

PATENT SPECIFICATION

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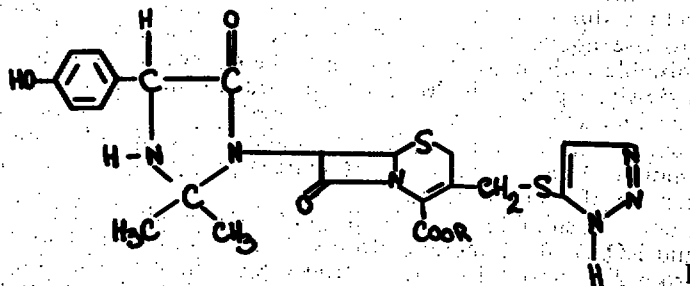


(54) WATER-SOLUBLE TRIAZOLYLTHIOMETHYL CEPHEM DERIVATIVES

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, having offices at 345 Park Avenue, New York, New York 10022, 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 novel water-soluble pharmaceutically acceptable forms of the antibiotic 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl - 3 - cephem - 4 - carboxylic acid which (1) upon the addition of water will give true solutions for parenteral administration, (2) have acceptable thermal stability in the solid state and (3) in aqueous solution have a useful life of at least several hours at room temperature. More particularly, the present invention provides alkali metal salts, especially sodium and potassium salts, of the acetone reaction product of 7 - [D - α - amino - α - (p - hydroxyphenyl) - acetamido] - 3 - (1,2,3 - triazol - 5 - ylthio-methyl) - 3 - cephem - 4 - carboxylic acid.

The alkali metal salts of the present invention have the formula



wherein R is an alkali metal, preferably sodium or potassium, and may be prepared by a process which comprises the consecutive steps of

(1) slurring 7 - [D - α - amino - α - (p - hydroxyphenyl) - acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid or a solvate or hydrate thereof in a solvent system comprising acetone and an acetone-miscible solvent, said acetone-miscible solvent being a solvent for the desired end-product of formula I and being present in an amount of at least about 20% of the volume of the total solvent system;

(2) adding to the acetone slurry an alkali metal base, preferably a sodium or potassium base to raise the pH to between about 5 and 7.5 and to form in solution the desired salt of formula I, said based being added in such a manner so as not to

allow the pH to exceed about 7.5; and

(3) precipitating the desired salt from the solution by addition of an organic solvent in which said salt is insoluble.

The starting material cephalosporin used in Step (1) of the above process may be any form of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamidol - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) 3 - cephem - 4 - carboxylic acid including the free acid zwitterion or a hydrate or solvate of said free acid zwitterion. The preferred starting materials for use in the present invention are the methanol and propylene glycol solvates disclosed in U.K. Patent Specification No. 447/75 and the sesquihydrate disclosed in U.K. Patent Specification No. 1,487,658; the entire disclosures of the two above-mentioned applications are incorporated herein by reference. The most preferred starting material is the crystalline propylene glycol solvate which may be prepared by the procedures described below in the section entitled "Preparation of Starting Materials".

Step (1) in the above process is believed to result in formation of a loosely bound physical complex of the acetone and cephalosporin. This complex is found to be more soluble in the solvent system of Step (1) than either the methanol or propylene glycol solvates used as preferred starting materials. As a result of this increased solubility, the complex in addition to its use in the present process may also be used in the purification of these solvates, e.g. by slurrying the impure propylene glycol solvate in an acetone-water system to form the physical complex, filtering to remove insoluble impurities and converting the complex to the purified propylene glycol solvate by addition of propylene glycol. The concentration of cephalosporin starting material in Step (1) is not critical, but best results are obtained when about 10 g. of cephalosporin are used per 50 ml. of solvent.

The solvent system used in Step (1) comprises a mixture of acetone and at least one acetone-miscible solvent in which the desired end-product is soluble. Examples of suitable acetone-miscible solvents include water and polar organic solvents such as methanol, dimethylsulfoxide and dimethylformamide. The acetone and acetone-miscible solvent are employed in proportions such that the acetone-miscible solvent makes up at least 20% of the total volume of the solvent system. The most preferred acetone-miscible solvents are water and methanol. The most preferred proportion of acetone to acetone-miscible solvent is about 60:40 (v/v).

The temperature at which Step (1) is carried out is not critical. For convenience, however, the optimum temperature is room temperature.

After obtaining the slurry the Step (1), the desired alkali metal salt of formula I is formed in solution by addition of a suitable alkali metal base. As the pH is raised to about 5-6 by addition of base, the acetone complex produced in Step (1) is converted into the soluble cyclic acetone reaction product of formula I. Because of the loss of the triazole moiety of the compound at alkaline pH, the base is added to the acetone slurry in such a manner that the pH of the reaction mixture is not allowed to rise above about 7.5. Preferably the base is dissolved in an organic solvent miscible with the solvent system of the slurry and then the solution of base is added slowly, e.g. over a 30 minute period, to the slurry with stirring until a solution or near solution is obtained. The pH at which this occurs will be in the range of about 5 to 7.5. Best results are obtained when the pH is adjusted to about 6. The amount of base used is not critical but preferably about 1 mole of base is used per mole of acetone complex.

Examples of suitable bases for Step (2) include alkali metal hydroxides (e.g. NaOH and KOH), alkali metal methoxides and alkali metal salts of organic acids having from 2 to about 18 carbon atoms and which are soluble in the solvent system used. Examples of such organic acids include 2-ethylhexanoic, caproic, oleic, ascorbic, glycolic, propionic and acetic acid. Preferred bases because of their desirable solubility properties are alkali metal hydroxides, most preferably solutions of sodium or potassium hydroxide in methanol. Other preferred bases are alkali metal salts of 2-ethyl-hexanoic acid; most preferably solutions of sodium or potassium 2-ethylhexanoate in a water-miscible organic solvent such as acetone or isopropanol. Best results are obtained in the present invention when the base is a solution of sodium or potassium hydroxide in methanol.

