

PATENT SPECIFICATION

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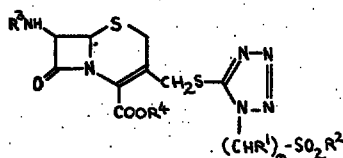
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(54) CEPHALOSPORIN COMPOUNDS

(71) We, SMITHKLINE CORPORATION, of 1500 Spring Garden Street, Philadelphia, Pennsylvania, 19101, United States of America, a corporation organized under the laws of the Commonwealth of Pennsylvania, one of the 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 new series of cephalosporin compounds which have antibacterial activity when administered parenterally. In particular, the structures of the biologically active cephalosporin compounds of this invention are characterized by having a sulfo- or sulfamoyl-alkyl substituted tetrazolyl-thiomethyl group at the 3-position of the cephem nucleus. The invention also includes pharmaceutical compositions and methods for treating bacterial infections using these new compounds and processes for preparing the compounds.

The cephalosporin compounds of this invention are those of the structural formula



FORMULA I

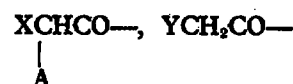
in which

each individual R¹ is hydrogen or lower alkyl;

n is from 1 to 10;

R² is hydroxy, amino, (lower alkyl)-amino; or di-(lower alkyl) amino;

R³ is an acyl group of the structure



or



where:

X is thienyl; dihydrophenyl; phenyl; phenyl mono-substituted with hydroxy, hydroxymethyl, formamido, ureido or carb-oxymethylamino; or 3 - fluoro - 4 - hydroxy-phenyl;

A is hydroxy, amino, COOR⁴ or SO₃H; or formyloxy when X is phenyl;

Y is thienyl, tetrazolyl, cyano, sydnon-3-yl or aminomethylphenyl;

Z is methyl, trifluoromethyl, trifluoroethyl, cyanomethyl or pyridyl; and

m is 0, 1 or 2; and each COOR⁴ is a carboxylic acid or esterified carboxylic acid group; or a non-toxic pharmaceutically-acceptable salt thereof.

By "lower alkyl" is meant an alkyl group having from 1 to 4 carbon atoms; preferably such an alkyl group is methyl or ethyl. Where COOR⁴ is an esterified carboxylic acid group, R⁴ can be, for example, an alkyl or aryl group or an esterifying group which is easily cleaved within the body to give a carboxylic acid group, such as an indanyl, pivaloyloxymethyl, acetoxymethyl, propionyloxymethyl, glycyloxymethyl, phenylglycyloxymethyl or thienylglycyloxymethyl group.

Preferably the compounds are those of Formula I where each R¹ is hydrogen and n is from 1 to 5; and preferably n is 1 or 2. Preferably R² is hydroxy or amino. Preferably where R³ is

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X is phenyl or hydroxyphenyl, and A is hydroxy or amino; where R³ is YCH₂, Y is thienyl or 2-aminomethylphenyl; and where

5 R³ is Z—S(O)_m—CH₂CO—, Z is trifluoromethyl and m is 0.

Examples of particularly suitable 7-acylamido substituents (R³NH—) of the compounds of Formula I are as follows:

- 10 α-hydroxyphenylacetamido
- α-aminophenylacetamido
- α-amino-4-hydroxyphenylacetamido
- trifluoromethylthioacetamido
- methylthioacetamido
- 15 2,2,2-trifluoroethylsulfinylacetamido
- thienylacetamido
- tetrazolylacetamido
- cyanoacetamido
- α-carboxythienylacetamido
- 20 α-carboxyphenylacetamido
- α-sulfophenylacetamido
- methylsulfonylacetamido
- cyanomethylthioacetamido
- α-amino-4-carboxymethylaminophenyl-
- 25 acetamido
- α-amino-3-fluoro-4-hydroxyphenyl-
- acetamido
- sydnon-3-ylacetamido
- 4-pyridylthioacetamido
- 30 2-aminomethylphenylacetamido.

Particularly suitable substituted tetrazolyl groups are the following:

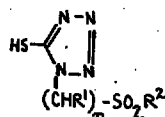
- 1-sulfomethyltetrazolyl
- 1-(2-sulfoethyl)tetrazolyl
- 35 1-(2-sulfamoyl)tetrazolyl
- 1-(3-sulfoethyl)tetrazolyl
- 1-(3-sulfamoyl)tetrazolyl
- 1-(5-sulfoethyl)tetrazolyl
- 1-(5-sulfamoyl)tetrazolyl.

- 40 Particularly preferred are the compounds 7-D - mandelamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 7 - D - mandelamido - 3 - [1 - (2 - sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid, 7 - D - mandelamido - 3 - [1 - (5 - sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid, 7 - D - mandelamido - 3 - [1 - (2 - sulfamoyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid, 7 - (2 - thienylacetamido) - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, 7 - (2 - thienylacetamido) - 3 - [1 - (2 - sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid, 7 - trifluoromethylthioacetamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid,

7 - (2 - thienylacetamido) - 3 - [1 - (2-sulfamoyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid and 7-trifluoromethylthioacetamido - 3 - [1 - (2-sulfamoyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

Cephalosporin derivatives having 7-acyl substituents as defined above are all documented in the prior art. Substitution by a substituted S-heterocyclic thiomethyl group (—CH₂SHet) at the 3-position of the cephem nucleus is also known and is disclosed in Netherlands Patent Specification No. 6916151 where Het is, among others, tetrazolyl substituted with, *inter alia*, carboxy, carbalkoxy, alkoxyalkylaminocarbonyl and dialkylaminoalkylaminocarbonyl and in Japanese Patent Specification No. 7205550 where Het includes tetrazolyl substituted with —(CH₂)_nR where n is 0 to 3 and R includes alkoxy-carbonyl, carboxy, N-alkoxyalkylcarbonyl and dialkylamino. Recently issued U.S. Patent Specification No. 3,819,623 discloses cephalosporins bearing a 7-heterocyclic-acetamido or 7-heterocyclic-thioalkylacetamido group and having in the 3-position, *inter alia*, thiomethyltetrazolyl substituted with carboxy, alkoxy-carbonyl, carboxyalkyl, alkoxy-carbonylalkyl and dialkylaminoalkylaminocarbonylalkyl. Cephalosporin compounds containing a 3-(sulfo- or sulfamoyl)-substituted tetrazolylthiomethyl moiety are believed to be unknown hitherto.

In a process of the invention compounds of Formula I where each COOR⁴ is a carboxylic acid group are prepared by acylating 7-aminocephalosporanic acid with an appropriately protected acylating agent capable of providing the acyl group R³ and the 3-acetoxy group in the acylation product is displaced by reaction with the appropriate substituted tetrazole-thiol of the structure



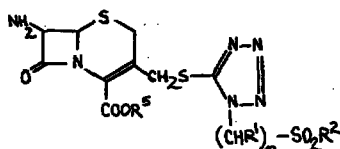
FORMULA II

and any protecting group is subsequently removed. Compounds of Formula II are described and claimed in British Patent Application 42761/77 (Serial No. 1547475).

The carboxylic acid group of the acylating agent can be activated by any of the standard methods such as conversion to the mixed anhydride, acid chloride, acid imidazole or activated ester. In a chemically equivalent process, a reagent such as dicyclohexylcarbodiimide can be used, provided that the carboxyl group on the cephem nucleus is protected with an easily removable protecting ester-forming group such as benzhydryl, *t*-butyl, trichloroethyl, benzyl, benzyloxymethyl, *p*-

nitrophenyl, *p*-methoxyphenyl, *p*-methoxybenzyl or *p*-nitrobenzyl. When R is NH₂, the α-amino group of the acylating agent is preferably protected prior to acylation with an easily removable protecting group known in the art such as *t*-butoxycarbonyl, trichloroethoxycarbonyl, benzyloxycarbonyl, or 1-methoxycarbonyl-2-propenyl (the methyl acetoacetate adduct).

