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(54) CEPHALOSPORIN DERIVATIVES AND A PROCESS FOR PREPARING THE SAME.

(71) We, **BRISTOL-MYERS COMPANY**, a Corporation existing under the laws 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, and the method by which it is to be performed, to be particularly described in and by the following statement:—

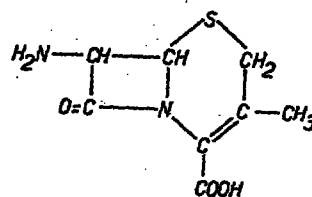
This invention relates to cephalosporin derivatives and to a process for preparing the same.

Antibacterial agents of the cephalosporin class have proven highly effective in the therapy of infections due to either Gram-positive or Gram-negative bacteria but few are effective against both, very few are effective in concentrations below 1.0 mcg./ml. and none are very effective in practical use against infections caused by *Salmonella* e.g. *S. enteritidis*.

We have now found that cephalosporin of formula I or a derivative thereof of formula V defined below are 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 and especially *Salmonella*.

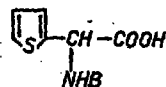
Accordingly the present invention provides a process for the preparation of the compound of the formula:—

or a nontoxic, pharmaceutically acceptable salt thereof, which process comprises acylating 3 - methyl - 7 - aminodecephalosporanic acid of the formula:



(II)

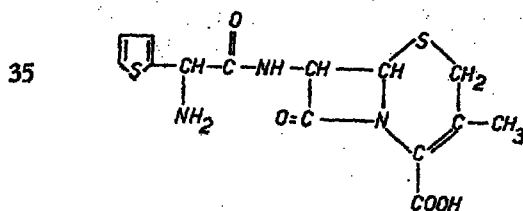
or a neutral salt thereof, such as the sodium salt or the triethylamine salt, with at least a substantially equimolar amount of an acid of the formula:—



(III)

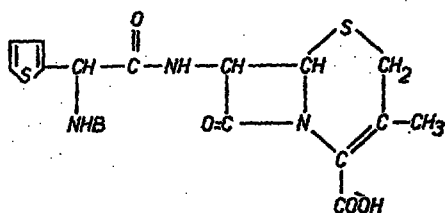
or an acylating derivative thereof, wherein B is hydrogen or a blocking group as herein-after defined, in an inert solvent at a temperature of from about $-50^{\circ}\text{C}.$ to about $50^{\circ}\text{C}.$; and, when B is a blocking group, removing said blocking group to produce the desired compound or salt thereof; and, if desired, converting the thus produced compound to a nontoxic pharmaceutically acceptable salt thereof.

The invention also provides a process for the preparation of the compound of the formula:—



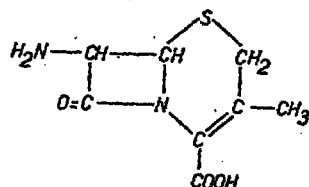
①

[Price 5s. 0d. (25p)]



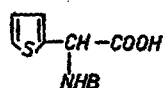
(IV)

wherein B is a blocking group, as herein-
after defined, or a nontoxic, pharmaceutically
acceptable salt thereof, which process com-
prises acylating 3 - methyl - 7 - amino-
decephalosporanic acid of the formula:—



(II)

or a neutral salt thereof, with at least a
substantially equimolar amount of acid of
the formula:—



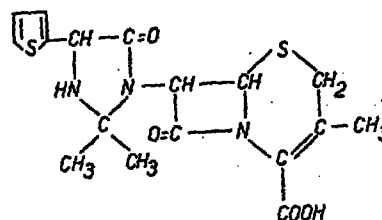
(III)

or an acylating derivative thereof, wherein B
is a blocking group, as hereinafter defined, in
an inert solvent at a temperature of from
about $-50^{\circ}\text{C}.$ to about $50^{\circ}\text{C}.$ to produce the
desired compound or salt thereof, and, if
desired, converting the thus produced com-
pound to a nontoxic pharmaceutically accept-
able salt thereof.

By "blocking group" is meant a group
which prevents or substantially inhibits the
inter-reaction of the carboxyl and amino
groups of molecules of the compound of
formula (III).

The invention further provides a compound
of the formula (I) or the formula (IV) or a
nontoxic, pharmaceutically acceptable salt
thereof.

The invention still further provides the
compound having the formula



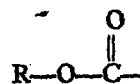
(V)

or a nontoxic pharmaceutically acceptable salt
thereof.

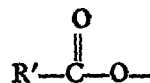
This compound or a salt thereof, 3 - methyl-
7 - [2',2' - dimethyl - 5' - oxo - 4' - (2''-
thienyl) - 1' - imidazolidinyl]decephalospor-
anic acid is prepared by reacting the compound
of formula (I) or a salt thereof with an excess
of acetone.