The addition of the base to the acetone slurry will result in formation of either a solution or near solution depending *inter alia* on the proportions of acetone and acetone-miscible solvent in the slurry. At the most preferred 60:40 (acetone:methanol) (v/v) proportions mentioned above, complete solution is usually achieved. With higher concentrations of acetone, however, some of the

desired cyclic product may precipitate out of the solution. The solution obtained in Step (2) is preferably stirred for about an hour after completion of the base addition so as to ensure complete reaction. The temperature for Step (2) is not critical. Most conveniently, the base addition may be performed at room temperature, but higher or lower temperatures may be used with the expected decrease or increase, respectively, in reaction times. For best results, the solution obtained in Step (2) is filtered to remove solid impurities prior to the recovery Step (3). Before filtration, the solution may optionally be carbon-treated with activated carbon to assist in removal of any colored impurities.

Steps (1) and (2) in the process of this invention are preferably carried out under a nitrogen atmosphere to prevent oxidation and resultant formation of colored impurities.

The desired product obtained in Step (2) is next recovered by precipitation from the reaction mixture. Precipitation is effected by addition of an organic solvent in which the cyclic salt is insoluble. Examples of such solvents include acetone, isopropanol, n-propanol, ethanol, t-butanol, acetonitrile, ethyl acetate and methyl isobutyl ketone. The preferred solvents to be employed as precipitating agents are acetone and isopropanol. The most preferred precipitating agent in Step (3) is acetone. In the preferred procedure the solution containing the desired cyclic salt is added with stirring to a large excess of the solvent used as the precipitating agent. The desired cyclic salt of formula I is then recovered by filtration, washed with a suitable organic solvent, e.g. acetone or isopropanol, and dried by conventional procedures, e.g. vacuum-drying at 50–56°C. for 24–48 hours or air drying at 60°C. for 48 hours. As an alternative procedure to recovering the end-product by filtration, the salt of formula I may also be recovered by lyophilization or evaporation of the reaction mixture of Step (2). Best quality product, however, has been found to be obtained when the above-described precipitation-recovery procedure is employed.

The alkali metal salts of the present invention may be used to provide pharmaceutical formulations of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid which have acceptable thermal stability in the solid state, high solubility in water, satisfactory aqueous stability and excellent *in vivo* and *in vitro* antibacterial activity against a variety of Gram-positive and Gram-negative bacteria.

The sodium salt which is the most preferred compound of the present invention may be dissolved in water up to 500 mg./ml. of activity. Concentrations of 250 mg./ml. of activity (pH 7–8) of the salt have acceptable aqueous stability, i.e. <10% loss in activity, for 2–4 hours at room temperature. Concentrations of 25 mg./ml. of activity (pH 7–8) have acceptable stability for 4–6 hours at room temperature. Concentrations of 10 mg./ml. of activity (pH 7–8) have acceptable stability for 6–8 hours at room temperature.

In dilute aqueous solution the compounds of the present invention hydrolyze to the parent cephalosporin, i.e. 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. Activities of the compounds of formula I are substantially equivalent to those of the parent cephalosporin. Blood levels of the present compounds upon oral and parenteral administration are somewhat lower than those of the parent cephalosporin but are sufficiently high as shown in the tables below to enable the compounds to be effective antibacterial agents when given either orally or parenterally.

In another aspect of the present invention provides a pharmaceutical composition suitable upon reconstitution with water for use as a parenterally-administrable antibiotic formulation, said composition comprising a compound of formula I and a solid pharmaceutically acceptable water-soluble organic acid, said organic acid being present in an amount such that the pH of the formulation upon reconstitution with water is between about 5 and 6.

The dry mixture of salt of formula I and organic acid in the above-mentioned composition may be reconstituted with water to provide a high concentration, i.e. up to about 500 mg./ml., solution suitable for parenteral administration. The composition provided by the present invention may be prepared by admixing a compound of formula I with a sufficient amount of a pharmaceutically acceptable water-soluble solid organic acid such that the pH of the composition upon reconstitution with water is between about 5 and 6. Glycine hydrochloride may also be used as a pharmaceutically acceptable pH regulator.

The organic acid used in the composition may be any non-toxic water-soluble solid organic acid. Examples of suitable acids include citric, tartaric, ascorbic, succinic, and the like. The most preferred acid for use in the composition of the present invention is citric acid.

The exact proportions of compound of formula I and solid organic acid are dependent on the physical and chemical properties of the acid selected, e.g. acidity, solubility, etc. Generally it is found that the desired pH range of 5-6 upon reconstitution with water is achieved when the solid organic acid is used in an amount of about 4-6% of the weight of the compound of formula I.

The alkali metal salt-organic acid mixtures provided according to this aspect of the present invention are found to have certain advantages over preparations containing compound I *per se*. The mixtures when reconstituted with water give solutions of enhanced aqueous stability. Thus, the addition of citric acid to the sodium salt of formula I extends the acceptable (<10% loss) stability of a 250 mg./ml. aqueous solution from about 4 hours to about 8 hours.

The compounds of formula I as well as the above-mentioned compositions are potent antibacterial agents useful in the treatment of infectious diseases in poultry and animals, including man, caused by many Gram-positive and Gram-negative bacteria. The compounds and compositions are also of value as nutritional supplements in animal feeds and as agents for the treatment of mastitis in cattle.

