

PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Cephalosporanic Acid Derivatives and a Process for the Preparation thereof

We, BRISTOL-MYERS COMPANY, a Corporation of the State of Delaware, United States of America, of Thompson Road, East Syracuse, 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:—

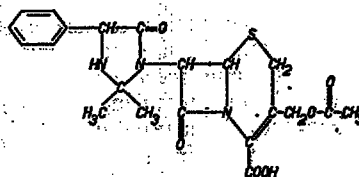
This invention relates to new synthetic compounds of value as antibacterial agents, as nutritional supplements in animal feeds, as agents for the treatment of mastitis in cattle, and as therapeutic agents in poultry and animals, including man, in the treatment of infectious diseases caused by Gram-positive and Gram-negative bacteria.

The preparation of the compound commonly called cephaloglycin or 7-(D- α -aminophenylacetamido)cephalosporanic acid has been described and claimed in British Patent Specification No. 985,747. This compound exhibits high activity *in vitro* against both Gram-positive and Gram-negative bacteria but suffers from the severe drawback of decomposing rapidly in aqueous solutions, as indicated by footnote 9 of Chauvette et al., J. Amer. Chem. Soc. 84, 3401—3402 (1962) and by Wick and Boniece, Applied Microbiology 13(2), 248—253 (March, 1965).

We have now found that cephaloglycin may be reacted with acetone in produce a new chemical compound which is substantially more stable in aqueous solution and yet is also nontoxic and exhibits good oral absorption and high antibacterial activity against both Gram-positive and Gram-negative bacteria.

According to the present invention there is provided 7-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)-3-aceoxymethyl-8-

oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-oic acid, which acid has the structural formula:



This acid has been given the trivial name hetasporin and may also be named 7-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)-cephalosporanic acid.

The invention includes the nontoxic, pharmaceutically acceptable salts of the above compound.

The invention also provides a process for the preparation of the compound of the formula (I) above and its nontoxic, pharmaceutically acceptable salts, which process comprises reacting cephaloglycin, or a salt thereof, with at least an equimolar weight of acetone in the absence of substantial amounts of water at a pH in the range of 5 to 9 and at a temperature in the range of -20°C. to +50°C.

The nontoxic, pharmaceutically acceptable salts include, for example, (1) nontoxic pharmaceutically acceptable salts of the acidic carboxylic acid group such as the sodium, potassium, calcium, aluminium and ammonium salts and nontoxic substituted ammonium salts with amines such as tri-(lower)-alkylamines, procaine, dibenzylamine, N-benzyl-beta-phenethylamine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietyl-enediamine, N-(lower)alkylpiperidines, such as

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N-ethylpiperidine and other amines which have been used to form salts of benzylpenicillin; and (2) nontoxic pharmaceutically acceptable acid addition salts (i.e. salts of the basic nitrogen) such as (a) the mineral acid addition salts such as the hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate, sulfonate and phosphate, and (b) the organic acid addition salts such as the maleate, acetate citrate, tartrate, oxalate, succinate, benzoate, fumarate, malate, mandelate, ascorbate, β -naphthalene sulfonate and p-toluenesulfonate.

The carbon bearing the free amino group of cephaloglycin and an asymmetric carbon atom and thus cephaloglycin and also hetasporin can exist in two optically active, isomeric forms (the D- and L-diastereoisomers) as well as in a mixture of the two optically active forms, all of which are included in the present invention.

The hetasporin of the present invention is prepared by the reaction of acetone with cephaloglycin. Although some reaction will occur no matter what molar proportion of reactants is used, it is preferable in order to obtain maximum yields to use a molar excess of the acetone. While the reaction may be conducted in any inert organic solvent (e.g. tetrahydrofuran, methylene chloride, dioxane or benzene), it is usually preferred to conduct the reaction in excess acetone as solvent. Water is split off during the reaction and it is thus desirable to have as small an amount of water as possible in the reaction medium. The pH of the reaction mixture should be from about 5 to 9 and preferably on the alkaline side. The pH may be adjusted to within this range, if necessary, by the addition of an alkaline material such as, for example, sodium hydroxide, sodium carbonate, potassium hydroxide, potassium carbonate, ammonium hydroxide, ammonium carbonate or organic amines (e.g. triethylamine).

The temperature during the reaction is not critical. The reaction will proceed satisfactorily at room temperature and may be hastened by heating.

The following example illustrates the best mode contemplated for carrying out this invention but it is not limited thereto.