The nontoxic, pharmaceutically acceptable
salt referred to above includes both salts of
the carboxylic acid group and of the amino
group. Salts of the carboxylic acid group
include salts with metallic cations such as the
sodium, potassium, calcium and aluminium
salts, and salts with organic amines such as
trialkylamines, e.g. triethylamine, procaine,
dibenzylamine, N - benzyl - β - phenethyl-
amine, 1 - ephenamine, N,N' - dibenzyl-
ethylenediamine, dehydroabietylamine, N,N'-
bis - dehydroabietyleneethylenediamine, N-
(lower)alkylpiperidines, e.g. N - ethyl-
piperidine, and other amines which have been
used to form salts with benzylpenicillin. The
salts of the amino group include both the
organic and inorganic acid addition salts, e.g.
those prepared from acids such as hydro-
chloric, sulfuric, sulfonic, tartaric, fumaric,
hydrobromic, hydriodic, glycolic, citric,
maleic, phosphoric, succinic, acetic, nitric,
cyclohexylsulfamic, naphthalenesulfonic or
methanesulfonic.

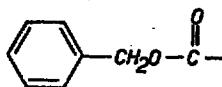
Blocking groups suitable for protecting the
amino group of the acid of formula III are
well-known to those skilled in the art. They
include, for example, groups of the formula



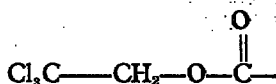
wherein R may be allyl, tertiary butyl, sub-
stituted alkyl, phenyl, substituted phenyl,
benzyl or substituted benzyl; the triphenyl-
methyl group; or



wherein R' may be the triphenylmethyl
group. Preferred blocking groups are in the
carbobenzoxy group of the formula



and the trichlorethoxycarbonyl group of the formula

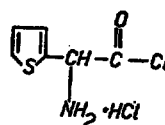


5 After completion of the acylation, the blocking group is removed by conventional means, e.g. the carbobenzoxy group may be removed by catalytic hydrogenation and the trichloroethoxycarbonyl group by treatment with zinc dust in glacial acetic acid.

10 The acylating derivatives of the acid of formula III above which are suitable for the acylation of compounds of formula II are well-known to those skilled in the art, and include the corresponding acid chlorides, acid bromides, acid azides, acid anhydrides, mixed acid anhydrides (and particularly the mixed anhydrides prepared from stronger acids such as the lower aliphatic monoesters of carbonic acid, of alkyl and aryl sulfonic acids and of more hindered acids such as diphenylacetic acid), active esters (e.g. the p-nitrophenyl ester, the 2,4 dinitrophenyl ester or N-hydroxysuccinimide ester), and active thioesters (e.g. with thiophenol or thioacetic acid). In addition, the free acid itself may be coupled with the compound of formula II after first reacting said free acid with N,N'-dimethylchloroformiminium chloride [cf. British Patent 1,008,170 and Novak and Weichert, *Experientia* XXI/6, 360 (1965)] or by the use of enzymes or an N,N'-carbonyldiimidazole or an N,N'-carbonyltriazole [cf. South African Patent 63/2684], or by the use of a carbodiimide reagent (especially N,N'-dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide or N-cyclohexyl-N'- (2-morpholinoethyl)carbodiimide [cf. Sheehan and Hess, *J. Amer. Chem. Soc.* 77, 1067 (1955)]). Use may also be made of an alkynylamine reagent [cf. R. Buijle and H. G. Viehe, *Angewandte Chemie International Edition* 3, 582 (1964)], a ketenimine reagent [cf. C. L. Stevens and M. E. Monk, *J. Amer. Chem. Soc.* 80, 4065 (1958)] or an isoxazolium salt reagent [cf. Woodward, Olofson and Mayer, *J. Amer. Chem. Soc.*, 83, 1010 (1961)]. Another acylating derivative of the acid of formula III is the corresponding azolide, i.e. an amide of the acid whose amide nitrogen is a member of a quasiaromatic five-membered ring containing at least two nitrogen atoms, i.e. imidazole, pyrazole, the triazoles, benzimidazole, benzotriazole and their substituted derivatives. As an example of the general

method for the preparation of an azolide, N,N'-carbonyldiimidazole is reacted with a carboxylic acid in equimolar proportions at room temperature in tetrahydrofuran, chloroform, dimethylformamide or a similar inert solvent to form the carboxylic acid imidazolidine in practically quantitative yield with liberation of carbon dioxide and one mole of imidazole. Dicarboxylic acids yield diimidazolides. The by-product, imidazole, precipitates and may be separated and the imidazolidine isolated, but this is not essential. The methods for carrying out these reactions to produce a penicillin or a cephalosporin and the methods used to isolate the penicillin or the cephalosporin so-produced are well-known in the art (cf. U.S. Patents Nos. 3,079,314; 3,117,126 and 3,129,224 and British Patents Nos. 932,644; 957,570 and 959,054).

When the acylating derivative of the acid III is the acid halide, it is often preferred to utilize the compound in the form of the acid halide hydrohalide, e.g. the acid chloride hydrochloride having the formula



(VI)

Use of the acid halide hydrohalide avoids the additional step of removing the blocking group from the product, such as is necessary when utilizing a blocking group, e.g. the carbobenzoxy group.