The salts and salt-organic acid mixtures of the present invention may be formulated as pharmaceutical compositions containing in addition to the active ingredient a pharmaceutically acceptable carrier or diluent. The compounds may be administered either orally or parenterally, but because of their high solubility in water, are especially useful for parenteral administration. In the treatment of bacterial infections in man, the compounds and compositions may be administered in an amount of from about 5 to 20 mg./kg./day in divided dosage, e.g. 3 or 4 times a day. They are administered in dosage units containing, e.g. 125, 250 or 500 mg. of active ingredient.

Preparation of Starting Materials

Preparation of 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid methanol solvate

In a three necked flask equipped with a reflux condenser, overhead stirrer and thermometer, there was placed a well mixed mixture of 8.36 g. (0.05 mole) of D-(-)-p-hydroxyphenylglycine and 3.02 g. (0.075 mole) of magnesium oxide in 120 ml. of 50% aqueous dioxane. The mixture was stirred for 1 hour and then treated with 10.74 g. (0.075 mole) of t-butoxycarbonylazide. The mixture was then stirred and heated at 45-50° for 17 hours under N₂. The solution was diluted with 400 ml. of H₂O and extracted twice with 300 ml. of ethyl acetate. The aqueous phase was acidified with 10% citric acid solution to pH 4 and saturated with NaCl. The aqueous mixture was extracted with 3 x 400 ml. of ethyl acetate. The solution was dried over Na₂SO₄ and the solvent evaporated. The residue was triturated with "Skellysolve B" to yield D- α -t-butoxycarbonylamino- α -(p-hydroxyphenyl)acetic acid as a solid weighing 10.4 g. (78.5%).

To a suspension of 7-amino-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (6.0 g., 19.0 mmole) in 100 ml. dry methylene chloride there was added 8.5 ml. of 1,1,1,3,3,3-hexamethyldisilazane (40.9 mmole). The mixture was stirred and heated at reflux for 4 hours at which time a clear solution was obtained. The solvent was evaporated and the residual oil was subjected to high-vacuum overnight at room temperature. The foamy residue was dissolved in 85 ml. of dry THF and cooled to about -15° before introduction into the subsequent reaction mixture.

D- α -t-Butoxycarbonylamino- α -(4-hydroxyphenyl)acetic acid, (4.4 g., 16.5 mmole) was dissolved in 145 ml. dry THF. The solution was stirred and cooled to -20°.

N-methylmorpholine (1.6 g., 16 mmole) and isobutylchloroformate (2.3 g., 16 mmole) were added in succession at such rate that the temperature of the mixture did not rise above about -10°. The resulting mixture was then stirred for 20 minutes at -12° to -15°. It was then cooled to -20° and the THF solution of silylated 7-amino-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid was added all at once. The temperature rose to about -12°. External cooling was discontinued until the temperature rose to 0°. At this point an ice-water bath was applied and the mixture stirred for three hours at 2-3°. This was followed by a period of one hour without

external cooling, the temperature rising to 20°. A total of 30 ml. methanol was added and the stirring continued for 15 minutes at room temperature. After evaporating the solvents under reduced pressure, the residue was suspended in 300 ml. ethyl acetate. The suspended solid was filtered off, (11.8 g.). The ethyl acetate solution was extracted three times with NaHCO₃ (5%) solution. The combined sodium bicarbonate extracts were cooled in an ice-bath, layered with ethyl acetate and acidified to a pH of 2.5 with 42.5% H₃PO₄. The phases were shaken and then separated. The ethyl acetate solution was then dried by passing it through sodium sulfate and then evaporated to about 15–20 ml. This solution was then added dropwise to stirred cyclohexane (~ 400 ml.) contained in an Erlenmeyer flask. After stirring for 1/2 hour the precipitated solid was collected by filtration. The collected, solid 7 - [D - α - t - butoxycarbonylamino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid was air dried. It weighed 1.75 g.

7 - [D - α - t - Butoxycarbonylamino - α - (p - hydroxyphenyl) - acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 3.5 g., was dissolved in 80 ml. HCOOH, 98–100%, and stirred for 2 hours at room temperature. The HCOOH was evaporated under reduced pressure (aspirator bath temperature not above 40°) and finally azeotroped 3 times with 30 ml. of toluene. The solid was dried overnight under high vacuum over P₂O₅. A total of 3.5 g. of foam was obtained. The foam, 2 g., was stirred with 300 ml. of H₂O: CH₃OH (8:2). The solvent was filtered from some solid (0.3 g.), charcoaled with 700 mg. of "Darco KB" ("Darco" is a registered Trade Mark), filtered through diatomaceous earth ("Celite" — registered Trade Mark) and freeze-dried to yield 0.9 g. of crude 7 - [D - α - amino - α - (p - hydroxyphenyl) - acetamido] - 3 - (1,2,3 - triazol - 5 - yl - thiomethyl) - 3 - cephem - 4 - carboxylic acid. To crystallize the following procedure was used. A suspension of 0.2 g. of the crude material in 6 ml. of 99% methanol was heated in a test tube to boiling. Immediately the heating was discontinued and the melt triturated with seeds. The melt solidified to a crystalline mass. In this manner a total of 0.211 g. of 7 - (D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid was obtained from 0.400 g. of crude material. The material was dried at 56°/0.1 mm over P₂O₅ for 20 hrs., m.p. > 200° dec. IR and NMR are consistent with structure. The NMR indicates also the presence of 1/3 mole of CH₃OH.

Anal. Calcd. for C₁₈H₁₈N₆O₅S₂·H₂O·1/3CH₃OH: C, 44.83; H, 4.38; N, 17.10; S, 13.09.

Found: C, 43.97; H, 4.36; N, 15.84; S, 6.18.