EXAMPLE

7-[D(-)-N-Carbobenzoxy-2-phenylglycyl]cephalosporanic Acid.—The mixed anhydride of N-carbobenzoxy-D(-)-2-phenylglycine was prepared by dissolving 6.00 g. (21.0 millimole) of the latter compound in 100 ml. of dry tetrahydrofuran, adding 2.96 ml. dry triethylamine (21.0 millimole), cooling to -5° C. adding 2.00 ml. ethyl chloroformate (21.0 millimole), and stirring at -5° C. for 10 minutes. To the resulting slurry was added a solution prepared as follows: 5.72 g. (21.0 millimole) of 7-aminocephalosporanic

acid was suspended in 50 ml. water and 1.5 ml. triethylamine was added, followed by 50 ml. tetrahydrofuran and more triethylamine dropwise until the pH of the solution reached 7.1 and only a small amount of the starting acid remained undissolved; the solution was then cooled to 3° C. The reaction mixture was stirred at -5° C. for one-half hour and then allowed to warm to $+20^{\circ}$ C. during one hour. It was diluted with 350 ml. water and 110 ml. wet tetrahydrofuran was distilled from it at 35° C. on a rotary evaporator. The resulting gelatinous mixture was found to have a pH of 6.0. It was shaken with 500 ml. ethyl acetate to give a stable emulsion, and then acidified to pH 2 with 10 ml. of 42% phosphoric acid. The product was quickly extracted into the ethyl acetate, the layers separated, and the aqueous phase washed twice more with 350 ml. portions of ethyl acetate. The combined ethyl acetate extracts were washed once with 300 ml. saturated sodium chloride, twice with 300 ml. portions water, dried briefly over sodium sulfate, filtered and evaporated to dryness at 35° C. The semi-crystalline residue was washed onto a filter with 200 ml. dry ether, dried, screened through a 40-mesh screen then rewashed with 200 ml. dry ether to give 6.05 g. (53.4%) of 7-[D(-)-N-carbobenzoxy-2-phenylglycyl]cephalosporanic acid as white, crystalline powder, m.p. $143-145^{\circ}$ C. Its infrared spectrum in KBr was characterized by the following absorption bands: 3.02 NH; 5.60 to 5.66, β -lactam; 5.72 to 5.85, ester, urethane and free acid carbonyls; 6.01, amide; 6.6, amide II; 8.0 to 8.2 microns, urethane and ester C-O-C; 14.4, monosubstituted phenyl.

7-[D(-)-2-Phenylglycyl]cephalosporanic Acid.—In a 500 ml. pressure bottle 1.50 g. of 7-[D(-)-N-carbobenzoxy-2-phenylglycyl]cephalosporanic acid was dissolved in 15 ml. dioxane, followed by 7.5 ml. water, 4.5 ml. saturated sodium bicarbonate and 71 ml. water in that order, care being taken at each addition to agitate vigorously so that the starting material remained in solution. The bottle was flushed with nitrogen, and 1.50 g. of 30% palladium on diatomaceous earth was added. The bottle was purged with hydrogen and filled to a pressure of 49.0 p.s.i.g. Shaking was started, and the pressure in the bottle was observed to fall rapidly to 42.5 p.s.i.g. during ca. 2.5 minutes, and no further decrease in pressure was noted during the total reduction time of 5.00 minutes. The solution was immediately acidified to pH 2.0 with 6 N hydrochloric acid and filtered to remove the catalyst, which was washed with a few ml. of acidified water. The filtrate was neutralized to pH 3.65 with saturated sodium bicarbonate solution and evaporated to dryness at 35° C. on a rotary evaporator. The crude product 7-[D(-)-2-phenylglycyl]-

cephalosporanic acid (also called cephaloglycin), weighed 1.02 g. and was shown to contain 45% by weight of sodium chloride. The yield of desired product was therefore 0.57 g. or 51%. Its infrared spectrum (KBr) had the following characteristic bands: 3.00 to 3.15, NH and NH₂; 5.69 to 5.77, β -lactam and ester; 5.97, amide carbonyl; 6.25 to 6.4, phenyl and carboxylate; 6.5, amide II; 14.4 microns, monosubstituted phenyl.