Alternatively, the compounds of Formula I where each COOR⁴ is a carboxylic acid group are prepared by acylating the appropriate 7-amino-3-substituted tetrazolythio-methyl cephalosporin of the structure



FORMULA III

in which

each individual R¹ is hydrogen or lower alkyl;

n is from 1 to 10;

R² is hydroxy, amino, (lower alkyl)amino or di(lower-alkyl)amino; and

R⁵ is hydrogen or a protecting ester-forming group, with an appropriate acylating agent, followed by removal of any protecting group. Certain compounds of Formula III are described and claimed in British Patent Application 42760/77 (Serial No. 1547474).

The protecting groups can be removed according to methods well known to the art, such as with trifluoroacetic acid when *t*-butyl or *t*-butoxycarbonyl protecting groups are used. A resulting salt may be converted to the zwitterionic product or to the free acid by means of a basic ion exchange resin such as polystyrene-amine ion exchange resin (Amberlite IR-45: Amberlite is a Registered Trade Mark) or by basification of an aqueous solution of the salt.

Where a compound of Formula I in which each COOR⁴ is an esterified carboxylic acid group is required, the corresponding compound where COOR⁴ is a carboxylic acid group can be readily esterified by methods well known in the art.

The acylating agents used as starting materials are either known or can be prepared by known methods.

The 7-amino-3-substituted tetrazolythio-methyl cephalosporin starting materials of Formula III where R² is hydroxy can be prepared by reaction of 7-formamidocephalosporanic acid, (itself prepared by reaction of 7-amidocephalosporanic acid with formic acid and acetic anhydride), and a substituted tetrazole-thiol of Formula II, followed by treatment with an acid such as hydrochloric acid to remove the formyl group.

The substituted tetrazole-thiols of Formula II where R² is hydroxy, (lower alkyl)amino or di-(lower alkyl)amino can be prepared by reaction of an N-substituted alkyl dithiocarbamate, such as methyl 2-sulfoethyldithiocarbamate or methyl 3-(N-*t*-butylsulfamoyl)-propyl-dithiocarbamate or its corresponding sodium or potassium salt with an azide such as sodium azide. The N-substituted alkyl dithiocarbamates can be prepared by treatment of an aminoalkanesulfonic acid, for example 2-aminoethanesulfonic acid, or an aminoalkane (N-alkyl or N,N-dialkyl)sulfonamide such as 3-aminopropane-N-*t*-butyl-sulfonamide or its corresponding salt with carbon disulfide and an alkyl halide such as methyl iodide in the presence of a base such as sodium or potassium hydroxide.

The aminoalkane (N-alkyl or N,N-dialkyl)sulfonamides can be prepared by reaction with hydrazine of an N-alkyl or N,N-dialkyl-phthalimidoalkylsulfonamide, obtained from treatment of a phthalimidoalkylsulfonyl halide, preferably chloride, with an alkyl- or dialkylamine. The phthalimidoalkylsulfonyl halides are known or can be prepared as described by Winterbottom *et al.*, *J. Amer. Chem. Soc.* 69:1393 (1947), and Griffin and Hey, *J. Chem. Soc.*, 3334 (1952).

When R² is amino, the compounds of Formula II can be prepared by removal of the N-alkyl group from the corresponding 1-(N-alkylsulfamoylalkyl)tetrazole-5-thiol, preferably a 1-(N-*t*-butylsulfamoylalkyl)tetrazole-5-thiol, with, for example, anisole and trifluoroacetic acid.

Certain compounds of this invention are capable of forming salts with, for example, the alkali metals such as sodium or potassium, the alkaline earth metals such as calcium or with the ammonium cation. When A is NH₂, the compounds can exist as the zwitterion or as either an acid or base addition salt. These salts can be prepared by standard methods using a wide variety of non-toxic pharmaceutically acceptable acids and bases known in the art.

It will be recognized that due to the asymmetric α-carbon atom in the 7-acetamido group of Formula I when R³ is



A

and the potentially asymmetric carbon atom in the tetrazole side-chain, optical isomers can exist. Racemic or resolved products are obtained depending upon whether a racemic or resolved side-chain acid is used as an acylating agent and whether a racemic or resolved tetrazole-thiol is used. The resolved side-chain acids are readily obtained from the racemic compounds by resolution according to well known methods, including frac-

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tional crystallization of a salt formed with an optically active acid or base. All of the isomers, including separated isomers and mixtures thereof, are included within the scope of this invention.

The compounds of Formula I have exceptional antibacterial activity against both Gram-positive and Gram-negative organisms.

Minimum inhibitory concentrations (MIC's) range from 0.2 to >200 µg./ml. in *in vitro* testing. These results are shown in Table 1 below for representative compounds of Formula I. *In vivo* mouse protection data are given in Table 2. Compound names corresponding to numbers are given in Table 3.

TABLE I
MIC (µg./ml.) *in vitro*

Compound	<i>S. aureus</i> HH 127	<i>S. aureus</i> SK 23390	<i>S. villaluz</i>	<i>Strep. Faecalis</i> HH 34358	<i>E. coli</i> SK 12140	<i>E. coli</i> HH 33779	<i>Kleb. pneumo.</i> SK 4200	<i>Kleb. pneumo.</i> SK 1200	<i>Pseudo. sp.</i> HH 63	<i>Salmonella</i> ATCC 12176	<i>Shigella</i> HH 117	<i>Enter. aerog.</i> ATCC 13048	<i>Serra. marc.</i> ATCC 13880	<i>Enter. cloacae</i> HH 31254	<i>Proteus morganii</i> 179
I	3.1, 6	0.8, 6	6.3, 50	50, 100	0.8, 0.8	3.1, 1.6	0.8, 0.8	0.2, 0.8	>200, >200	0.8, 0.4	0.4, 0.4	3.1, 13	12.5, 13	0.8, 3	3.1, 3
II	1.6	0.8	6.3	50	0.8	1.6	0.8	0.4	>200	0.8	0.4	6.3	25	3.1	6.3
III	1.6	0.2	3.1	12.5	0.8	1.6	1.6	0.8	>200	0.4	0.2	3.1	6.3	0.8	3.1
IV	3.1	0.8	12.5	50	1.6	6.3	1.6	1.6	>200	3.1	0.8	6.3	100	0.8	3.1
V	0.8, 0.8	0.4, 0.4	3.1, 12.5	25, 50	1.6, 1.6	6.3, 6.3	1.6, 0.8	0.4, 0.4	>200, >200	0.8, 0.8	1.6, 1.6	12.5, 12.5	100, 200	1.6, 1.6	>200, >200
VI	1.6	0.8	3.1	50	3.1	12.5	1.6	0.8	>200	0.8	3.1	25	>200	6.3	>200
VII	0.8, 0.8	0.8, 0.4	50, 15	50, 50	0.8, 0.4	3.1, 1.6	0.4, 0.2	0.4, 0.2	>200, >200	0.4, 0.2	0.8, 0.8	6.3, 3.1	200, 100	3.1, 0.8	100, 100
VIII	0.4	0.1	1.6	6.3	0.8	1.6	1.6	0.4	>200	0.8	0.4	3.1	200	0.8	>200
IX	0.4	0.2	6.3	12.5	0.8	1.6	0.8	0.4	>200	0.4	0.2	1.6	100	0.8	200

TABLE 2

ED₅₀ *in vivo* (mg./kg.)