The acylation reaction may be conducted in an inert organic solvent or in a mixture of such solvent and water. Suitable inert organic solvents include acetone, tetrahydrofuran, dioxane, dimethylformamide, dimethylacetamide, chlorinated aliphatic hydrocarbons (e.g. methylene chloride), dimethylsulfoxide, methyl isobutyl ketone and dialkyl ethers of ethylene- or di-ethylene glycol. In some instances it may be desirable to add a solution of the acylating agent in a solvent such as benzene to a solution of a salt of the acid of formula II in an aqueous organic solvent (e.g. acetone-water). In such an instance the reaction medium may be either one phase or two phases, depending on the relative amount of water and acetone. Vigorous stirring is, of course, preferred when using a two-phase reaction medium.

While the acylating reaction may be conducted over a temperature of from about -50° C. to 50° C., the preferred reaction temperature is from about -10° C. to about 15° C.

3 - Methyl - 7 - aminodecephalosporanic acid (also called 7 - amino - 3 - methyl - 3 -

(12)

cephem - 4 - carboxylic acid) of formula II, used as one starting material in the process of this invention is prepared by catalytic reduction of cephalosporin C followed by hydrolytic removal of the 5-aminoadipoyl side chain, as described in Specification No. 957,569.

In the acylating acid of formula III the carbon atom bearing the free amino group is an asymmetric carbon atom and thus the reagents and final products 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 within the scope of the present invention.

Starting Reagents

α - (2 - Thienyl)glycine [also called α -amino - (2 - thienyl)acetic acid] is prepared according to Ber. 19, 2115 (1886). (—)- α -(2-Thienyl)glycine and (—)- α - carbobenzoxyamino - α - (2 - thienyl)acetic acid are prepared in the following manner:

A mixture of 25 gms. (0.157 mole) of d,1- α -amino(2-thienyl)acetic acid and 43.6 gms. (0.188 mole) of d-10-camphorsulfonic acid in 250 ml. of acetone is heated to boiling to bring the solids into solution. The hot solution is filtered to remove any insoluble material, and the filtrate is cooled in an ice bath. The resulting crystalline precipitate is recovered by filtration, washed with acetone, and air-dried to give 27.2 gms. of the diastereoisomeric camphorsulfonate salt of the amino acid. This salt is suspended in a small amount of water, the pH is adjusted to 6.3 with concentrated NH_4OH , and the mixture is cooled in an ice bath. The crystalline product, (—)- α -amino(2-thienyl)acetic acid, is recovered by filtration, washed with water and acetone, recrystallized twice from isopropanol, and air-dried. It is found to weigh 4.5 gms., to melt with decomposition at 189–191° C., and to have an optical rotation of $[\alpha]_D^{25} = -73.7^\circ$ (C=1.0% in water).

(—)- α -Amino(2-thienyl)acetic acid (10 gms.; 0.063 mole) is dissolved in 75 mls. of water at 5° C. Carbobenzoxy chloride (12 gms.; 0.07 mole) is added, the mixture is stirred and a solution of NaOH (5.0 gms.) in 30 mls. of water is added. The mixture, which has a pH of 5, is stirred for 25 minutes, extracted twice with ether and acidified to pH 2 by the addition of H_2SO_4 . The acid is then extracted with ether and the extract is washed with water and dried over magnesium sulfate to give a clear solution. The ether is evaporated and an oil is obtained which is slurried with petroleum ether. Crystals are thereby formed which are collected by filtration. The product, (—)- α - carbobenzoxyamino(2 - thienyl)acetic acid (13 gms.), is dried overnight *in vacuo* over P_2O_5 . The product is determined to have a melting point of 85–86° C.

α - (2,2,2 - Trichloroethoxycarbonylamino)-

α - (2 - thienyl)acetic acid is prepared as illustrated below in the examples from α -(2-thienyl)glycine and 2,2,2-trichloroethyl chloroformate.

The following examples will serve to illustrate this invention without limiting it thereto.

EXAMPLE 1

3 - Methyl - 7 - [D(—) - α - carbobenzoxyaminothienyl - 2' - acetamido] - decephalosporanic acid — To a stirred solution of 17.49 g. (0.06 mole) (—)-N-carbobenzoxy-2-thienylglycine, 8.4 ml. (0.06 mole) triethylamine and 300 ml. tetrahydrofuran at 0° C., there is added 6.13 ml. (0.06 mole) of trimethylacetyl chloride. After 10 min., a solution of 12.85 g. (0.06 mole) 3-methyl-7-aminodecephalosporanic acid, 16.8 ml. (0.12 mole) triethylamine, 150 ml. tetrahydrofuran and 150 ml. water is added. The reaction mixture is stirred at 0° for 30 min., at 0–10° for 1 hr., and then diluted with 200 ml. H_2O . The tetrahydrofuran is evaporated with the use of a rotary evaporator to give a thick syrup which is shaken with 400 ml. ethyl acetate. H_3PO_4 (17 ml. of 42%) is added and the product is extracted into the ethyl acetate. The aqueous solution is extracted with an additional 200 ml. ethyl acetate and the combined ethyl acetate extracts are dried briefly with sodium sulfate and filtered. The ethyl acetate is concentrated and gives, after dilution with petroleum ether, crystalline 3-methyl - 7 - [D(—) - α - carbobenzoxyaminothienyl - 2' - acetamido]decephalosporanic acid. It is characterized by its infrared spectrum: amide NH , 3300 cm^{-1} ; carboxyl OH and absorbed H_2O , 2800–3500 cm^{-1} ; β -lactam C , 1780 cm^{-1} ;