A total 6.5 g. (11.55 mmole) of 7 - [D - α - t - butoxycarbonylamino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid was dissolved in 175 ml. 98–100% formic acid under anhydrous conditions. The mixture was stirred at room temperature for 2.5 hours. Part of the solution, 125 ml., was evaporated under reduced pressure to an amber oil. The oil was then azeotroped 3 times with 70 ml. of toluene under reduced pressure. The residue was suspended in an 80:20 H₂O—CH₃OH solution (700 ml.) and stirred for 0.5 hour until most of the solid dissolved, then filtered. The filtration was treated with 1.5 g of ("Darco") charcoal for about 20 minutes. The charcoal was filtered off through a "Celite" pad. The solution was then freeze-dried in 9 separate 100 ml. round bottom flasks. The freeze-dried material weighed 2.415 g. It was recrystallized in batches of 0.200 g. as described above to yield a total of 0.923 g. 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. NMR was consistent, indicating the presence of a 1/3 mole of CH₃OH.

Anal. Calcd. for C₁₈H₁₈N₆O₅S₂·H₂O·1/3CH₃OH: C, 44.83; H, 4.38; N, 17.10; S, 13.09.

Found: C, 45.77, 44.36; H, 4.44, 4.34; N, 16.61, 16.52; S, 13.01, 13.01.

Preparation of Crystalline Methanol Solvate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

1. Fifty grams of 7 - (D - α - amino - α - (p - hydroxyphenyl) - acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid is slurried in 250 ml. of 95% V/V methanol/water (95% methanol) solution, at 22–25° C.

2. Concentrated hydrochloric acid is added with rapid stirring to a pH of 1.3–1.5. A solution or near solution is obtained.

3. Adjust the pH to 1.7 with triethylamine.
4. Add 7.5 grams of activated charcoal ("Darco G-60") and slurry for 0.5 hours.

5. The carbon is removed by filtration and washed with 75 ml. of methanol which is added to the filtrate. Steps 2, 3 and 4 should be completed within 5 hours.

6. The combined wash and filtrate of Step 5 is rapidly stirred. Triethylamine is added over a 5 minute period to pH 4.5. Crystallization starts in about 1-3 minutes. The mixture is slurried for one hour.

7. The crystals are collected by filtration, washed with 100 ml. of methanol and vacuum dried at 56° C. — 24 hours. Bio yield 75-90%; bio-assay = 850-900 mcg./mg.; NMR-IR = Consistent for 1 mole of methanol; % H₂O, KF = 2-4.0.

Preparation of Crystalline 1,2-Propylene Glycol Solvate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

1. Twenty-five grams of the methanol solvate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid prepared above is slurried in 150-200 ml. of 75% V/V propylene glycol-water solution at 20-25° C.

2. Concentrated hydrochloric acid is added to a pH of 1-1.2 to obtain a solution or near solution.

3. Triethylamine (TEA) is slowly added with rapid stirring to obtain a pH of 1.7-1.8.

4. Five grams of "Darco G-60" is added and the mixture is slurried for 0.5 hour. The carbon is removed by filtration (filtration is slow, an 18.5 cm. SS No. 576 paper is suggested). The carbon filter cake is washed with 40 ml. of 75% V/V propylene glycol-water solution. The wash is added to the filtrate. Steps 2, 3 and 4 above should be completed within 5 hours.

5. Triethylamine is added to pH 4.5 over a 10 minute period to the rapidly stirring filtrate — wash mixture of Step 4. Crystals form in about 1-3 minutes. The mixture is slurried for one hour.

6. The crystals of the propylene glycol solvate of 7 - (D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid are collected by filtration. Filtration is slow (a 12.5-15.0 cm. SS No. 604 paper is suggested). The crystals are washed consecutively with 50 ml. of 75% propylene glycol, 50 ml. of methanol, 50 ml. of acetone and vacuum dried at 56° C. for 24 hours. Biological yield: 80-95%.

Properties of 7 - (D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid propylene glycol solvate.

a. Bio-assay = 750-790 mcg./mg.

b. IR-NMR = Consistent for a structure containing 1.3-1.5 MOLES of Propylene glycol (17-19% propylene glycol). No loss of the 3-triazole side chain evident.

c. % Water, K.F. = 1-3.0.

d. Crystal morphology = 100% crystalline microcrystals, triangular shaped.

e. M.P. = 182-184° C. (D, hot stage).

f. $[\alpha]_D^{25}$ (C = 1%; 1N-HCl) = +53°.

g. Water solubility = Approximately 10 mg./ml. in water at 23° C.

h. Loss of bioactivity on storage at elevated temperatures: 100° C., 24 hours = <6%; 48 hours = <12%; 56° C., 1 month = <10%.

Preparation of Crystalline Methanol Solvate from Crystalline Propylene Glycol Solvate.

The propylene glycol solvate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid (50 g.) as prepared above is slurried in 250 ml. of 95% (volume/volume) methanol-water solution at 22-25° C. Concentrated HCl is added with rapid stirring to a pH of 1.1-1.5 whereupon a solution or near solution is obtained. The pH is adjusted to 1.7 with triethylamine and 7.5 g. of activated charcoal is added with slurring for 0.5 hours. The charcoal is removed by filtration and washed with 75 ml. of methanol. The wash solution is then added to the filtrate. (The steps from addition of the HCl to this point should be completed within 5 hours). The combined wash and filtrate is rapidly stirred and triethylamine added over a 5 minute period until a pH of 4.5 is reached. Crystallization starts in

about 1—3 minutes. The mixture is slurried for 1 hour, and the crystals are removed by filtration, washed with 100 ml. methanol and vacuum dried at 56°C. for 24 hours. Bio Yield 75—90%; bio-assay = 850—900 mcg./mg.; NMR—IR = consistent for 1 mole of methanol; % H₂O, K.F. = 2—4.0.

