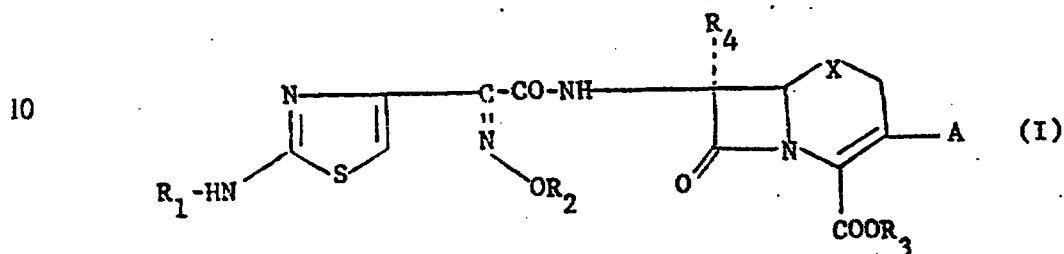


Description.

The present invention relates to new derivatives of cephalosporins, to a process for preparing them and to pharmaceutical compositions containing said derivatives of cephalosporin as active ingredients.

More particularly, the present invention relates to new cephalosporins substituted in 3 position by a pyridinium thiomethyl group.

Belgian Patent 866 038 describes a series of sulfoxides and sulfones of cephalosporins of general formula:

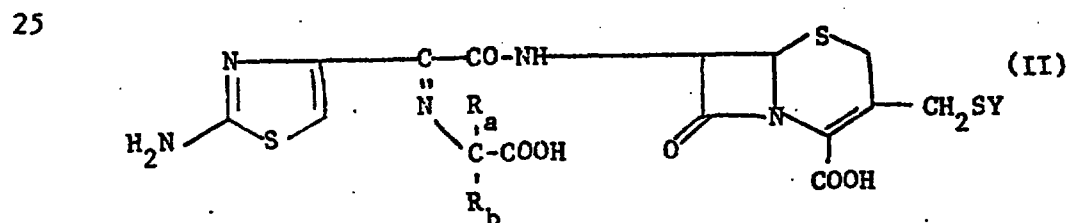


15 X = SO, SO₂.

Among the radicals indicated for A, this Patent mentions in particular CH₂SR₅ groups where R₅ may be a possibly substituted pyridyl.

The cephalosporins of formula (I) are generally supposed to possess a very strong bacterial activity against gram positive and gram negative bacteria and to be highly effective against the penicillinase-producing staphylococci.

Furthermore, West German Patent Application 2 921 332 describes a family of cephalosporins of general formula:



30

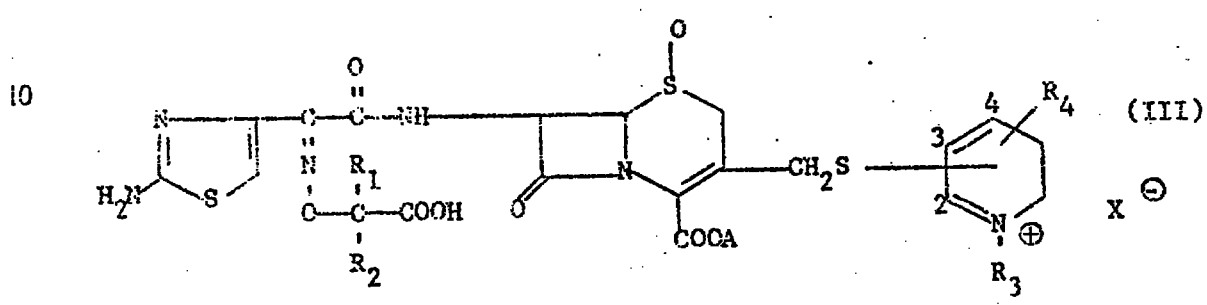
in which Y may in particular be



These cephalosporins are presented as broad-spectrum antibiotics.

It is an object of the present invention to produce new cephalosporins which have a bacterial profile very different from that of the compounds of the Patents mentioned hereinabove. In fact, the compounds of the invention have remarkable activity on enterobacteria, including those producing β -lactamases, good activity on Pseudomonas and weak activity on staphylococci.