1690–1750 cm^{-1} , carboxyl C , amide C ;
amide NH deformation, 1525 cm^{-1} .

3 - Methyl - 7 - [D(—) - α - aminothienyl-2' - acetamido]decephalosporanic acid—The 3 - methyl - 7 - [D(—) - α - carbobenzoxyaminothienyl - 2' - acetamido]decephalosporanic acid prepared above (12.0 g.) is hydrogenated in four batches, in a Parr hydrogenation apparatus at an initial pressure of 48 pounds per square inch for exactly 5.0 minutes. Each batch contains a solution of 3.0 g. (0.00617 mole) of this material, 30 ml. 1,4-dioxane, 10 ml. of a saturated sodium bicarbonate solution, 165 ml. of water and 3.0 g. of 30% palladium on diatomaceous earth. The combined reaction mixture is acidified to pH 2.0 with 6N hydrochloric acid, and the catalyst is removed by filtration through a bed of diatomaceous earth. The filtrate is adjusted to pH 3.6 with a saturated

solution of sodium bicarbonate and diluted with an equal volume of n-butanol. The solution is concentrated at 33° at reduced pressure to approximately 10 ml. The product is collected to give about 6.0 g., containing about 45% NaCl. The filtrate is diluted with diethyl ether to give an additional 0.5 g. of crystalline product as the zwitter-ion. The total yield of product as the amino acid is about 3.5 g. (44%). The zwitter-ion is characterized by its infrared spectrum: amide NH, and (strong, broad) NH₃⁺, 3600—2400 cm⁻¹; β-lactam C, 1760 cm⁻¹; amide C,

$\begin{array}{c} \text{O} \\ \parallel \\ \text{C} \end{array}$
 $\begin{array}{c} \text{O} \\ \parallel \\ \text{C} \end{array}$

1690 cm⁻¹; carboxylate, 1600 and 1400 cm⁻¹.

The compound prepared above exhibited *in vitro* antibacterial activity against *S. aureus* Smith, against the benzylpenicillin-resistant *S. aureus* BX-1633—2, against *S. enteritidis*, against *S. typhosa* and against *Shig. sonnei*.

3 - Methyl - 7 - [2',2' - dimethyl - 5'-oxo - 4' - (2'' - thienyl) - 1' - imidazolidinyl]decephalosporanic acid—A solution containing 4.0 g. (containing about 45% sodium chloride; 0.0062 mole) of 3 - methyl - 7 - [D(-) - α - aminothieryl - 2' - acetamido]decephalosporanic acid, 0.77 ml. triethylamine, and 70 ml. acetone is stirred for 15 hours at 22°. The insoluble NaCl is filtered off, and the filtrate is evaporated on a rotary evaporator to give a brittle foam of the triethylammonium salt. The foam is dissolved in 17 ml. of H₂O, acidified to pH 2.4 with 42% H₃PO₄, and quickly extracted with 75 ml. of ethyl acetate. The aqueous solution is again extracted with 50 ml. of ethyl acetate. The ethyl acetate extracts are combined, dried briefly with Na₂SO₄ and evaporated to dryness to give about 63 mg. of crystalline 3 - methyl - 7 - [2',2' - dimethyl - 5' - oxo - 4' - (2'' - thienyl) - 1' - imidazolidinyl]decephalosporanic acid after washing with anhydrous diethyl ether. The infrared spectrum of this material shows absorption attributed to: NH and absorbed H₂O, 3400 cm⁻¹ (broad); acid OH, 2800—2700 cm⁻¹; β-lactam C, 1780 cm⁻¹; and acid

$\begin{array}{c} \text{O} \\ \parallel \\ \text{C} \end{array}$

C, 1720 cm⁻¹ (broad).

The compound prepared above exhibited *in vitro* antibacterial activity against *S. aureus* Smith, against the benzyl penicillin-resistant *S. aureus* BX-1633—2, against *S. enteritidis*, against *S. typhosa* and against *Shig. sonnei*.