Preparation of Crystalline 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid sesquihydrate.
 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid methanol solvate (15 g.) was slurried in 60 ml. of water. The pH was raised to 6.5 by addition of 4N NaOH and the mixture was passed through a 200 mesh screen. The reaction mixture was slurried at room temperature for 2 hours, the pH being maintained at 6.5 during this period. The crystals were removed by filtration, washed with 20 ml. of water and air dried at 37°C. for 24 hours to give 11.5 g. of title crystalline product. Bio assay = 924 mcg./mg. (average). % H₂O, K.F. = 5.26. NMR and IR were consistent for the proposed structure and indicated that the product contained no methanol but did have a trace of propylene glycol.

Preparation of Crystalline 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid sesquihydrate and formation of other crystalline hydrates.

7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid methanol solvate (200 mesh; 10.0 g) substantially free of propylene glycol is slurried in 30—40 ml. of deionized water at ambient room temperature (20—25°C.) to give a pH 3—4 aqueous suspension. NaOH (40%) is slowly added with rapid stirring to bring the pH to 6.3—6.7. The mixture is slurried at pH 6.3—6.7 for 2 hours. The crystals are removed by filtration, washed with water and air dried at room temperature for 24 hours to give a 75—80% weight yield of 950—1000 mcg./mg. crystals of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid dihydrate. IR and NMR analyses were consistent for the proposed structure and indicated that the product contained no methanol but did have a trace of propylene glycol. H₂O K.F. = 6.56.

A sample of the crystalline dihydrate was air dried at 37°C. for 24 hours giving the crystalline sesquihydrate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - carboxylic acid. H₂O, K.F. = 4.26.

A second sample of the dihydrate was air dried at 45°C. for 24 hours to give the crystalline sesquihydrate. H₂O, K.F. = 5.5.

A sample of dihydrate was air dried at 56°C. for 24 hours to give the crystalline monohydrate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. H₂O, K.F. = 4.38 (theoretical % H₂O for monohydrate — 3.75).

A sample of the dihydrate was vacuum dried over P₂O₅ at room temperature for 24 hours giving the crystalline hemihydrate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. H₂O, K.F. = 2.63 (theoretical % H₂O for hemihydrate — 1.91).

A sample of the dihydrate was vacuum dried at 56°C. for 24 hours giving the crystalline hemihydrate. H₂O, K.F. = 1.6—2.0.

The following examples are given in illustration of, but not in limitation of, the present invention.

Description of the Preferred Embodiments

Example 1

These grams of crystalline methanol solvate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid was slurried in 20 ml. of a solvent system comprising 60% acetone and 40% methanol. To the slurry was added 9 ml. of 40% sodium 2-ethylhexonate (SEH) in isopropanol (IPA), and a solution was obtained in 10 minutes. The solution was stirred at room temperature for 2.5 hours. The solution was then added with rapid stirring to 300 ml. of acetone. The amorphous precipitate which formed was removed by filtration, washed with 75 ml. of acetone and vacuum dried at room temperature for 24 hours to yield 3 g. of product which was confirmed by IR and NMR to be sodium 7 - (2,2 - dimethyl - 4 - p - hydroxyphenyl -

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5 - oxo - 1 - imidazolidinyl - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate.

Bio-assay = 694—703 mcg./mg.

% H₂O (K.F.) = 2.18

% Na by = 5.73

ash

Example 2

To a slurry of one gram of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid methanol solvate in 15 ml. of a 60% acetone—40% methanol solvent system was added over a 5 minute period 0.9 ml. of a solution of 10% NaOH in methanol. The near solution which was obtained was slurried for 1 hour at room temperature, heated to 50°C. for 10 minutes, cooled to 25°C. and kept at room temperature for 1 hour. A small amount of solids was removed by filtration. The filtrate was then added with rapid stirring to 100 ml. of acetone. The amorphous solids which formed were removed by filtration, washed with 25 ml. of acetone and vacuum dried at 56°C. for 24 hours to yield 0.75 g. of sodium 7 - (2,2 - dimethyl - 4 - p - hydroxyphenyl - 5 - oxo - 1 - imidazolidinyl - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate which was confirmed by IR and NMR analyses.

Bio-assay = 720—744 mcg./mg.

% H₂O (K.F.) = 1.92

% Na by = 4.12

ash

Example 3

Four grams of the crystalline propylene glycol solvate of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid was slurried in a mixture of 20 ml. of an acetone-methanol (80:20) (v/v) solvent and 12 ml. of a solution of 40% SEH in IPA. A solution was obtained in 20 minutes. The solution was stirred for an additional hour at room temperature. To the solution was added 0.8 g. of "Darco G—60" activated charcoal and the mixture was slurried for 0.5 hour. The carbon was removed by filtration and washed with 10 ml. of 80% acetone-20% methanol. The wash was then added to the filtrate. The filtrate was stirred an additional hour.

One-half of the filtrate was added with rapid stirring to 200 ml. acetone. The amorphous precipitate which formed was removed by filtration, washed with 10 ml. acetone and vacuum dried at 56°C. for 24 hours to yield 1.6 g. of the sodium salt of the, acetone reaction product of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

Bio-assay = 674 mcg./ml.

% H₂O (K.F.) = 2.52

% Na by = 6.77

ash

IR and NMR indicated the presence of small amounts of SEH and propylene glycol in the sample.

The other half of the filtrate above was added to 200 ml. of isopropanol. The precipitate was collected, washed and dried as above to yield 1.6 g. of the desired sodium salt.

Bio-assay = 656—710 mcg./ml.