These new cephalosporins correspond to general formula:



in which:

- R_1 represents hydrogen or a methyl group, R_2 represents a methyl group, or R_1 and R_2 taken together represent a 1,3-propylene group;
- R_3 represents an alkyl group, an alkenyl group or an alkynyl group containing up to 4 carbon atoms, or a CH_2COO Alk group (Alk representing an alkyl group containing up to 4 carbon atoms).
- R_4 denotes H or OH occupying a free position of the cycle of the pyridine, the atom of sulfur of the thiomethyl group being bonded in ortho or para position of the nitrogen of the cycle of pyridine,
- 25 - A represents hydrogen, a cation or an ester which is easily hydrolysable or metabolically labile and pharmaceutically acceptable,
- $X^\ominus$ represents an anion derived from a pharmaceutically acceptable mineral or organic acid such as chloride, bromide, acetate, trifluoro-
- 30 acetate, formate...,

Under certain conditions, it is also possible to salify the quaternary ammonium by the carboxylic function carried by the cepHEME ring. In this case, $X^{\ominus}$ does not exist. These inner salts form an integral part of the invention.

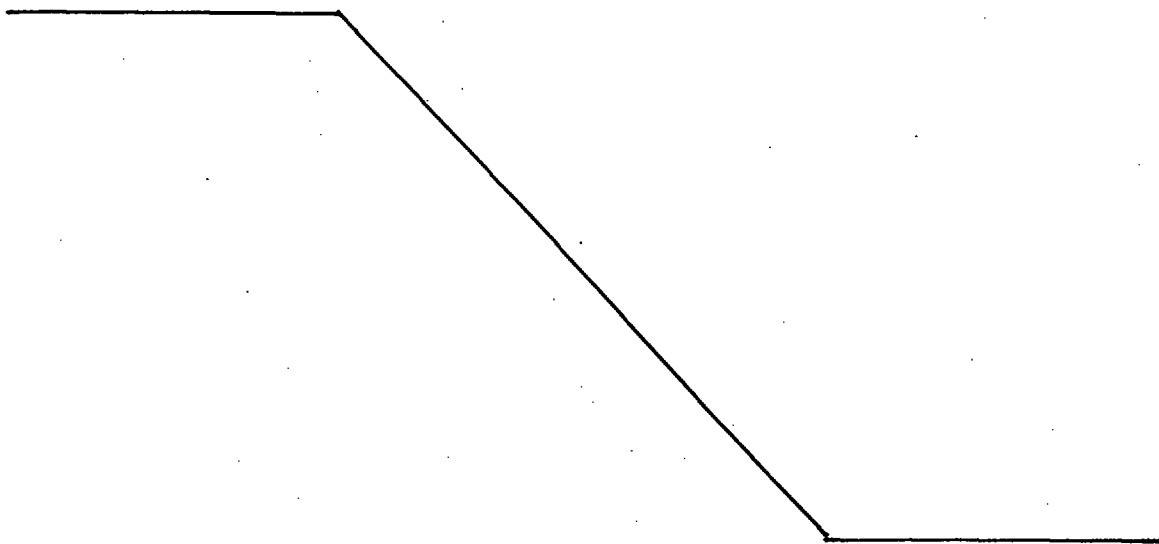
In the present Application :

- The term "cation" denotes an alkaline or alkaline-earth ion, preferably the sodium, potassium or calcium ions or the "ammonium" derivative resulting by protonation of a pharmaceutically acceptable organic amine such as ethylene-diamine, ethanolamine, tromethamide and the like to form addition salts.