7 - (2,2 - Dimethyl - 5 - oxo - 4 - phenyl - 1 - imidazolidinyl) - 3 - acetoxymethyl - 8 - oxo - 5 - thia - 1 - azabicyclo - [4.2.0]oct-2-ene-2-oic Acid, Triethylamine Salt.—A mixture of 4.92 g. of 7-[D(-)-2-phenylglycyl]-cephalosporanic acid (containing 50% sodium chloride) and 0.69 ml. dry triethylamine in 69 ml. anhydrous acetone was stoppered and stirred at 25° C. for 14.5 hours. The insoluble sodium chloride was removed by filtration and solvent was removed from the clear, light brown filtrate on a rotary evaporator at 35° C. The residue was evaporated again with about 50 ml. dry ethyl acetate and rinsed from the flask with 200 ml. dry ether. The slightly hygroscopic crystalline product (triethylamine salt of hetasporin) weighed 2.40 g. (90.5% yield). An attempt to determine the melting point showed that it gradually decomposes at any temperature above 100° C. Its infrared spectrum (KBr) had intense bands at 2.95 (NH), 3.66, 3.75 and 4.02 (triethyl-ammonium), 5.67 (β -lactam), 5.78 (ester), 5.86 and 5.91 (imidazolidinone), 6.26 (carboxylate), and 7.12 microns (ester).

Anal. Calc'd for C₂₁H₂₃N₃O₆S.1/2 H₂O: C, 57.42; H, 7.14; N, 9.92. Found: C, 57.34; H, 7.98; N, 9.85.

7 - (2,2 - Dimethyl - 5 - oxo - 4 - phenyl - 1 - imidazolidinyl) - 3 - acetoxymethyl - 8 - oxo - 5 - thia - 1 - azabicyclo - [4.2.0]oct-2-ene-2-oic Acid.—The triethylamine salt of hetasporin, 2.40 g., was dissolved in 110 ml. water, filtered, layered with 110 ml. ethyl acetate, and acidified to pH 2 with 42% phosphoric acid. The free acid was quickly extracted into the solvent, and the aqueous layer was extracted twice more with 60 ml. portions of ethyl acetate. The combined extracts were washed once with 50 ml. of saturated sodium chloride and twice with 40 ml. portions of water. The solution was dried briefly over sodium sulfate, filtered, and evaporated to about 3 ml. at 35° C. on a rotary evaporator. The concentrated solution was diluted with light petroleum, b.p. 60—70° C., and the solid product, hetasporin, was isolated by filtration, 450 mg. (23.4% yield). Its infrared spectrum (KBr) had the following bands: 2.94 (NH and H₂O), 3.35 and 3.40 (aliphatic CH), 4.0 (acid hydroxyl), 5.62 (β -lactam), and a broad band centered at 5.75 microns (includes carbonyls of free acid, ester, and imidazolidinone). The infrared spectrum

showed the total absence of an amide II band near 6.5 microns. The NMR spectrum of this product in D₂O revealed only the expected protons: 7.4 ppm, aromatic H; 5.8 ppm, NH, H₂O, and acid OH; 3.0 ppm, β -lactam H, 4.75 ppm, =C—CH₂—O; 5.0 ppm, N—CH(C₆H₅)C=O; 3.4 ppm, S—CH₂—; 2.0 ppm, CH₃C=O; 1.4 ppm, (CH₃)₂C.

Anal. Calc'd for C₂₁H₂₃N₃O₆S.1/2 H₂O: C, 55.49; H, 5.32; N, 9.24; H₂O, 1.98. Found: C, 55.37; H, 5.37; N, 8.95; H₂O 2.44.

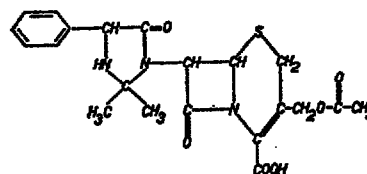
The hetasporin prepared above exhibited *in vitro* Minimum Inhibitory Concentrations versus *S. aureus* Smith of about 1.0 mcg./ml., versus the benzylpenicillin-resistant *S. aureus* Bx-1633-2 of about 6.2 mcg./ml., versus *S. enteritidis* of about 3.1 mcg./ml., versus *S. typhosae* of about 6.2 mcg./ml. and versus *Shig. sonnei* of about 3.1 mcg./ml. and, upon intramuscular injection (i.m.) or oral administration (p.o.) to mice exhibited CD₅₀'s of about 5.2 mgm./kg. i.m. and 6.8 mgm./kg. p.o. versus *Staph. aureus* Smith, of about 45 mgm./kg. i.m. and about 26 mgm./kg. p.o. versus *S. aureus* Bx-1633-2 and of about 38 mgm./kg. p.o. versus *S. enteritidis*.