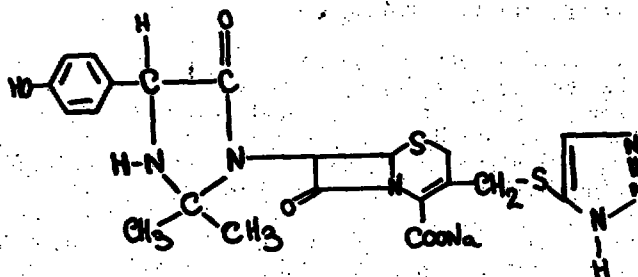
% H₂O (K.F.) = 2.35

% Na by = 6.20

ash

IR and NMR indicated small amounts of IPA and propylene glycol impurities but no SEH.

Example 4



PROCEDURE

1. Ten grams of crystalline 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid propylene glycol solvate is vigorously slurried under nitrogen in 50 ml. of a 60% acetone-40% methanol (v/v) mixture (container is optional).

2. Eight and 0.75 ml. of a 10% (v/v) sodium hydroxide in methanol solution is added under nitrogen, with vigorous stirring over a 30-45 minute time period. A solution or near solution is obtained.

3. The solution or near solution is slurried an additional 1 hour at ambient room temperature and polish filtered (i.e. subjected to a fine filtration to remove colloids).

4. The solution is sterilely filtered to remove pyrogens and bacteria.

The time required for completion of step 3 and 4 should not be under 3 hours or greater than 4.5 hours.

5. Using aseptic conditions in a sterile area, and the sterile solution of step 4 over a 10 minute period to 350 ml. of rapidly stirring sterile acetone. An amorphous precipitate forms.

6. Stir for 5 minutes. Remove the precipitate by filtration (the filter cake tends to channel and crack and must be tamped flat) and wash with 75 ml. of sterile acetone.

7. Vacuum-dry the product at 50-56°C. for 24 hours in a stile oven. Yield: approximately 8.8 grams of the sodium salt of the cyclic reaction product of acetone and 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

Bio-assay = 720-770 mcg./mg.

% H₂O (K.F.) = 2-4.0

% Na by = 4-4.7

ash

Solubility in water — greater than 400 mg./ml. (pH 7.4-8.0). β-lactam and 3-triazole side chain are intact.

Example 5

Sodium 7 - (2,2 - Dimethyl - 4 - p - hydroxyphenyl - 5 - oxo - 1 - imidazolidinyl - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate with citric acid for parenteral use.

(Label claim is 250 mg./ml. of 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid activity)

Formula

Sodium 7 - (2,2 - dimethyl - 4 - p - hydroxyphenyl - 5 - oxo - 1 - imidazolidinyl) - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate, milled 100 mesh, sterile

*0.250 g.

Sterile anhydrous citric acid, 100 mesh

**0.050g.

*Calculate the amount of sodium salt to use as follows:

**This is an approximate weight required to obtain the desired pH of 5.5. An acceptable pH range is 5.2-5.9.

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0.250 gram $\times$ 1000

= Grams of sodium salt
per single 1 ml. dose

Potency of sodium salt in mcg./mg.

- 1) Double check all weights before using.
- 2) Use sterile lint free procedures.
- 3) Spray blender room with peracetic acid before starting.
- 4) Relative humidity should be 50% or less.
- 5) Aseptically mill the sterile monosodium salt through a suitable sterile mill such as a Fitzmill using a sterile mesh screen to obtain approximately 100 mesh particle size.

Note: The sodium salt is amorphous, does not readily pass through a 60 mesh screen and becomes highly charged upon screening and handling.

- 6) Pass the sterile milled monosodium salt into a suitable sterile blender (blender may have to be earthed to dissipate charge on the powder).
- 7) Aseptically add the sterile, anhydrous 100 mesh citric acid.
- 8) Blend for at least 5 hours to assure complete mixing.
- 9) Drop into suitable sterile container for storage.
- 10) Fill into officially designated sterile flint glass vials, capped with designated stoppers.

Example 6

A blend was made according to the procedure of Example 5 of 0.360 g. of sodium 7 - (2,2 - dimethyl - 4 - p - hydroxyphenyl - 5 - oxo - 1 - imidazolidinyl) - 3 - (1,2,3 - triazole - 5 - ylthiomethyl) - 3 - cephem - 4 carboxylate and 0.018 g. of d-tartaric acid. Upon addition of 0.8 ml. deionized water, a clear amber solution was obtained having a pH of 5.0. The 1 ml. reconstituted solution has a concentration of 250 mg./ml.

Example 7

A blend was made according to the procedure of Example 5 of 1.08 g. sodium 7 - (2,2 - dimethyl - 4 - p - hydroxyphenyl - 5 - oxo - 1 - imidazolidinyl) - 3 - (1,2,3 - triazole - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate and 0.05 g. glycine hydrochloride. Upon addition of 2.25 ml. deionized water, a clear amber solution was obtained having a pH of 5.5.

TABLE I

Antibiotic Activity In Nutrient Broth.

Organism		MIC (μ g./ml)			
		Sodium Salt Of Ex. 4	BL-S640 $\pm$	Cepha- lexin	Cepha- lothin
<i>D. pneumoniae</i> *	(10 ⁻⁵)**				
	A9585	0.03	0.03	0.13	0.03
<i>Str. pyogenes</i>	(10 ⁻⁵)**				
	A9604	0.06	0.03	0.13	0.03
<i>S. aureus</i> Smith	(10 ⁻⁴)				
	A9537	0.25	0.5	0.13	0.06
<i>S. aureus</i> + 50% serum	(10 ⁻⁴)				
	A9537	2	1	0.5	0.25
<i>S. aureus</i> BX1633	(10 ⁻⁵)				
	A9606	1	1	1	0.13
<i>S. aureus</i> BX1633	(10 ⁻³)				
	A9606	2	2	4	0.25