Compound	E. coli 12140		Kleb. pneumo. 4200	
	s.c.	p.o.	s.c.	p.o.
I	1.56	—	0.28	—
II	0.58	35	0.39	18.6
III	0.70	25	0.68	21.5
IV	18	>50	15.7	—
V	4	>50	0.26	—
VI	4.4	—	1.56	—
VII	0.86	—	0.24	—
VIII	1.32	>50	2	—
IX	1.56	44	—	—

TABLE 3

Compound Number	Compound Name
I	7-D-Mandelamido-3-(1-sulfomethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid
II	7-D-Mandelamido-3-[1-(2-sulfoethyl)-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
III	7-D-Mandelamido-3-[1-(2-sulfamoyl-ethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
IV	7-D-Mandelamido-3-[1-(5-sulfopentyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
V	7-(2-Thienylacetamido)-3-(1-sulfomethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid
VI	7-(2-Thienylacetamido)-3-[1-(2-sulfoethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
VII	7-Trifluoromethylthioacetamido-3-(1-sulfomethyltetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid
VIII	7-(2-Thienylacetamido)-3-[1-(2-sulfamoyl-ethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid
IX	7-Trifluoromethylthioacetamido-3-[1-(2-sulfamoyl-ethyl)tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylic acid

In addition, the active compounds of this invention exhibit broad spectrum activity and shown advantageously high blood serum levels and half-life values.

Pharmaceutical compositions of the invention are those comprising a compound of Formula I of non-toxic, pharmaceutically-acceptable salt thereof and a pharmaceutically-acceptable carrier. The invention includes a method of combatting bacterial infection in a non-human animal which comprises administering to the animal such a pharmaceutical composition in a non-toxic amount sufficient to combat such infection. The administration may be by parenteral injection such as subcutaneously, intramuscularly or intravenously. The injection of suitably prepared sterile solutions or suspensions containing an effective, toxic amount of the new cephalosporin compound is the preferred route of administration.

The compounds of Formula I are formulated and administered in the same manner as other cephalosporins. The dosage regimen comprises administration, preferably by injection, of an active but non-toxic quantity of a compound of Formula I selected from the dosage unit range of from 100 to 1000 mg., with the total daily dosage regimen being from 400 mg. to 6 g. The precise dosages are dependent upon the age and weight of the subject and on the infection being treated and can be determined by those skilled in the art, based on the data disclosed herein compared with that available to the art attained with known cephalosporins.

The following Examples illustrate the invention. Temperatures are in degrees Centigrade unless otherwise stated. "Amberlite" is a Registered Trade Mark.

EXAMPLE 1.

7 - D - Mandelamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

To a solution of 112 g. (2.0 mol.) of potassium hydroxide and 111 g. (1.0 mol.) of aminomethanesulfonic acid in 250 ml. of water at 25° was added 71 ml. of carbon disulfide. The reaction mixture was stirred for 12 hours and 250 ml. of ethanol was added. The reaction vessel was fitted with a reflux condenser and 62 ml. (1.0 mol.) of methyl iodide was added. After an exothermic reaction the mixture was allowed to cool to ambient temperature, the solid product was collected by filtration. The solid was extracted with hot methanol and the extract was concentrated to give methyl sulfomethyldithiocarbamate as the potassium salt.

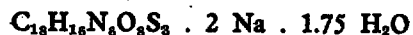
A mixture of 45.3 g. (0.19 mol.) of methyl sulfomethyldithiocarbamate potassium salt and 16.9 g. (0.26 mol.) of sodium azide in 425 ml. of water was heated at 80° for 4.75 hours. The reaction mixture was passed

through an Amberlite IR—120H ion exchange resin column and eluted with water until the pH of the eluant became 3.5. The eluant was extracted with ether and the aqueous solution was evaporated to dryness to give 1-sulfomethyltetrazole-5-thiol.

1 - Sulfomethyltetrazole - 5 - thiol was dissolved in acetone and a 30% solution of sodium 2-ethylhexanoate in isopropanol was added. 1 - Sulfomethyltetrazole - 5 - thiol sodium salt precipitated and was collected by filtration.

A mixture of 27.4 g. (0.062 mol.) of 7 - D - mandelamidocephalosporanic acid methanolate, 10.2 g. (0.047 mol.) of 1-sulfomethyltetrazole-5-thiol sodium salt and 9.2 g. (0.109 mol.) of sodium bicarbonate in 300 ml. of water was heated at 70° for one hour. The reaction mixture was cooled (ice bath) and acidified to pH 1.8 with 3N hydrochloric acid. The mixture was extracted with ethyl acetate, filtered and chromatographed on an Amberlite XAD—8 resin column with water containing increasing amounts of methanol as the eluant to give the title compound.

The title compound was dissolved in methanol and a 5% solution of sodium methoxide in methanol was added until pH 7.0. Addition of ethanol precipitated the product salt which was collected, dissolved in water and lyophilized to give 7 - D-mandelamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid disodium salt.



Calculated:

34.98% C; 3.18% H; 13.59% N

Found:

35.09% C; 3.17% H; 13.27% N

EXAMPLE 2.

7 - (D - α - Aminophenylacetamido) - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

A solution of 7.58 g. (0.015 mol.) of 7 - (D - α - t - butoxycarbonylaminophenylacetamido)cephalosporanic acid, 1.96 g. (0.01 mol.) of 1 - sulfomethyltetrazole - 5 - thiol and 2.52 g. (0.03 mol.) of sodium bicarbonate in 125 ml. of water is stirred at 60° for five hours while maintaining the pH at 7.0—7.2 by addition of sodium bicarbonate. The mixture is cooled and extracted with ethyl acetate. The aqueous phase is acidified to pH 2.5 with 3N hydrochloric acid and the acidic solution is extracted again with ethyl acetate. The aqueous phase is brought to pH 7.1 by addition of 5% sodium carbonate solution, then passed through an Amberlite XAD—4 ion exchange resin column and eluted with water and methanol to give 7-

(D - α - *t* - butoxycarbonylamino-phenylacetamido) - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid disodium salt.

- 5 7 - (D - α - *t* - butoxycarbonylamino-phenylacetamido) - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid disodium salt is stirred at 25° with 25 ml. of trifluoroacetic acid and 25 ml. of 1,3-dimethoxybenzene for 2.25 hours. The mixture is evaporated to dryness, ether is added to the residue and the precipitate is collected, washed with ether, stirred in acetonitrile for two hours, then collected and dried *in vacuo* to give the title compound.

EXAMPLE 3.

- 20 7 - D - Mandelamido - 3 - [1 - (2 - sulfamoyl-ether)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

A solution of 2.73 g. (0.01 mol.) of 2-phthalimidoethanesulfonyl chloride in 20 ml. of chloroform was added dropwise to a solution of 2.19 g. (0.03 mol.) of *t*-butylamine in 20 ml. of chloroform at 5°. The reaction mixture was warmed to ambient temperature and stirred for three hours. The precipitate was removed by filtration and the filtrate was evaporated to dryness to give a residue which was purified by chromatography on silica with 19:1 chloroform-methanol as eluant to give N - *t* - butyl - 2 - phthalimidoethanesulfonamide.

- 30 N - *t* - Butyl - 2 - phthalimidoethanesulfonamide (2.10 g., 6.78 mmol.) was suspended in 20 ml of ethanol and 0.344 g. of hydrazine hydrate was added. The reaction mixture was refluxed for three hours, then evaporated to dryness. The residue was suspended in 45 ml. of water and acidified to pH 3.0 by addition of dilute hydrochloric acid. The acid mixture was filtered and the filtrate evaporated to dryness to give 2-aminoethane - N - *t* - butylsulfonamide hydrochloride.