The invention includes a pharmaceutical composition comprising the compound of the above formula (I), or a nontoxic pharmaceutically acceptable salt thereof, and an inert diluent or carrier.

The invention further includes a method of treating infectious diseases in poultry and animals excluding man, caused by Gram-positive or Gram-negative bacteria, such as mastitis in cattle, which method comprises administering an effective amount of the compound of the above formula (I), a nontoxic pharmaceutically acceptable salt thereof, or a composition as defined above.

WHAT WE CLAIM IS:—

1. A compound of the formula:



2. A nontoxic, pharmaceutically acceptable salt of the compound of claim 1.
3. The calcium, aluminium, or ammonium salts of the compound of claim 1.
4. The sodium salt of the compound of claim 1.
5. The potassium salt of the compound of claim 1.
6. The procaine, dibenzylamine, N-benzyl-beta-phenethyl-amine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietyl-



cephalosporanic acid (also called cephaloglycin), weighed 1.02 g. and was shown to contain 45% by weight of sodium chloride. The yield of desired product was therefore 0.57 g. or 51%. Its infrared spectrum (KBr) had the following characteristic bands: 3.00 to 3.15, NH and NH₂; 5.69 to 5.77, β -lactam and ester; 5.97, amide carbonyl; 6.25 to 6.4, phenyl and carboxylate; 6.5, amide II; 14.4 microns, monosubstituted phenyl.

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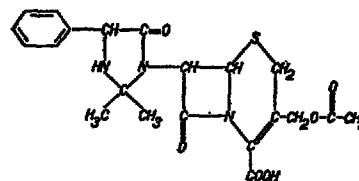
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The invention includes a pharmaceutical composition comprising the compound of the above formula (I), or a nontoxic pharmaceutically acceptable salt thereof, and an inert diluent or carrier.

The invention further includes a method of treating infectious diseases in poultry and animals excluding man, caused by Gram-positive or Gram-negative bacteria, such as mastitis in cattle, which method comprises administering an effective amount of the compound of the above formula (I), a nontoxic pharmaceutically acceptable salt thereof, or a composition as defined above.

WHAT WE CLAIM IS:—

1. A compound of the formula:



2. A nontoxic, pharmaceutically acceptable salt of the compound of claim 1.

3. The calcium, aluminium, or ammonium salts of the compound of claim 1.

4. The sodium salt of the compound of claim 1.

5. The potassium salt of the compound of claim 1.

6. The procaine, dibenzylamine, N-benzyl-beta-phenethyl-amine, 1-phenamine, N,N'-dibenzylethylenediamine, dehydroabietyl-

amine, N,N'-bis-dehydroabietyl ethylenediamine, or N-ethylpiperidine salt of the compound of claim 1.

5 7. The triethylamine salt of the compound of claim 1.

8. The hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate, sulfonate, or phosphate, salt of the compound of claim 1.

10 9. The maleate, acetate, citrate, tartrate, oxalate, succinate, benzoate, fumarate, malate, mandelate, ascorbate, β -naphthalene sulfonate, or p-toluenesulfonate salt of the compound of claim 1.

15 10. A process for the preparation of the compound of the formula (I) defined in claim 1, and its non-toxic, pharmaceutically acceptable salts, which process comprises reacting cephaloglycin, or a salt thereof, with at least an equimolar weight of acetone in the absence of substantial amounts of water at a pH in the range of 5 to 9 and at a temperature in the range of -20°C. to $+50^{\circ}\text{C.}$

20 11. A process as claimed in claim 10 wherein the reaction is carried out with excess acetone as solvent.

25 12. A process as claimed in claim 10 or claim 11 when carried out at an alkaline pH.

13. A process as claimed in claim 10 and substantially as hereinbefore described with reference to the specific Example. 30

14. A pharmaceutical composition comprising the compound claimed in claim 1, or a salt thereof as claimed in any one of claims 2 to 9, and an inert diluent or carrier. 35

15. A method of treating infectious diseases in poultry and animals excluding man caused by Gram-positive or Gram-negative bacteria, which method comprises administering an effective amount of the compound claimed in claim 1, a salt thereof as claimed in any one of claims 2 to 9, or a composition as claimed in claim 14. 40

16. A method of treating mastitis in cattle which method comprises administering an effective amount of the compound claimed in claim 1, a salt thereof as claimed in any one of claims 2 to 9, or a composition as claimed in claim 14. 45

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