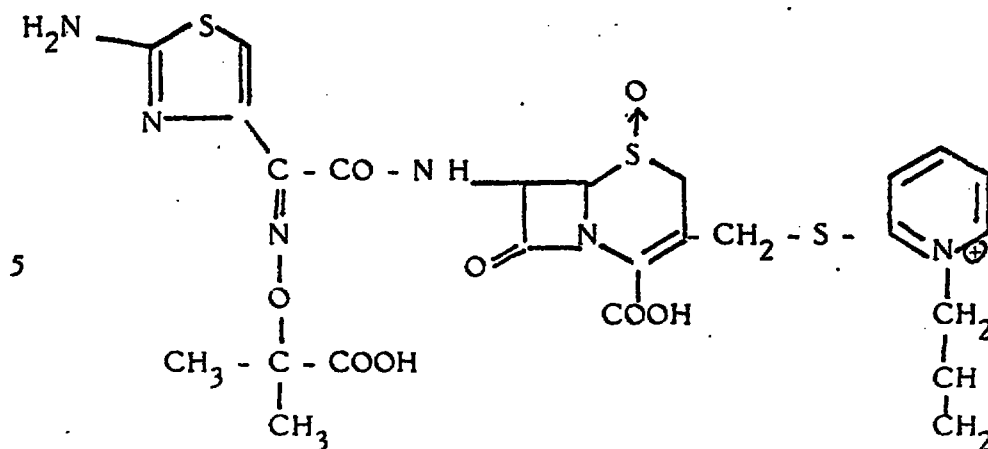
- The term ester of hemiacetal which is easily hydrolyzable or metabolically labile and pharmaceutically acceptable denotes radicals such as phthalidyl, pivoloyloxymethyl, acetoxymethyl, ethoxycarbonyloxymethyl 1-(ethoxycarbonyloxy)ethyl, acetonyl, α -methoxy α -carbomethoxymethyl, carbomethoxymethyl, carbethoxymethyl and the like.

The invention relates in particular to the compounds of formula III which are selected from :

- a pharmaceutically acceptable mineral or organic quaternary salt of the syn isomer of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyminino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepHEME 4-carboxylic S-oxide-1 acid of formula :

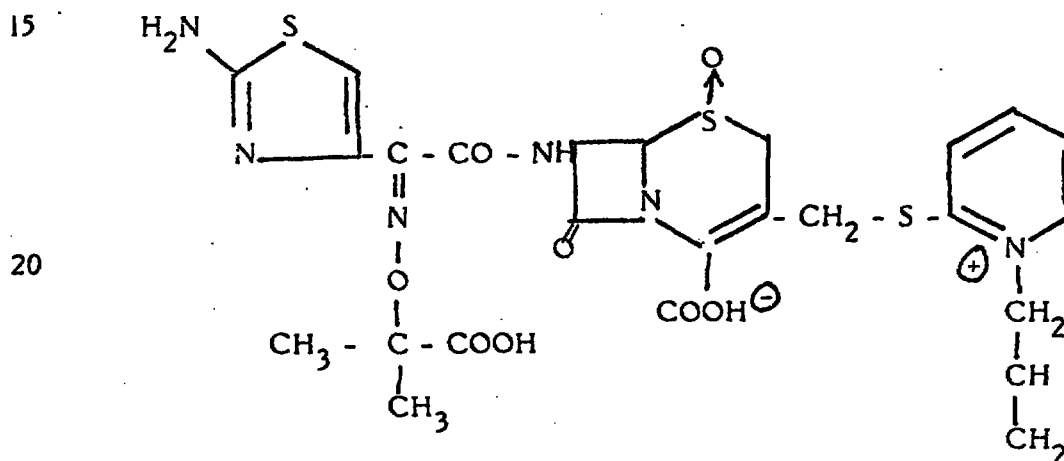


-4-



10 - and the products obtained by salification, or esterification
of at least one carboxylic function of said salt and possible
salification of the amine function of said salt.

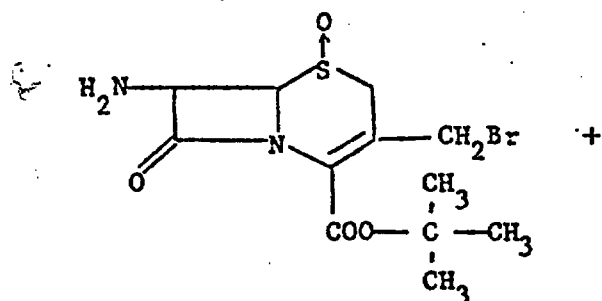
- and the inner quaternary salt of the syn isomer of the above-
mentioned acid, said product having as formula:



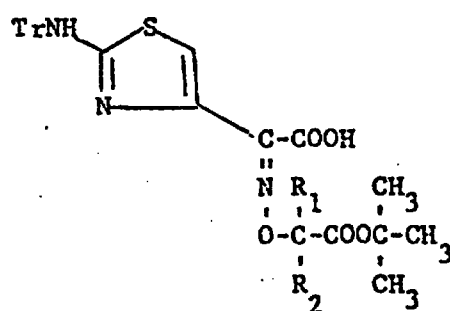
25 - and the products obtained by salification or esterification or hemiaceta-
lization of the acid function of said inner salt and possible salification
of the amine function of said salt.

The invention relates more particularly to the trifluoroacetate
of the syn isomer of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl
30 oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme
4-carboxylic S-oxide-I acid, or the bromide of the hydrochloride of
said acid or the chloride of the hydrochloride of said acid.

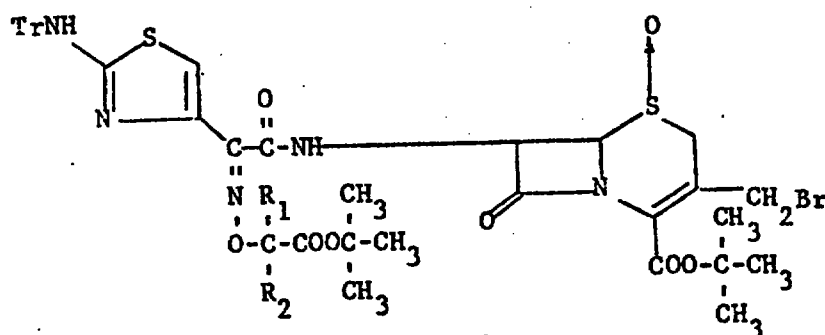
The invention also relates to a process for preparing the
compounds of formula (III)



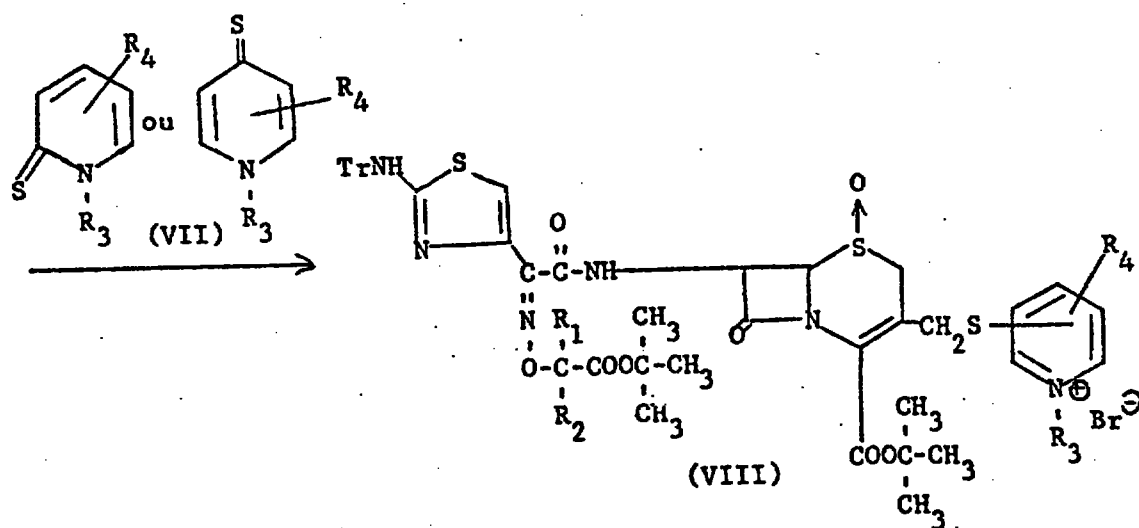
(IV)



(V) Tr = protector group
(for example trityl)



(VI)



(VIII)



(III)

The first step consists in acylating the 7-amino 3-bromomethyl 3-cepheme carboxylate of tertibutyl S-oxide-I (IV) by the acid (V). Before effecting the reaction of acylation, it is desirable to substitute the amino group of the acid (V) by a protector group easy to eliminate subsequently. The groups usually used in organic synthesis may be used for the protection of the amines and, in particular, the trityl group. To effect the reaction of acylation, it is necessary to proceed with activation of the carboxyl group of compound (V), preferably by transformation into anhydride with the aid of a carbodiimide, generally dicyclohexylcarbodiimide.