- 35 2 - Aminoethane - N - *t* - butylsulfonamide hydrochloride (1.25 g., 5.78 mmol.) was added to a solution of 1.17 g. (11.56 mmol.) of triethylamine in 20 ml. of ethanol. Carbon disulfide (0.44 g., 5.78 mmol.) was added, the mixture was stirred at 25° for 1.5 hours, then 0.82 g. (5.78 mmol.) of methyl iodide in 5 ml. of ethanol was added and the resulting mixture was stirred for 1.5 hours. The mixture was evaporated to dryness and the residue was dissolved in water and acidified to pH 2.0 with dilute hydrochloric acid. The aqueous mixture was extracted with ethyl acetate and the extract was dried (MgSO₄) and evaporated to dryness to give methyl-2-(N - *t* - butylsulfamoyl) - ethyldithiocarbamate.

Methyl 2 - (N - *t* - butylsulfamoyl)-

ethyldithiocarbamate was treated with sodium azide as described in the procedure of Example 1 for 35 minutes to give 1 - (2 - N - *t* - butylsulfamoyl-ethyl)tetrazole - 5 - thiol. 65

1 - (2 - N - *t* - Butylsulfamoyl-ethyl)-tetrazole - 5 - thiol (1.0 g.) was suspended in 10 ml. of anisole and 20 ml. of trifluoroacetic acid is added. The solution was heated at 56° for 3.5 hours, then cooled. The precipitate was collected by filtration and washed with petroleum ether to give 1 - (2 - sulfamoyl-ethyl)tetrazole - 5 - thiol. 70 75

A solution of 0.210 g. (2.5 mmol.) of sodium bicarbonate in 5 ml. of water was added to a suspension of 0.272 g. (1 mmol.) of 7-aminocephalosporanic acid in 5 ml. of water and 2.5 ml. of acetone at 15°. The solution was heated to 45°, a solution of 0.314 g. (1.5 mmol.) of 1 - (2 - sulfamoyl-ethyl)tetrazole - 5 - thiol in 10 ml. of acetone was added and the reaction mixture was refluxed for two hours while maintaining the pH at 7.4—7.6 by addition of aqueous sodium bicarbonate solution. The mixture was cooled and acidified to pH 4.0 with dilute hydrochloric acid. The precipitate was collected by filtration to give 7 - amino - 3 - [1 - (2 - sulfamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid. 80 85 90

To a solution of 1.26 g. (15 mmol.) of sodium bicarbonate in 75 ml. of acetone and 50 ml. of water at 5° was added 2.1 g. (5 mmol.) of 7 - amino - 3 - [1 - (2 - sulfamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid. The solution was cooled to -10° and a solution of 1.55 g. (5.5 mmol.) of D - O - dichloroacetyl-mandeloyl chloride in 25 ml. of acetone was added. The reaction mixture was stirred for 30 minutes in the cold while maintaining the pH at 7.2 by addition of aqueous sodium bicarbonate, then for 1.5 hours at 25°C. The mixture was extracted with ether and the aqueous phase was brought to pH 9.3 with 5% sodium carbonate and stirred for 1.5 hours at 25°. The aqueous mixture was extracted with ether, the pH adjusted to 4.5 and the solution was again extracted with ether. The aqueous phase was acidified to pH 1.5 with dilute hydrochloric acid and it was extracted with ethyl acetate. Evaporation of the extract to dryness gave a solid which was suspended in ethyl acetate and filtered. The filtrate was diluted with ether and petroleum ether to precipitate the title compound. 95 100 105 110 115

EXAMPLE 4.

- 120 7 - D - Mandelamido - 3 - [1 - (2 - N - *t* - butylsulfamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

When an equivalent amount of 1 - (2 - N - *t* - butylsulfamoyl-ethyl)tetrazole - 5 - thiol 125

is reacted with 7 - aminocephalosporanic acid as described in Example 3, 7 - amino - 3 - [1 - (2 - N - *t* - butylsulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid is obtained.

Acylation of 7 - amino - 3 - [1 - (2 - N - *t* - butylsulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid with D - O - dichloroacetylmandeloyl chloride according to the procedure of Example 3 gives the title compound.

EXAMPLE 5.

7 - D - Mandelamido - 3 - [1 - (2 - N - methylsulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

Use of methylamine in the reaction with 2 - phthalimidoethanesulfonyl chloride described in Example 3, followed by the subsequent synthetic steps described therein gives 1 - (2 - N - methylsulfamoyl)tetrazole - 5 - thiol.

When 1 - (2 - N - methylsulfamoyl)ethyl]tetrazole - 5 - thiol is reacted with 7 - aminocephalosporanic acid as described in Example 3 and the product, 7 - amino - 3 - [1 - (2 - N - methylsulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid, is acylated with D - O - dichloroacetylmandeloyl chloride as described therein, the title compound is obtained.

EXAMPLE 6.

7 - (D - α - Amino - 4 - hydroxyphenylacetamido) - 3 - [1 - (2 - sulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

A solution of 7.82 g. (0.015 mol.) of 7 - (D - α - *t* - butoxycarbonyl - 4 - hydroxyphenylacetamido)cephalosporanic acid, 4.6 g. (0.022 mol.) of 1 - (2 - sulfamoyl)ethyl]tetrazole - 5 - thiol and sodium bicarbonate is reacted according to the procedure described in Example 2. After cooling, the reaction mixture is extracted with ethyl acetate. Fresh ethyl acetate is added to the aqueous phase and it is acidified with stirring to pH 2.8 with 6N sulfuric acid. The layers are separated and the aqueous phase is again extracted with ethyl acetate. The combined extracts are washed with water, dried (MgSO_4) and the solvent evaporated to give 7 - (D - α - *t* - butoxycarbonyl - 4 - hydroxyphenylacetamido) - 3 - [1 - (2 - sulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

7 - (D - α - *t* - Butoxycarbonyl - 4 - hydroxyphenylacetamido) - 3 - [1 - (2 - sulfamoyl)ethyl]tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid is treated with trifluoroacetic acid as described in Example 2 to give the title compound.

EXAMPLE 7.

7 - D - Mandelamido - 3 - [1 - (2 - sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

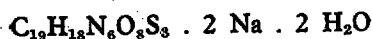
2 - Aminoethanesulfonic acid (50 g., 0.4 mol.) was added to a solution of 45 g. (0.8 mol.) of potassium hydroxide in 100 ml. of water at 25°. Carbon disulfide (24.4 ml., 0.4 mol.) was added and the reaction mixture was refluxed for 2.5 hours. Ethanol was added to the warm solution, the mixture was cooled to ambient temperature, 57 g. (0.4 mol.) of methyl iodide was added and the resulting mixture was stirred for 1.5 hours. The mixture was evaporated *in vacuo* and the residue recrystallized from hot ethanol containing 3% water to give methyl 2-sulfoethylthiocarbamate potassium salt.

A mixture of 21.5 g. (0.087 mol.) of methyl 2-sulfoethylthiocarbamate potassium salt (0.5 hydrate) and 7.16 g. (0.11 mol.) of sodium azide in 200 ml. of water was refluxed for two hours. The solution was cooled to 25° and extracted with ethyl acetate. The aqueous phase was treated with Amberlite IR-120H resin, washed with ether and evaporated to give an oil. The oil was taken up in acetone, the mixture was filtered and the filtrate was evaporated to dryness to give 1 - (2 - sulfoethyl) - tetrazole - 5 - thiol. The thiol was dissolved in isopropanol, cyclohexylamine was added until pH 8-9 and acetonitrile was added to give 1 - (2 - sulfoethyl)tetrazole - 5 - thiol as the bis-cyclohexylamine salt.

1 - (2 - Sulfoethyl)tetrazole - 5 - thiol bis-cyclohexylamine salt was dissolved in water and treated with Amberlite IR-120H resin to give 1 - (2 - sulfoethyl)tetrazole - 5 - thiol.