EXAMPLE 2

2,2,2 - Trichloroethyl chloroformate [Ref.: German Pat. 358,125; C.A. 17, P2172²]

To a stirred and cooled solution of 74.7 g. (0.5 mole) of 2,2,2-trichloroethanol, 64.58 g. (0.5 mole) of quinoline in 1,000 ml. of benzene at 8° C., was added a solution of 49.46 g. (0.5 mole) of phosgene in 500 ml. of benzene over a one-hour period. The resulting thick slurry was stirred at 6° to 10° C. for one hour and then two hours with the ice bath removed. The slurry was then filtered and the cake of quinoline hydrochloride washed with two 250 ml. portions of benzene. The combined filtrates were concentrated *in vacuo* to an oil at a temperature not exceeding 60° C. The oil was then filtered under a nitrogen atmosphere through glass wool and weighed 95 g. (90%). The infrared spectrum was consistent with the desired structure showing the O—C—Cl

$\begin{array}{c} \text{O} \\ \parallel \\ \text{C} - \text{Cl} \end{array}$

carbonyl at 1785 cm⁻¹.

D - (-) - α - (2,2,2 - Trichloroethoxycarbonylamino) - α - (2 - thienyl)acetic acid

—To an ice cold (5° C.) stirred solution of 8 g. (0.2 mole) of NaOH in 200 ml. of water, was added 15.7 g. (0.1 mole) of D(-) - α - amino - α - (2-thienyl)acetic acid and, when solution had been obtained, 21.2 g. (0.1 mole) of 2,2,2-trichloroethyl chloroformate in 50 ml. of CH₂Cl₂ was added all at once with vigorous stirring. After 30 minutes the ice bath was removed and the vigorous stirring continued for one hour. The CH₂Cl₂ layer was separated and discarded and a second 100 ml. CH₂Cl₂ extract taken and discarded. The aqueous phase was then cooled, stirred, layered with 100 ml. of CH₂Cl₂ and acidified to pH 2 with 40% H₃PO₄. The CH₂Cl₂ layer was separated and combined with a second CH₂Cl₂ (100 ml.) extract and then washed once with 100 ml. of water. The combined CH₂Cl₂ layers were filtered through anhydrous Na₂SO₄ and the Na₂SO₄ was washed with three 25 ml. portions of CH₂Cl₂. The combined filtrate and washings were concentrated *in vacuo* to an oil which crystallized slowly. Recrystallization from benzene gave 14.4 g., m.p. 147—149° C. and a second crop 7 g., m.p. 130—133° C. which was less pure. The combined yield was 64% of theory. [α]_D^{25.00} = -80.45° (1st crop) C=1% in 95% ethanol.

Analysis for C₉H₈Cl₃NO₄S:

Calculated:

C, 32.53%; H, 2.43%.

Found:

C, 33.38%; 33.21%; H, 2.63%, 2.52%.

Sodium 3 - Methyl - D - (-) - 7 - [α - (2 - thienyl) - α - (trichloroethoxycarbonylamino)acetamido]decephalosporanate — To a stirred, -10° C. solution of 8.3 g. (0.025 mole) of D - (-) - α - (2,2,2 - trichloroethoxycarbonylamino) - α - (2 - thienyl)acetic

acid, 3.5 ml. (0.025 mole) of triethylamine (TEA) and 50 ml. of tetrahydrofuran (THF), there is added 3.03 g. (0.025 mole) of pivalyl chloride (Aldrich Chemical Co.) all at once.

5 Fifteen minutes later an ice cold solution of 5.35 g. (0.025 mole) of 3-methyl-7-amino-decephalosporanic acid, 7 ml. (0.050 mole) of triethylamine, 25 ml. of tetrahydrofuran and 25 ml. of water is added all at once with

10 vigorous stirring. The temperature is kept at or below 0° C. for 30 minutes and then the ice-salt-acetone bath is removed and stirring continued for one hour. The tetrahydrofuran is then removed *in vacuo* at 20° C. and

15 the remaining solution diluted with 50 ml. of water. Two 75 ml. ether extracts are taken and discarded. The aqueous solution is then layered with 100 ml. of ethyl acetate and cooled and stirred while being acidified to

20 pH 2 with 40% phosphoric acid. The ethyl acetate layer is then combined with a second 100 ml. ethyl acetate extract and washed two times with 100 ml. of water and once with a 100 ml. portion of saturated NaCl

25 solution. The extracts are dried over Na₂SO₄, filtered and the Na₂SO₄ washed with three 25 ml. portions of ethyl acetate. The combined filtrates are then treated with 8.5 ml. of a solution of sodium 2-ethylhexanoate in

30 n-butanol (3.4 ml.=0.01 mole; total=0.025 mole). Approximately half of the ethyl acetate is then removed *in vacuo* at 20° C. and crystallization begins. There is thereby obtained crystalline sodium 3 - methyl - D -

35 (—) - 7 - [α - (2 - thienyl) - α - (trichloroethoxy - carbonylamino)acetamido]decephalosporanate. The infrared and NMR spectra are entirely consistent with the desired structure.