TABLE I

Antibiotic Activity In Nutrient Broth

Organism		MIC ($\mu\text{g}/\text{ml}$)			
		Sodium Salt Of Ex. 4	BL-S640 $\ddagger$	Cepha- lexin	Cepha- lothin
<i>S. aureus</i>	(10^{-3})				
Meth-Res	A15097	4	4	32	0.5
<i>Sal. enteritidis</i>	(10^{-4})				
	A9531	0.5	0.5	2	0.25
<i>E. coli</i> Juhl	(10^{-4})				
	A14119	1	2	8	16
<i>E. coli</i>	(10^{-4})				
	A9675	4	8	16	63
<i>K. pneumoniae</i>	(10^{-4})				
	A9977	1	1	4	2
<i>K. pneumoniae</i>	(10^{-4})				
	A15130	2	2	16	16
<i>Pr. mirabilis</i>	(10^{-4})				
	A9900	0.5	0.5	4	1
<i>Pr. morgani</i>	(10^{-4})				
	A15153	32	32	>125	>125
<i>Ps. aeruginosa</i>	(10^{-4})				
	A9843A	>125	>125	>125	>125
<i>Ser. marcescens</i>	(10^{-4})				
	A20019	>125	>125	>125	>125
<i>Ent. cloacae</i>	(10^{-4})				
	A9656	>125	>125	>125	>125
<i>Ent. cloacae</i>	(10^{-4})				
	A9657	0.5	1	4	8
<i>Ent. cloacae</i>	(10^{-4})				
	A9659	63	32	>125	>125

* 45% AAB + 5% serum + 50% broth listed above.

** Dilution of overnight broth culture.

$\ddagger$ BL-S640 = 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-
3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid

TABLE II

Mouse Blood Levels After Oral
Administration Of 100 mg /kg. Body Weight

Compound	Blood Level (μ /ml.)			
	0.5	1	2	3.5
Hrs. After Administration				
Compound of Ex. 4	19	29.5	21.4	11.3
‡BL-S640	53.4	49.2	24.1	10.7
Cephalexin	41.2	21.6	7.0	<3.3

The compounds were prepared in Tween-carboxymethyl cellulose solution and 8 mice were used for each compound. ("Tween" is a registered Trade Mark).

‡BL-S640 = 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid

TABLE III

Mouse Blood Levels After Intramuscular
Administration Of 10 mg./kg. Body Weight

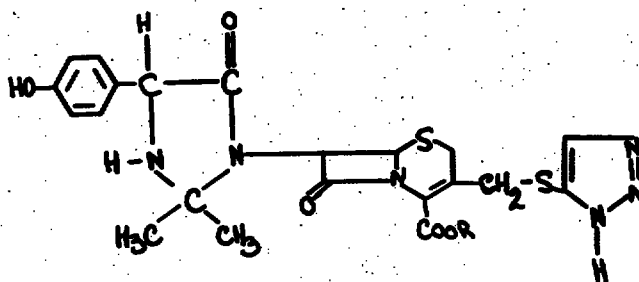
Compound	Blood Level (μ /ml.)			
	15	30	60	90
Minutes After Administration				
Compound of Ex. 4	12.2	8.8	6.7	5.6
‡BL-S640	16.9	15.5	8.9	6.1
Cephalothin	7.3	3.5	<2.1	<2.1

The compounds were prepared in 0.001% phosphate buffer and 16 mice were used for each compound.

‡BL-S640 = 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid

WHAT WE CLAIM IS:—

A process for the preparation of a compound of the formula



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TABLE II

Mouse Blood Levels After Oral
Administration Of 100 mg /kg. Body Weight

Compound	Blood Level (μ /ml.)			
	0.5	1	2	3.5
Hrs. After Administration				
Compound of Ex. 4	19	29.5	21.4	11.3
†BL-S640	53.4	49.2	24.1	10.7
Cephalexin	41.2	21.6	7.0	<3.3

The compounds were prepared in Tween-carboxymethyl cellulose solution and 8 mice were used for each compound. ("Tween" is a registered Trade Mark).

†BL-S540 = 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid

TABLE III

Mouse Blood Levels After Intramuscular
Administration Of 10 mg./kg. Body Weight

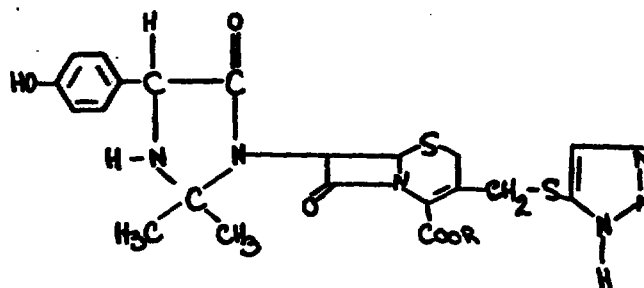
Compound	Blood Level (μ /ml.)			
	15	30	60	90
Minutes After Administration				
Compound of Ex. 4	12.2	8.8	6.7	5.6
†BL-S640	16.9	15.5	8.9	6.1
Cephalothin	7.3	3.5	<2.1	<2.1

The compounds were prepared in 0.001% phosphate buffer and 16 mice were used for each compound.

†BL-S640 = 7-[D- α -amino- α -(p-hydroxyphenyl)acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid

WHAT WE CLAIM IS:—

A process for the preparation of a compound of the formula





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wherein R is an alkali metal; which process comprises the consecutive steps of

(1) slurring 7 - [(D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid or a solvate or hydrate thereof in a solvent system comprising acetone and an acetone-miscible solvent, said acetone-miscible solvent being a solvent for the desired end-product of formula I and being present in the an amount of at least about 20% of the volume of the total solvent system;

(2) adding to the acetone slurry an alkali metal base to raise the pH to between about 5 and 7.5 and to form in solution the desired salt of formula I, said base being added in such a manner so as not to allow the pH to exceed about 7.5; and

(3) precipitating the desired salt from the solution by addition of an organic solvent in which said salt is insoluble.