To a solution of 2.1 g. (0.01 mol.) of 1 - (2 - sulfoethyl)tetrazole - 5 - thiol in 100 ml. of water was added 6.4 g. (0.015 mol.) of 7 - D - mandelamidocephalosporanic acid sodium salt and 1.68 g. (0.02 mol.) of sodium bicarbonate. The mixture was stirred at 70° for 2.5 hours, then cooled and acidified to pH 1.8 with 3N hydrochloric acid. The acid solution was extracted with ethyl acetate and ether, acidified to pH 0.9 and chromatographed on an Amberlite XAD-8 resin column with water as eluant to give the title compound.

The title compound was converted to the corresponding disodium salt by treatment with sodium methoxide as described in the procedure of Example 1.



Calculated:

35.85% C; 3.48% H; 13.20% N

Found:

36.21% C; 3.29% H; 12.96% N

EXAMPLE 8.

7 - D - Mandelamido - 3 - (1 - sulfamoylmethyltetrazol - 5 - ylthiomethyl) - 3-cephem - 4 - carboxylic acid.

5 A suspension of 15.1 g (0.136 mol.) of aminomethane sulfonic acid and 14.2 g. (0.145 mol.) of anhydrous potassium acetate in 48 ml. of acetic acid is refluxed for ten minutes. Phthalic anhydride (21.4 g., 0.145 mol.) is then added and the resulting mixture is re-

10 fluxed for 2-5 hours. The product is collected by filtration and washed with acetic acid and ethanol to give phthalimidomethanesulfonic acid potassium salt.

15 To 41.7 g. (0.15 mol.) of phthalimidomethanesulfonic acid potassium salt in 220 ml. of dry benzene is added 22.5 g. (0.132 mol.) of phosphorus pentachloride. The reaction mixture is refluxed on a steam bath for one hour, then an additional 22.5 g. of phosphorus pentachloride is added and heating is continued for 1.5 hours. The reaction mixture is evaporated to dryness, crushed ice is added to the residue and the slurry is

25 filtered. The product is washed with water to give phthalimidomethanesulfonyl chloride. When phthalimidomethanesulfonyl chloride is substituted in the procedure of Example 3 for 2-phthalimidoethanesulfonyl chloride, N-t-butylphthalimidomethanesulfonamide is prepared and is converted to 1 - N - t-butylsulfamoylmethyltetrazole - 5 - thiol as described therein. Treatment of 1 - N - t-butylsulfamoylmethyltetrazole - 5 - thiol with trifluoroacetic acid as described in Example

35 3 gives 1 - sulfamoylmethyltetrazole - 5 - thiol.

40 Reaction of 1 - sulfamoylmethyltetrazole - 5 - thiol with 7-aminocephalosporanic acid and treatment of the product, 7 - amino - 3 - (1 - sulfamoylmethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, with D-O-dichloroacetylmandeloyl chloride as described in Example 3 gives the title compound.

EXAMPLE 9.

7 - (D - α - Amino - 4 - hydroxyphenylacetamido) - 3 - [1 - (2 - N,N-dimethylsulfamoyl)ethyl]-tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

50 1 - (2 - N,N - Dimethylsulfamoyl)ethyl-tetrazole - 5 - thiol is prepared by reaction of dimethylamine and 2-phthalimidoethanesulfonyl chloride, followed by conversion of the product thus obtained to the tetrazole-thiol by the reaction sequence described in the procedure of Example 3.

55 When 1 - (2 - N,N - dimethylsulfamoyl)ethyl-tetrazole - 5 - thiol is reacted with 7 - (D - α - t - butoxycarbonyl - 4 - hydroxyphenylacetamido)cephalosporanic acid as described in Example 6 and the product is deblocked as described in Example 2, the title compound is obtained.

EXAMPLE 10.

7 - D - Mandelamido - 3 - [1 - (3 - sulfopropyl)tetrazol - 5 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid.

When an equivalent amount of 3-aminopropanesulfonic acid was substituted in the procedure of Example 7 for 2-aminoethanesulfonic acid, methyl 3-sulfopropylidithiocarbamate potassium salt was prepared.

Reaction of methyl 3-sulfopropylidithiocarbamate potassium salt with sodium azide, as described in Example 7, gave 1 - (3-sulfopropyl)tetrazole - 5 - thiol.

Substitution of an equivalent amount of 1 - (3 - sulfopropyl)tetrazole - 5 - thiol in the procedure of Example 7 in place of 1 - (2 - sulfoethyl)tetrazole - 5 - thiol in the reaction with 7 - D - mandelamidocephalosporanic acid sodium salt gives the title compound.

The title compound is converted to the corresponding sodium salt as described in the procedure of Example 1.

EXAMPLE 11.

7 - D - Mandelamido - 3 - [1 - (3 - sulfamoylpropyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

When 3 - phthalimidopropanesulfonyl chloride is substituted in the procedure of Example 3 for 2-phthalimidoethanesulfonyl chloride, N - t - butyl - 3 - phthalimidopropanesulfonamide is prepared, and is converted to 1 - (3 - N - t - butylsulfamoylpropyl)tetrazole - 5 - thiol as described therein. Treatment of 1 - (3 - N - t - butylsulfamoylpropyl) - tetrazole - 5 - thiol with trifluoroacetic acid as described in Example 3 gives 1 - (3 - sulfamoylpropyl)tetrazole - 5 - thiol.

Reaction of 1 - (3 - sulfamoylpropyl)tetrazole - 5 - thiol with 7-aminocephalosporanic acid and treatment of the resulting 7 - amino - 3 - [1 - (3 - sulfamoylpropyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid with D - O - dichloroacetylmandeloyl chloride as described in Example 3 gives the title compound.

EXAMPLE 12.

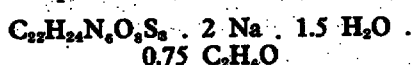
7 - D - Mandelamido - 3 - [1 - (5 - sulfopentyl)tetrazol - 5 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid.

Substitution of an equivalent amount of 5-aminopentanesulfonic acid in the procedure of Example 7 for 2-aminoethanesulfonic acid gave methyl 5-sulfopentylidithiocarbamate potassium salt.

Reaction of methyl 5 - sulfopentylidithiocarbamate potassium salt with sodium azide, as described in Example 7, gave 1 - (5-sulfopentyl)tetrazole - 5 - thiol.

A mixture of 4.55 g. (0.010 mol.) of 7 - D - mandelamidophthalosporanic acid sodium salt and 3.25 g. (0.011 mol.) of 1 - (5-

sulfopentyl)tetrazole - 5 - thiol disodium salt, prepared as previously described in Example 1, in 80 ml. of water at pH 7.2 (adjusted by addition of sodium bicarbonate) was heated at 65° for 4.5 hours. The reaction mixture was cooled, acidified to pH 1.6 with 3N hydrochloric acid and extracted with ethyl acetate. The pH of the aqueous phase was brought to 7.0 by addition of sodium bicarbonate and the solution was chromatographed on an Amberlite XAD-4 resin column, eluting with water and then methanol. The product was dissolved in methanol and ethanol was added to the solution to precipitate the title compound as its disodium salt.



Calculated:

40.08% C; 4.50% H; 11.93% N;
13.65% S

Found:

40.01% C; 4.20% H; 10.71% N;
13.54% S

7 - D - Mandelamido - 3 - [1 - (5 - sulfopentyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid disodium salt is converted to the title compound as described in Example 1.

EXAMPLE 13.

7 - D - Mandelamido - 3 - [1 - (5 - sulfamoylpentyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

Use of 5-aminopentanesulfonic acid in the procedure of Example 8 in place of aminomethanesulfonic acid, followed by reaction of the product thus obtained with phosphorus pentachloride, gives 5-phthalimidopentane-sulfonyl chloride.