40 Sodium 3 - Methyl - D - (—) - 7 - [α - amino - α - (2 - thienyl)acetamido]decephalosporanate—To a stirred solution of 3.04 g. (0.0055 mole) of sodium 3 - methyl - 7 - [α - (2 - thienyl) - α - (trichloroethoxycarbonyl-

45 amino)acetamido]decephalosporanate in 27 ml. of glacial acetic acid and 3 ml. of water at 0° C., is added all at once 10 g. of Zn dust and stirring is continued for two and a half hours. The slurry is then filtered

50 and the cake washed with 25 ml. of 1:1 (by volume) acetic acid-water and finally with 15 ml. of water. The combined filtrates are concentrated to an oil under reduced pressure at 20° C. Twenty-five ml. of water is then

55 added and the resulting solution acidified to pH 2 with 40% H₃PO₄. At this point some crystalline material (presumably Zn salts) crystallize out and are filtered off, washed with a little water and the combined filtrates

60 treated with solid NaHCO₃ until pH 4.5 is

reached. More crystalline material precipitates. The slurry is filtered again, the cake washed with a little water and the combined filtrate concentrated *in vacuo* at 20° C. to dryness. The residues are triturated with

65 dry ether and the resulting solids filtered off. The air dried material, which weighs about 2.5 g. is then slurried in 150 ml. of CH₂Cl₂ and 1.4 ml. of triethylamine. After three

70 minutes stirring, the inorganic salts are filtered off and the filtrate is treated with 2 ml. of 50% sodium 2-ethylhexanoate in n-butanol. The mixture is then concentrated

75 under reduced pressure to a small volume and the sodium 3 - methyl - D - (—) - 7 - [α - amino - α - (2 - thienyl)acetamido]decephalosporanate crystallizes and is collected by

80 filtration, washed with ethyl acetate and air dried. After drying 18 hours at 0.1 mm. Hg. over P₂O₅ there is obtained about 400 mg. The IR and NMR spectra are entirely consistent with the desired structure.

EXAMPLE 3

3 - Methyl - 7 - [D - (—) - α - amino-thienyl - 2' - acetamido]decephalosporanic

85 Acid — To a stirred, 0° C. solution of 3-methyl - 7 - aminodecephalosporanic acid (2.14 gm.; 0.01 mole) dissolved in a mixture of 25 ml. acetone and 25 ml. water containing 3 ml. of 6N H₂SO₄, there is added

90 in one portion solid (—) - α - amino - 2-thienylacetyl chloride hydrochloride [(—) - α - (2 - thienyl)glycyl chloride hydrochloride] (3.19 gm.; 0.015 mole). The reaction mixture is stirred for an additional thirty minutes

95 at 0–5° C., after which the pH is adjusted to 4.5 by the addition of 10% NaOH. A small amount of precipitate is removed by filtration and the filtrate is evaporated *in vacuo* to small volume, to yield amorphous

100 3 - methyl - 7 - [D - (—) - α - aminothieryl-2' - acetamido]decephalosporanic acid. The product is recovered by filtration and air dried. Its infrared spectrum is entirely consistent with the desired structure. The product

105 exhibits *in vitro* antibacterial activity against *S. aureus* Smith, against the benzylpenicillin resistant *S. aureus* BX-1633—2, against *S. enteritidis*, against *S. typhosa* and against *Shig. sonnei*.

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The invention also includes a pharmaceutical composition comprising a compound of the formula I or formula V defined above, or a nontoxic pharmaceutically acceptable salt thereof, and a pharmaceutical carrier

115 or diluent.

WHAT WE CLAIM IS:—

1. A process for the preparation of the compound of the formula:—

acid, 3.5 ml. (0.025 mole) of triethylamine (TEA) and 50 ml. of tetrahydrofuran (THF), there is added 3.03 g. (0.025 mole) of pivalyl chloride (Aldrich Chemical Co.) all at once.

5 Fifteen minutes later an ice cold solution of 5.35 g. (0.025 mole) of 3-methyl-7-amino-decephalosporanic acid, 7 ml. (0.050 mole) of triethylamine, 25 ml. of tetrahydrofuran and 25 ml. of water is added all at once with vigorous stirring. The temperature is kept at or below 0° C. for 30 minutes and then the ice-salt-acetone bath is removed and stirring continued for one hour. The tetrahydrofuran is then removed *in vacuo* at 20° C. and the remaining solution diluted with 50 ml. of water. Two 75 ml. ether extracts are taken and discarded. The aqueous solution is then layered with 100 ml. of ethyl acetate and cooled and stirred while being acidified to pH 2 with 40% phosphoric acid. The ethyl acetate layer is then combined with a second 100 ml. ethyl acetate extract and washed two times with 100 ml. of water and once with a 100 ml. portion of saturated NaCl solution. The extracts are dried over Na₂SO₄, filtered and the Na₂SO₄ washed with three 25 ml. portions of ethyl acetate. The combined filtrates are then treated with 8.5 ml. of a solution of sodium 2-ethylhexanoate in n-butanol (3.4 ml.=0.01 mole; total=0.025 mole). Approximately half of the ethyl acetate is then removed *in vacuo* at 20° C. and crystallization begins. There is thereby obtained crystalline sodium 3 - methyl - D - (-) - 7 - [α - (2 - thienyl) - α - (trichloroethoxy - carbonylamino)acetamido]decephalosporanate. The infrared and NMR spectra are entirely consistent with the desired structure.

