 ml.) and added to
 a stirred, ice-cold mixture of 6-aminopenicil-
 lanic acid (30 g.), water (275 ml.), ether (150
 ml.), and N sodium hydroxide solution (135
 ml.). The mixture is stirred for 30 minutes,
 75 the aqueous phase adjusted to pH 2 by addi-
 tion of dilute hydrochloric acid, and the layers
 separated. The ether solution is washed with
 ice-cold water, saturated with ether (8×300
 ml.), and then extracted with water to which
 sufficient dilute sodium hydroxide has been
 80 added to bring the aqueous phase to pH 7.
 Evaporation of the aqueous solution at low
 temperature and pressure, followed by drying
 the residual white powder in a vacuum desic-
 85 cator, gives the crude disodium salt of α -
 carboxybenzylpenicillin (33.3 g.).

WHAT WE CLAIM IS:—

1. A process for making a crystalline alkali
 metal salt of α -carboxybenzylpenicillin which
 comprises admixing a solution of α -carboxy-
 90 benzylpenicillin in a solvent selected from
 ethylacetate, n - butanol, methylethyl ketone
 or methyl isobutyl ketone, said solution con-
 taining from 3 per cent to 20 per cent by
 weight of water, with an alkali metal salt
 95 selected from:

(a) an alkali metal salt of an organic acid
 selected from 2 - ethyl hexanoic, caproic,
 oleic, ascorbic, glycolic, propionic, acetic, suc-
 100 cinic, cinnamic, caprylic and citric acids;

(b) an alkali metal hydroxide; and

(c) an alkali metal methoxide, wherein the
 alkali metal is of sodium or potassium, and
 the product is isolated.

2. The process according to claim 1, where-
 105 in the solvent is selected from n - butanol or
 methyl isobutyl ketone.

3. The process according to claim 1, where-
 in at least a 2:1 molar ratio of an alkali metal
 salt of group (a) to α -carboxybenzylpenicillin
 110 is used and the crystalline dialkali metal salt
 separated therefrom.

4. The process according to claim 1 or 2,
 wherein a molar ratio of an alkali metal salt
 of the group (a) to α -carboxybenzylpenicillin
 115 of about 1:1 is used and the crystalline mono-
 alkali metal salt separated therefrom.

5. The process according to claim 3, where-
 in the alkali metal salt is selected from an
 alkali metal salt of acetic acid and an alkali
 120 metal salt of 2 - ethyl hexanoic acid.

6. The process according to claim 4, wherein
 the alkali metal salt of 2-ethyl hexanoic acid
 is used in a solvent selected from acetone, ethyl
 acetate, n-butanol and isopropanol.
 125

7. The process according to claim 5, wherein
 an n-butanol solution of α -carboxybenzylpeni-
 cillin containing at least 5 per cent α -carboxy-



benzylpenicillin by weight and from 3 per cent to 15 per cent by weight of water is admixed with at least a 2:1 molar proportion of sodium 2-ethyl hexanoate in acetone and the crystalline disodium salt is separated therefrom.

8. The process according to claim 6, wherein an n-butanol solution of α -carboxybenzylpenicillin containing at least 5 per cent α -carboxybenzylpenicillin by weight and from 3 per cent to 15 per cent by weight of water is admixed with from 0.8 to 1.2 molar proportions of sodium 2-ethyl hexanoate in n-butanol and the crystalline disodium salt is separated therefrom.

9. A process for making a crystalline alkali metal salt of α -carboxybenzylpenicillin according to any one of the preceding claims, which comprises extracting an aqueous solution of the acid form of α -carboxybenzylpenicillin having a pH of from 2 to 3.5 with a solvent selected from n-butanol and methyl isobutyl ketone, drying the thus-produced extract to a water content of from 3 per cent to 20 per cent water by weight, then admixing said extract with an alkali metal 2-ethyl hexanoate wherein the alkali metal is selected from sodium and potassium.

10. A process for making a crystalline alkali metal salt of α -carboxybenzylpenicillin according to any one of claims 1 to 9, which comprises:

(a) adjusting the pH of an aqueous alkaline solution of α -carboxybenzylpenicillin to 2 to 3.5;

(b) extracting the acid solution thus produced with a water-immiscible organic solvent selected from ethylacetate, n-butanol, methyl-ethyl ketone and methyl isobutyl ketone;

(c) back-extracting the water-immiscible organic solvent extract with water at a pH of from 6.5 to 8.0;

(d) adjusting the pH of the aqueous extract thus produced to a value of from 2.5 to 3.5;

(e) extracting the aqueous acid solution thus produced with a water-immiscible organic solvent selected from n-butanol and methyl isobutyl ketone;

(f) drying said water-immiscible organic solvent extract to a water content of from 3 per cent to 20 per cent by weight;

(g) admixing said dried extract with an alkali metal 2-ethyl hexanoate, and

(h) separating the crystalline alkali metal salt.

11. The process according to claim 10, wherein the solvent is selected from n-butanol and methyl isobutyl ketone.

12. The process according to claims 10 and 11, wherein the concentration of α -carboxybenzylpenicillin in the aqueous alkaline solution is from 0.5 per cent to 10 per cent by weight.

13. The process according to claim 12, wherein the water-immiscible organic solvent of step (b) is methyl isobutyl ketone.

14. The process according to claim 13, wherein the back-extraction is conducted at a pH of from 6.8 to 7.5.

15. The process according to claim 14, wherein the water-immiscible organic solvent of step (e) is n-butanol.

16. The process according to claim 15, wherein the n-butanol extract is dried to a water content of from 7 per cent to 15 per cent by weight.

17. The process according to claim 16, wherein the dried n-butanol extract is admixed with at least a two molar proportion of sodium 2-ethyl hexanoate dissolved in a solvent selected from acetone, ethyl acetate, n-butanol, and isopropanol.

18. The process according to claim 16, wherein the dried n-butanol extract is admixed with a 1:1 molar proportion of sodium 2-ethyl hexanoate dissolved in a solvent selected from acetone, ethyl acetate, n-butanol, and isopropanol.

19. The process according to claim 17, wherein an n-butanol solution of sodium 2-ethyl hexanoate is used.

20. The process according to claim 18, wherein an n-butanol solution of sodium 2-ethyl hexanoate is used.

21. A process for making a crystalline alkali metal salt of α -carboxybenzylpenicillin according to claim 1, substantially as described.

22. A crystalline alkali metal salt of α -carboxybenzylpenicillin whenever prepared by the process as claimed in any one of the preceding claims substantially as described.

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