When 5-phthalimidopentanesulfonyl chloride is used as a starting material in the sequence described in Example 3, 1 - (5 - N - t - butyl-sulfamoylpentyl)tetrazole - 3 - thiol is obtained. Treatment of 1 - (5 - N - t - butyl-sulfamoylpentyl) - tetrazole - 5 - thiol with trifluoroacetic acid as described above gives 1 - (5 - sulfamoylpentyl)tetrazole - 5 - thiol.

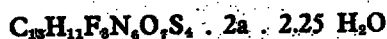
Reaction of 1 - (5 - sulfamoylpentyl)tetrazole - 5 - thiol with 7 - aminocephalosporanic acid and treatment of the resulting 7 - amino - 3 - [1 - (5 - sulfamoylpentyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid with D - O - dichloroacetyl-mandeloyl chloride as described in Example 3 gives the title compound.

EXAMPLE 14.

7 - Trifluoromethylthioacetamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - cephem - 4 - carboxylic acid.

A solution of 2.18 g. (10.0 mmol.) of 1-sulfomethyltetrazole - 5 - thiol sodium salt,

prepared as described in Example 1, 0.840 g. of sodium bicarbonate and 5.45 g. (12.5 mmol.) of 7 - trifluoromethylthioacetamidocephalosporanic acid sodium salt in 60 ml. of water was stirred at 70—75° for five hours while maintaining the pH at 6.8 by addition of 5% aqueous sodium carbonate solution. The reaction mixture was cooled and diluted with water. Ethyl acetate was added and the mixture was acidified to pH 2.0 with 6N hydrochloric acid. The aqueous phase was extracted with ethyl acetate, then brought to pH 6.8 by addition of sodium bicarbonate and chromatographed on Amberlite XAD-4 resin with water and methanol as eluents. The product-containing fractions were evaporated to dryness to give a residue which was dissolved in water and lyophilized to give the title compound as the disodium salt.



Calculated:

24.59% C; 2.46% H; 13.23% N

Found:

24.54% C; 2.18% H; 12.85% N

An aqueous solution of 7 - trifluoromethylthioacetamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid disodium salt is treated with Amberlite IR-120H ion exchange resin as described in Example 1 to give the title compound.

EXAMPLE 15.

7 - Trifluoromethylthioacetamido - 3 - [1 - (3 - sulfamoylpropyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

7 - Trifluoromethylthioacetamido - 3 - [1 - (3 - sulfamoylpropyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid sodium salt is prepared by substitution of an equivalent amount of 1 - (3 - sulfamoylpropyl)tetrazole - 5 - thiol sodium salt, prepared as described in Example 1, in the procedure of Example 14.

The title compound is obtained from the salt as described above.

EXAMPLE 16.

7 - Trifluoromethylthioacetamido - 3 - [1 - (2 - sulfamoylethyl)tetrazole - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

A mixture of 2.18 g. (5 mmol.) of 7 - trifluoromethylthioacetamidocephalosporanic acid sodium salt and 1.75 g. (8 mmol.) of 1 - (2 - sulfamoylethyl)tetrazole - 5 - thiol in 50 ml. of water, maintained at pH 7.0 by addition of sodium bicarbonate, was heated at 60—70° for three hours. The reaction mixture was cooled, acidified to pH 3.5 and extracted with ethyl acetate. The extract was

washed with water, dried (MgSO_4) and evaporated to dryness to give a residue which was dissolved in ethyl acetate. Ether was added, the solution was filtered, petroleum ether was added to the filtrate and the resulting precipitate was collected by filtration and dissolved in methanol. A 5% solution of sodium methoxide in methanol was added to the methanol solution until pH 7.1. Ether was added and the precipitate was collected and dried *in vacuo* to give 7-trifluoromethylthioacetamido - 3 - [1 - (2 - sulfamoyl-ethyl)-tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid sodium salt.

7 - Trifluoromethylthioacetamido - 3 - [1 - (2 - sulfamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid sodium salt is converted to the title compound by methods described in Example 1.

EXAMPLE 17.

7 - D - Mandelamido - 3 - [1 - (10 - sulfodecyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

A suspension of 56 g. (1.0 mol.) of potassium hydroxide and 118.7 g. (0.5 mol.) of 10-aminodecanesulfonic acid in 170 ml. of water is stirred for 30 minutes at 25°, then 40 g. (0.52 mol.) of carbon disulfide and 80 ml. of ethanol are added and the reaction mixture is stirred at 25° for 12 hours. The mixture is refluxed gently for two hours and cooled. Methyl iodide (71 g., 0.3 mol.) and 130 ml. of ethanol are added to the mixture and it is stirred at 25° for 12 hours. The mixture is evaporated to remove the ethanol and the solid residue is collected by filtration to give methyl 10-sulfodecylidithiocarbamate.

Methyl 10-sulfodecylidithiocarbamate (31.4 g., 0.096 mol.) is reacted with 6.5 g. (0.1 mol.) of sodium azide as described in Example 1 to give 1 - (10 - sulfodecyl)tetrazole - 5 - thiol.

1 - (10 - Sulfodecyl)tetrazole - 5 - thiol (4.84 g., 15 mmol.) is slowly added to a solution of 3.36 g. (40 mmol.) of sodium bicarbonate in 100 ml. of water. 7 - D - Mandelamidocephalosporanic acid (4.20 g., 10 mmol.) is then added and the mixture is heated at 65° for 3.5 hours. The mixture is filtered, the filtrate is extracted with ethyl acetate and the aqueous layer is acidified to pH 4 and extracted again with ethyl acetate. The extract is dried (MgSO_4) and evaporated to dryness to give the title compound.

EXAMPLE 18.

7 - D - Mandelamido - 3 - [1 - (10 - sulfamoyldecyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

Use of 10-amidodecanesulfonic acid in the procedure of Example 8 in place of aminomethanesulfonic acid, followed by reaction of the product thus obtained with phosphorus

pentachloride, gives 10-phthalimidodecanesulfonyl chloride.

When 10-phthalimidodecanesulfonyl chloride is used as a starting material in the sequence described in Example 3, 1 - (10 - N - t-butylsulfamoyldecyl)tetrazole - 5 - thiol is obtained. Treatment of 1 - (10 - N - t-butylsulfamoyldecyl)tetrazole - 5 - thiol with trifluoroacetic acid gives 1 - (10 - sulfamoyldecyl)tetrazole - 5 - thiol.

Reaction of 1 - (10 - sulfamoyldecyl)tetrazole - 5 - thiol with 7 - aminocephalosporanic acid and treatment of the resulting 7 - amino - 3 - [1 - (10 - sulfamoyldecyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid with D - O - dichloroacetylmandeloyl chloride as described in Example 3 gives the title compound.

EXAMPLE 19.

7 - D - Mandelamido - 3 - [1 - (2 - sulfo - 1 - methylethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

Substitution of an equivalent amount of 2-aminopropanesulfonic acid in the procedure of Example 7 for 2-aminoethanesulfonic acid gives methyl (2 - sulfo - 1 - methylethyl)-dithiocarbamate potassium salt.

Treatment of methyl (2 - sulfo - 1 - methylethyl)dithiocarbamate potassium salt with sodium azide also as described in Example 7 gives 1 - (2 - sulfo - 1 - methylethyl)tetrazole - 5 - thiol.

7 - D - Mandelamidocephalosporanic acid and 1 - (2 - sulfo - 1 - methylethyl)tetrazole - 5 - thiol are reacted in the presence of excess sodium bicarbonate as described in Example 7 to give the title compound.

EXAMPLES 20 and 21.