2. The process according to Claim 1 wherein the acetone-miscible solvent is methanol or water.

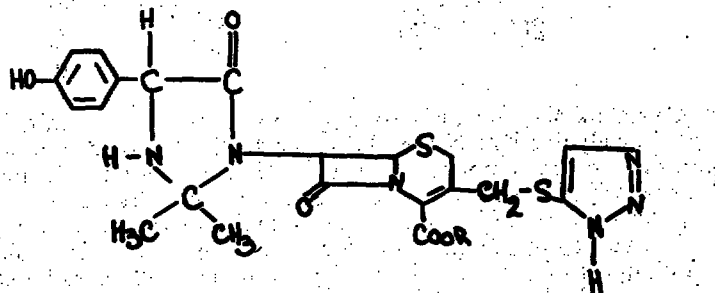
3. The process according to Claim 1 or 2 wherein the 7 - [(D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid starting material is in the form of the methanol or propylene glycol solvate or sesquihydrate.

4. The process of any one of Claims 1 to 3 wherein R is sodium or potassium.

5. The process of Claim 4 wherein the base used in Step (2) is sodium hydroxide, potassium hydroxide, sodium 2-ethylhexanoate or potassium 2-ethylhexanoate.

6. The process of any one of Claims 1 to 5 wherein the organic solvent in Step (3) is acetone or isopropyl alcohol.

7. A process for the preparation of a compound of the formula



wherein R is sodium; which process comprises the consecutive steps of

(1) slurring 7 - [(D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid in the form of the methanol or propylene glycol solvate or sesquihydrate in a solvent system comprising acetone and methanol, the methanol being present in an amount of at least 20% of the total volume of the solvent system;

(2) slowly adding to the acetone slurry with stirring a preformed solution of sodium hydroxide in methanol to raise the pH to between about 5 and 7.5 and to form in solution the desired compound of formula I; and

(3) precipitating the desired sodium salt from the solution by addition of acetone.

8. The process of Claim 7 wherein the 7 - [(D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1,2,3 - triazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid starting material is in the form of the methanol or propylene glycol solvate.

9. The process of any one of the preceding Claims wherein the solvent system in Step (1) is a 60% acetone-40% methanol (v/v) mixture.

10. The process of any one of the preceding Claims wherein the solution obtained in Step (2) is filtered prior to addition of the acetone in Step (3).

11. The process of any one of the preceding Claims wherein the solution obtained in Step (2) is treated with activated carbon prior to the filtration step.

12. The process of any one of the preceding Claims wherein the reaction steps (1) and (2) are carried out under nitrogen.

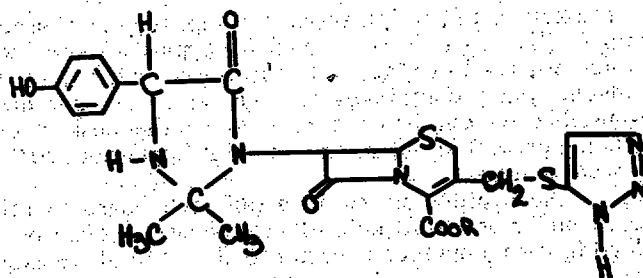
13. Compounds of the formula



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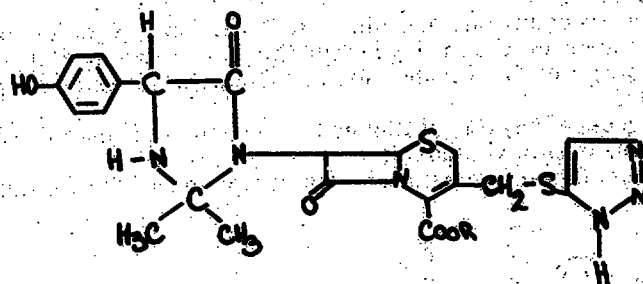
I

wherein R is an alkali metal.

14. A compound of Claim 13 wherein R is sodium.

15. A compound of Claim 13 wherein R is potassium.

16. A pharmaceutical composition suitable upon reconstitution with water for use as a parenterally-administrable antibiotic formulation, said composition comprising a compound of the formula



wherein R is an alkali metal and a solid pharmaceutically acceptable water-soluble organic acid or glycine hydrochloride, said organic acid or glycine hydrochloride being present in an amount such that the pH of the formulation upon reconstitution with water is between about 5 and 6.

17. A composition of Claim 16 wherein the solid organic acid is present in an amount of about 4-6% of the weight of the compound of formula I.

18. A composition of Claim 16 or 17 wherein the compound I is sodium 7-(2,2-dimethyl-4-p-hydroxyphenyl-5-oxo-1-imidazolidinyl-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylate.

19. A composition of Claim 16, 17 or 18 wherein the solid organic acid is citric acid, or tartaric acid.

20. A composition of Claim 19 wherein the solid organic acid is citric acid.

21. A composition of any one of Claims 16 to 20 wherein R is sodium or potassium.

22. A pharmaceutical composition according to any one of Claims 16 to 21 further comprising a pharmaceutically-acceptable diluent or carrier.

23. A pharmaceutical composition comprising a compound of Formula I substantially as hereinbefore described.

24. A pharmaceutical composition comprising a compound of Formula I substantially as hereinbefore described in any one of the Examples.

25. A process for the preparation of a compound of Formula I substantially as hereinbefore described.

26. A process for the preparation of a compound of Formula I substantially as hereinbefore described in any one of the Examples.

27. A compound of Formula I whenever prepared by the process of any one of Claims 1 to 12, Claim 25, or Claim 26.



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