40 Sodium 3 - Methyl - D - (-) - 7 - [α - amino - α - (2 - thienyl)acetamido]decephalosporanate—To a stirred solution of 3.04 g. (0.0055 mole) of sodium 3 - methyl - 7 - [α - (2 - thienyl) - α - (trichloroethoxycarbonylamino)acetamido]decephalosporanate in 27 ml. of glacial acetic acid and 3 ml. of water at 0° C., is added all at once 10 g. of Zn dust and stirring is continued for two and a half hours. The slurry is then filtered and the cake washed with 25 ml. of 1:1 (by volume) acetic acid-water and finally with 15 ml. of water. The combined filtrates are concentrated to an oil under reduced pressure at 20° C. Twenty-five ml. of water is then added and the resulting solution acidified to pH 2 with 40% H₃PO₄. At this point some crystalline material (presumably Zn salts) crystallize out and are filtered off, washed with a little water and the combined filtrates treated with solid NaHCO₃ until pH 4.5 is

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reached. More crystalline material precipitates. The slurry is filtered again, the cake washed with a little water and the combined filtrate concentrated *in vacuo* at 20° C. to dryness. The residues are triturated with dry ether and the resulting solids filtered off. The air dried material, which weighs about 2.5 g. is then slurried in 150 ml. of CH₂Cl₂ and 1.4 ml. of triethylamine. After three minutes stirring, the inorganic salts are filtered off and the filtrate is treated with 2 ml. of 50% sodium 2-ethylhexanoate in n-butanol. The mixture is then concentrated under reduced pressure to a small volume and the sodium 3 - methyl - D - (-) - 7 - [α - amino - α - (2 - thienyl)acetamido]decephalosporanate crystallizes and is collected by filtration, washed with ethyl acetate and air dried. After drying 18 hours at 0.1 mm. Hg. over P₂O₅ there is obtained about 400 mg. The IR and NMR spectra are entirely consistent with the desired structure.

EXAMPLE 3

3 - Methyl - 7 - [D - (-) - α - amino-thienyl - 2' - acetamido]decephalosporanic Acid — To a stirred, 0° C. solution of 3-methyl - 7 - aminodecephalosporanic acid (2.14 gm.; 0.01 mole) dissolved in a mixture of 25 ml. acetone and 25 ml. water containing 3 ml. of 6N H₂SO₄, there is added in one portion solid (-) - α - amino - 2-thienylacetyl chloride hydrochloride [(-) - α - (2 - thienyl)glycyl chloride hydrochloride] (3.19 gm.; 0.015 mole). The reaction mixture is stirred for an additional thirty minutes at 0—5° C., after which the pH is adjusted to 4.5 by the addition of 10% NaOH. A small amount of precipitate is removed by filtration and the filtrate is evaporated *in vacuo* to small volume, to yield amorphous 3 - methyl - 7 - [D - (-) - α - aminothienyl-2' - acetamido]decephalosporanic acid. The product is recovered by filtration and air dried. Its infrared spectrum is entirely consistent with the desired structure. The product exhibits *in vitro* antibacterial activity against *S. aureus* Smith, against the benzylpenicillin resistant *S. aureus* BX-1633—2, against *S. enteritidis*, against *S. typhosa* and against *Shig. sonnei*.

The invention also includes a pharmaceutical composition comprising a compound of the formula I or formula V defined above, or a nontoxic pharmaceutically acceptable salt thereof, and a pharmaceutical carrier or diluent.

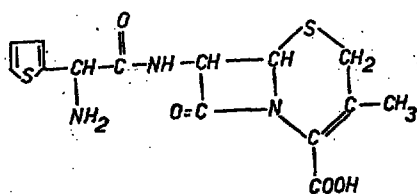
WHAT WE CLAIM IS:—

1. A process for the preparation of the compound of the formula:—



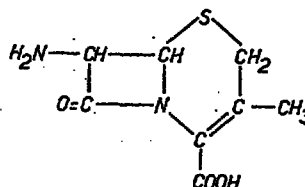


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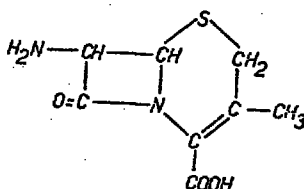
(I)

or a nontoxic, pharmaceutically acceptable salt thereof, which process comprises acylating 3-methyl-7-amino-decephalosporanic acid of the formula:—



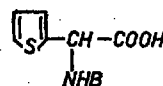
(II)

or a neutral salt thereof, with at least a substantially equimolar amount of an acid of the formula:—



(III)

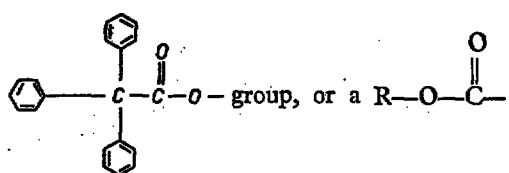
or a neutral salt thereof, with at least a substantially equimolar amount of an acid of the formula:—



(IV)

or an acylating derivative thereof, wherein B is a blocking group, as hereinafter defined, in an inert solvent at a temperature of from about $-50^{\circ}\text{C}.$ to about $50^{\circ}\text{C}.$ to produce the desired compound or salt thereof; and, if desired, converting the thus produced compound to a nontoxic, pharmaceutically acceptable salt thereof.