Reaction of the sodium salt of a cephalosporanic acid listed below:

7 - (2,2,2 - trifluoroethylthioacetamido)-cephalosporanic acid,

7 - methylthioacetamidoccephalosporanic acid

with 1 - (2 - sulfamoyl-ethyl)tetrazole - 5 - thiol sodium salt by the procedure of Example 14 gives the 7 - substituted - 3 - [1 - (2 - sulfamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acids listed below:

7 - (2,2,2 - trifluoroethylthioacetamido)-3 - [1 - (2 - sulfamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid (Example 20).



Calculated:

30.24% C; 3.11% H; 15.92% N

Found:

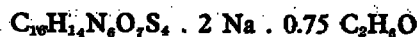
30.62% C; 3.00% H; 15.32% N

7 - methylthioacetamido - 3 - [1 - (2-sulfamoylthioethyl) - tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid (Example 21).

EXAMPLE 22.

7 - (2 - Thienylacetamido) - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

Reaction of 2.18 g. (0.01 mol.) of 1-sulfomethyltetrazole - 5 - thiol sodium salt, 5.23 g. (0.012 mol.) of 7 - (2 - thienylacetamido)cephalosporanic acid sodium salt and 0.84 g. (0.01 mol.) of sodium bicarbonate as described in the procedure of Example 14 gave the title compound as the corresponding disodium salt.



Calculated:

34.81% C; 3.09% H; 13.56% N

Found:

34.41% C; 3.03% H; 13.76% N

7 - (2 - Thienylacetamido) - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid disodium salt is converted to the title compound as described in Example 1.

EXAMPLE 23.

7 - (2 - Thienylacetamido) - 3 - [1 - (2-sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

Reaction of 5.68 g. (0.01 mol.) of 1 - (2-sulfoethyl)tetrazole - 5 - thiol cyclohexylamine salt and 5.23 g. (0.0125 mol.) of 7 - (2 - thienylacetamido)cephalosporanic acid sodium salt in 80 ml. of water containing sufficient sodium bicarbonate to maintain the pH at 7.0, as described in the procedure of Example 14, gave the title compound.

Preparation of 7 - amino - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid (starting material in Example 24).

To a mixture of 97 g. (200 ml., 2.1 mol.) of formic acid, distilled from anhydrous copper sulfate, and 37.5 ml. (0.4 mol.) of acetic anhydride was added 25.0 g. (0.1 mol.) of 7-aminocephalosporanic acid. The mixture was stirred at ambient temperature for 0.5 hour, then evaporated to dryness. The residue was dissolved in ethyl acetate and the ethyl acetate solution was filtered and evaporated to dryness to give a residue which was recrystallized from ether-petroleum ether to give 7-formamidocephalosporanic acid.

A mixture of 1.0 g. (3.3 mmol.) of 7-formamidocephalosporanic acid and 0.7 g. (2.6 mmol.) of 1 - sulfomethyltetrazole - 5 - thiol disodium salt in 15 ml. of water was stirred at 65-70° for 3 hours while main-

taining the pH at 7.0 by addition of sodium bicarbonate and/or hydrochloric acid. The mixture was cooled, acidified to pH 1.0 with hydrochloric acid and extracted with ethyl acetate. The extract was filtered and the filtrate was evaporated to dryness to give a residue which was dissolved in 30 ml. of methanol. The methanol solution was filtered, 30-40 ml. of isopropanol and ethanol were added, the solution was filtered again and 100 ml. of ether was added. The precipitate was collected by filtration and dried to give the title compound.



Calculated:

27.0 % C; 3.50% H; 16.95% N

Found:

26.96% C; 3.23% H; 16.55% N

EXAMPLE 24.

7 - (4 - Pyridylthioacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

(4-Pyridylthio)acetyl chloride (0.53 g., 2.8 mmol.) was dropwise added to a mixture of 1.0 g. of 7 - amino - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid (mixture of sodium salt, free acid and sodium chloride) and 0.9 g. (9.0 mmol.) of triethylamine in 10 ml. of dry dimethylformamide. The reaction mixture was stirred for 1.5 hour at -10°, then it was warmed to ambient temperature and stirred for 1 hour. The mixture was filtered and the filtrate was diluted with 200 ml. of ether-petroleum ether. The precipitate was collected by filtration and dissolved in warm ethanol. The methanol solution was filtered and the filtrate was concentrated to 10 ml. Ethanol (50 ml.) was added and the precipitate was collected by filtration and washed with ether to give the title compound as the sodium salt. Additional amounts of the salt were obtained by addition of ether to the filtrate.



Calculated:

37.23% C; 4.21% H; 15.10% N

Found:

36.84% C; 4.48% H; 15.43% N

7 - (4 - Pyridylthioacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid sodium salt is converted to the title compound by procedures described in Example 1.

EXAMPLES 25 to 29.

Using the procedures described in Examples 7, 12, 14 and 24 for the preparation of compounds of this invention, the following compounds were prepared:

7 - (1 - tetrazolylacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3-cephem - 4 - carboxylic acid (Example 25).



- 5 Calculated:
27.76% C; 2.15% H; 24.90% N
Found:
27.55% C; 3.21% H; 23.00% N

10 7 - sydon - 3 - ylacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3-cephem - 4 - carboxylic acid (Example 26).



- 15 Calculated:
27.59% C; 3.72% H; 16.08% N
Found:
27.59% C; 3.02% H; 15.67% N

20 7 - trifluoromethylthioacetamido - 3 - [1-(4 - sulfobutyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid (Example 27).



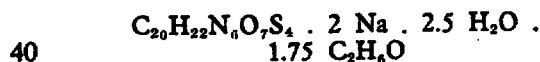
- 25 Calculated:
29.36% C; 2.93 % H; 12.84%N
Found:
29.31% C; 3.33% H; 12.76% N

7 - (2 - thienylacetamido) - 3 - [1 - (4-sulfobutyl)tetrazol - 5 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid (Example 28).



- 30 Calculated:
34.85% C; 3.69% H; 12.83% N;
19.59% S
Found:
34.52% C; 3.88% H; 13.54% N;
19.09% S

7 - (2 - thienylacetamido - 3 - [1 - (5-sulfopentyl)tetrazol - 5 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid (Example 29).



- 45 Calculated:
36.33% C; 4.77% H; 11.29% N;
17.23% S
Found:
36.46% C; 4.30% H; 11.63% N;
17.30% S

EXAMPLE 30.

7 - (2 - Thienylacetamido) - 3 - [1 - (2-sulphamoyl-ethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid. 50

Reaction of 2-thiopheneacetyl chloride and 7 - amino - 3 - [1 - (2 - sulphamoyl-ethyl)-tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid according to the procedure of Example 24 yielded the title compound. 55

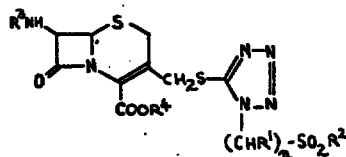
EXAMPLE 31.

An injectable pharmaceutical composition is formed by adding sterile water or sterile saline solution (2 ml) to 500 mg. of 7 - D-mandelamido - 3 - [1 - (2 - sulphamoyl-ethyl) - tetrazol - 4 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid sodium salt. 60

Pharmaceutical compositions of the other antibacterial compounds disclosed above may be formulated in a similar manner. 65

WHAT WE CLAIM IS:—

1. A cephalosporin compound of the structural formula:



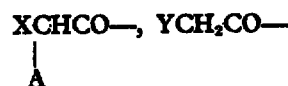
in which:

each individual R^1 is hydrogen or lower alkyl;

n is from 1 to 10;

R^2 is hydroxy, amino, (lower alkyl)amino or di(lower alkyl)amino; 75

R^3 is an acyl group of the structure



or



where:

X is thienyl; dihydrophenyl; phenyl; phenyl mono-substituted with hydroxy, hydroxymethyl, formamido, ureido or carboxymethyl-amino; or 3-fluoro-4-hydroxyphenyl; 85

A is hydroxy, amino, $COOR^4$ or SO_3H ; or formyloxy when X is phenyl;

Y is thienyl, tetrazolyl, cyano, sydn-3-yl or aminomethylphenyl;

Z is methyl, trifluoromethyl, trifluoroethyl, cyanomethyl or pyridyl; and 90

m is 0, 1 or 2;

and each $COOR^4$ is a carboxylic acid or esterified carboxylic acid group;

or a non-toxic pharmaceutically-acceptable salt thereof. 95

2. A compound according to Claim 1 in which each $COOR^4$ is a carboxylic acid group.



7 - (1 - tetrazolylacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3-cephem - 4 - carboxylic acid (Example 25).



Calculated:

27.76% C; 2.15% H; 24.90% N

Found:

27.55% C; 3.21% H; 23.00% N

7 - sydon - 3 - yacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3-cephem - 4 - carboxylic acid (Example 26).



Calculated:

27.59% C; 3.72% H; 16.08% N

Found:

27.59% C; 3.02% H; 15.67% N

7 - trifluoromethylthioacetamido - 3 - [1 - (4 - sulfobutyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid (Example 27).



Calculated:

29.36% C; 2.93 % H; 12.84%N

Found:

29.31% C; 3.33% H; 12.76% N

7 - (2 - thienylacetamido) - 3 - [1 - (4-sulfobutyl)tetrazol - 5 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid (Example 28).



Calculated:

34.85% C; 3.69% H; 12.83% N;

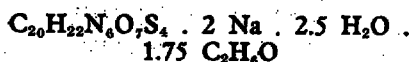
19.59% S

Found:

34.52% C; 3.88% H; 13.54% N;

19.09% S

7 - (2 - thienylacetamido - 3 - [1 - (5-sulfopentyl)tetrazol - 5 - ylthiomethyl] - 3-cephem - 4 - carboxylic acid (Example 29).



Calculated:

36.33% C; 4.77% H; 11.29% N;

17.23% S

Found:

36.46% C; 4.30% H; 11.63% N;

17.30% S

EXAMPLE 30.

7 - (2 - Thienylacetamido) - 3 - [1 - (2-sulphamoyl-ethyl)tetrazol - 5 - ylthio-methyl] - 3 - cephem - 4 - carboxylic acid.

Reaction of 2-thiopheneacetyl chloride and 7 - amino - 3 - [1 - (2 - sulphamoyl-ethyl)-tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid according to the procedure of Example 24 yielded the title compound.

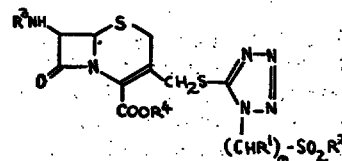
EXAMPLE 31.

An injectable pharmaceutical composition is formed by adding sterile water or sterile saline solution (2 ml) to 500 mg. of 7 - D-mandelamido - 3 - [1 - (2 - sulphamoyl-ethyl) - tetrazol - 4 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid sodium salt.

Pharmaceutical compositions of the other antibacterial compounds disclosed above may be formulated in a similar manner.

WHAT WE CLAIM IS:—

1. A cephalosporin compound of the structural formula:



in which:

each individual R¹ is hydrogen or lower alkyl;

n is from 1 to 10;

R² is hydroxy, amino, (lower alkyl)amino or di(lower alkyl)amino;

R³ is an acyl group of the structure



A

or



where:

X is thienyl; dihydrophenyl; phenyl; phenyl mono-substituted with hydroxy, hydroxymethyl, formamido, ureido or carboxymethyl-amino; or 3-fluoro-4-hydroxyphenyl;

A is hydroxy, amino, COOR⁴ or SO₃H; or formyloxy when X is phenyl;

Y is thienyl, tetrazolyl, cyano, sydnon-3-yl or aminomethylphenyl;

Z is methyl, trifluoromethyl, trifluoroethyl, cyanomethyl or pyridyl; and

m is 0, 1 or 2;

and each COOR⁴ is a carboxylic acid or esterified carboxylic acid group;

or a non-toxic pharmaceutically-acceptable salt thereof.

2. A compound according to Claim 1 in which each COOR⁴ is a carboxylic acid group.

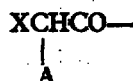


3. A compound according to Claim 1 or Claim 2 in which each R^1 is hydrogen and n is from 1 to 5.

4. A compound according to Claim 3, in which n is 1 or 2.

5. A compound according to any preceding claim in which R^2 is hydroxy or amino.

6. A compound according to any preceding claim in which R^3 is



where X is phenyl or hydroxyphenyl and A is hydroxy or amino.

7. A compound according to any one of Claims 1 to 5 in which R^3 is $\text{YCH}_2\text{CO—}$ where Y is thienyl.

8. A compound according to any one of Claims 1 to 5 in which R^3 is $\text{YCH}_2\text{CO—}$ where Y is 2-aminomethylphenyl.

9. A compound according to any one of Claims 1 to 5 in which R^3 is



where Z is trifluoromethyl and m is 0.

10. 7 - D - mandelamido - 3 - (1 - sulfo-methyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

11. 7 - D - mandelamido - 3 - [1 - (2-sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

12. 7 - D - mandelamido - 3 - [1 - (5-sulfopentyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

13. 7 - D - mandelamido - 3 - [1 - (2-sulfamoylthyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

14. 7 - (2 - Thienylacetamido) - 3 - (1-sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

15. 7 - (2 - Thienylacetamido) - 3 - [1 - (2 - sulfoethyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

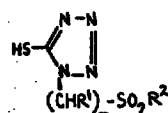
16. 7 - (2 - Thienylacetamido) 3 - [1 - (2 - sulfamoylthyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

17. 7 - Trifluoromethylthioacetamido - 3 - (1 - sulfomethyltetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

18. 7 - Trifluoromethylthioacetamido - 3 - [1 - (2 - sulfamoylthyl)tetrazol - 5 - ylthiomethyl] - 3 - cephem - 4 - carboxylic acid.

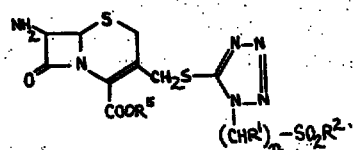
19. The compound according to Claim 1 of any one of Examples 2, 4 to 6, 8 to 11, 13, 15, 17 to 21 and 24.

20. A process for preparing a compound according to any one of Claims 1 to 19, where each COOR^4 is a carboxylic acid group, in which 7-aminocephalosporanic acid is acylated with an appropriately protected acylating agent capable of providing the acyl group R^3 and the 3-acetoxy group in the acylation product is displaced by reaction with the appropriate tetrazole-thiol of the structure



and any protecting group is subsequently removed.

21. A process for preparing a compound according to any one of Claims 1 to 19, where each COOR^4 is a carboxylic acid group, in which the appropriate 7-amino-3-substituted tetrazolythiomethyl cephalosporin of the structure



where R^1 and R^2 are as defined in Claim 1 and R^5 is hydrogen or a protecting ester-forming group, is acylated with an appropriate acylating agent, followed by removal of any protecting group.

22. A process for preparing a compound according to Claim 1 substantially as described in any one of Examples 1 to 24 and 30.

23. A compound according to Claim 1 whenever prepared by a process according to any one of Claims 20 to 22.

24. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 19 and 23 and a pharmaceutically-acceptable carrier.

25. A pharmaceutical composition according to Claim 24 and substantially as described in Example 31.

26. A method of combatting bacterial infection in a non-human animal which comprises administering to the animal a pharmaceutical composition as claimed in Claim 24 or Claim 25 in a non-toxic amount sufficient to combat such infection.

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Chartered Patent Agent.