3. A process as claimed in claim 1 or claim 2, wherein the blocking group is a triphenylmethyl group, a



group, wherein R is allyl, tertiary butyl, substituted alkyl, phenyl, substituted phenyl, benzyl or substituted benzyl.

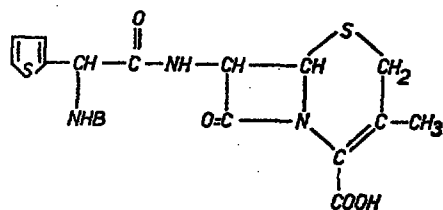
4. A process as claimed in claim 3, wherein R is benzyl, or 2,2,2-trichloroethyl.

5. A process as claimed in any one of the preceding claims, wherein the acylating derivative of the acid of formula (III) is an acid halide.

6. A process as claimed in claim 5, wherein the acid halide is an acid chloride or an acid chloride hydrochloride.

7. A process as claimed in any one of the preceding claims, wherein the acylating reaction is carried out at a temperature of from about $-10^{\circ}\text{C}.$ to $15^{\circ}\text{C}.$

8. A process for the preparation of a compound of the formula (I) or formula (IV) or a nontoxic, pharmaceutically acceptable

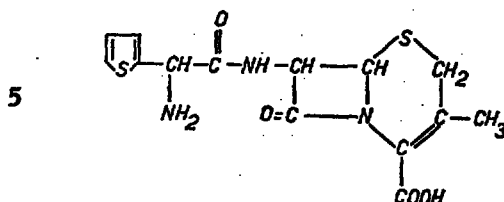


(VI)

wherein B is a blocking group, as hereinbefore defined, or a nontoxic, pharmaceutically acceptable salt thereof, which process comprises acylating 3-methyl-7-amino-decephalosporanic acid of the formula:—

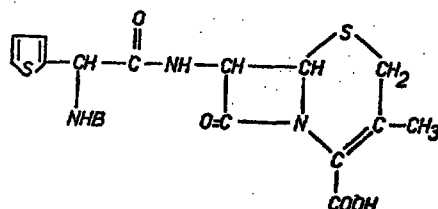
salt thereof, substantially as hereinbefore described with reference to Examples 1 to 3.

9. A compound of the formula:—



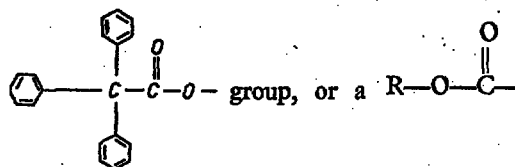
or a nontoxic pharmaceutically acceptable salt thereof.

10. A compound of the formula:—



10 wherein B is a blocking group as hereinbefore defined; or a nontoxic, pharmaceutically acceptable salt thereof.

11. A compound as claimed in claim 10, wherein the blocking group is a triphenylmethyl group, a



group, wherein R is allyl, tertiary butyl, substituted alkyl, phenyl, substituted phenyl, benzyl or substituted benzyl.

12. A compound as claimed in claim 11 wherein R is benzyl or 2,2,2-trichloroethyl.

13. A compound or nontoxic pharmaceutically acceptable salt thereof as claimed in claim 9 when prepared by a process as claimed in any one of claims 1 or 5 to 8.

14. A compound or nontoxic pharmaceutically acceptable salt thereof as claimed in any one of claims 10 to 12 when prepared by a process as claimed in any one of claims 2 to 8.

15. 3 - Methyl - 7 - [2',2' - dimethyl - 5'-oxo - 4' - (2'' - thienyl) - 1' - imidazolidinyl]decephalosporanic acid or a nontoxic pharmaceutically acceptable salt thereof.

16. A process for the preparation of the compound or salt as claimed in claim 15, which process comprises reacting a compound or salt as claimed in claim 9 with an excess of acetone.

17. A process for the preparation of 3-methyl - 7 - [2',2' - dimethyl - 5' - oxo - 4' - (2'' - thienyl) - 1' - imidazolidinyl]decephalosporanic acid substantially as hereinbefore described in Example 1.

18. A pharmaceutical composition comprising a compound or a nontoxic pharmaceutically acceptable salt thereof, as claimed in any one of claims 9, 13 or 15 and a pharmaceutical carrier or diluent.

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