The reaction of activation is effected within a suitable organic solvent such as tetrahydrofuran at a temperature of between 0 and 50°C and preferably at ambient temperature. The reaction of activation is possibly facilitated by addition of a hydroxylated derivative such as 1-hydroxy benzotriazole.

The solution of the reagent of acylation thus obtained, from which the dicyclohexylurea formed is removed by filtration, is added to a solution of compound (IV) in a solvent such as dimethylformamide. The addition of the two reagents may also be effected in the reverse order.

By action on the compound (VI) thus obtained of a pyridine 2-thione or of a pyridine 4-thione (VII) carrying on the nitrogen the substituent R_3 and possibly substituted on the cycle by R_4 , compound (VIII) is obtained in the form of bromide of quaternary ammonium.

The reaction is carried out within a suitable aprotic-polar solvent such as dimethylformamide or N,N-dimethylacetamide at a temperature of between 0 and 50°C and preferably at ambient temperature.

The product (VIII) is isolated by precipitation by addition of a solvent where it is slightly soluble, such as isopropyl ether, then purified by the conventional methods and, in particular, by chromatography over silica gel.

The series of operations which allow passage from the brominated derivative (IV) to compound (VIII) may also be carried out in the reverse order: substitution of the brominated derivative (IV) by

١٠

5

10

10

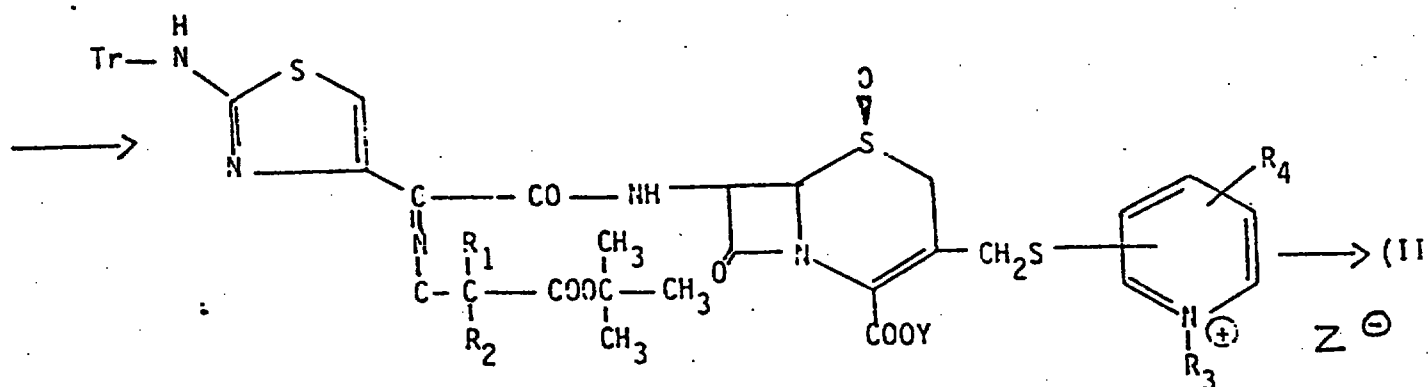
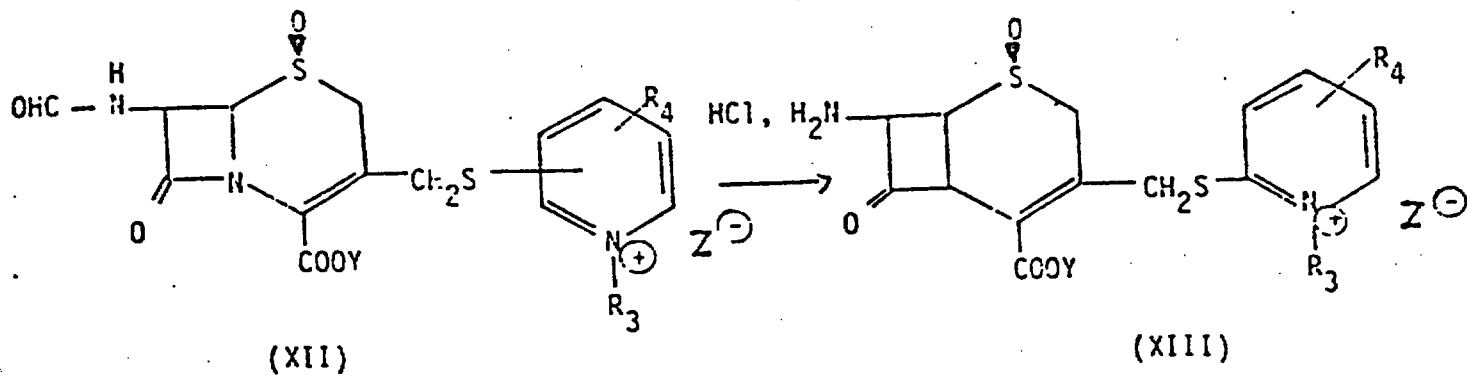
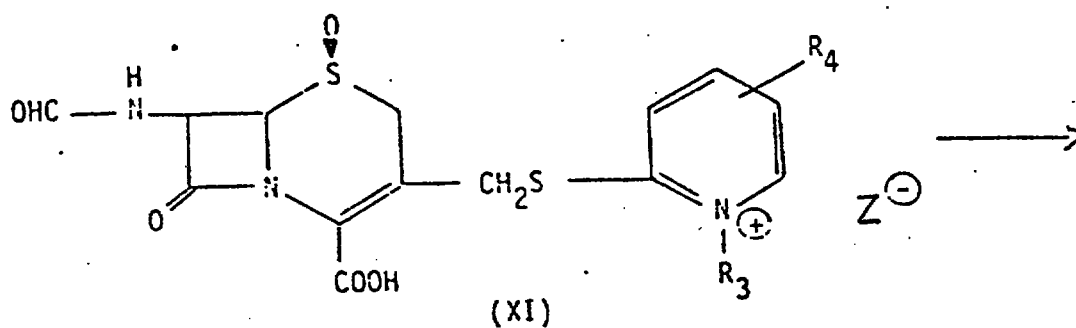
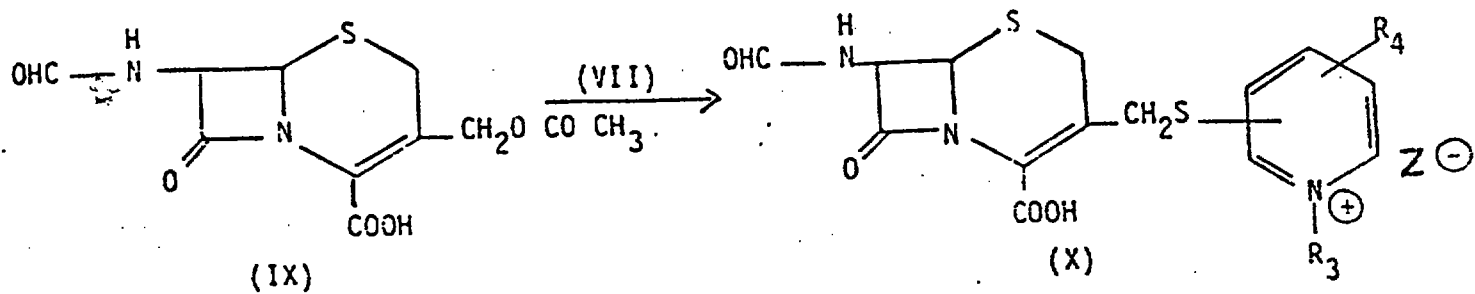


20

25

Compounds (III) according to the invention may also be prepared from the 7-formylamino cephalosporanic acid according to the following reaction scheme:

-8-



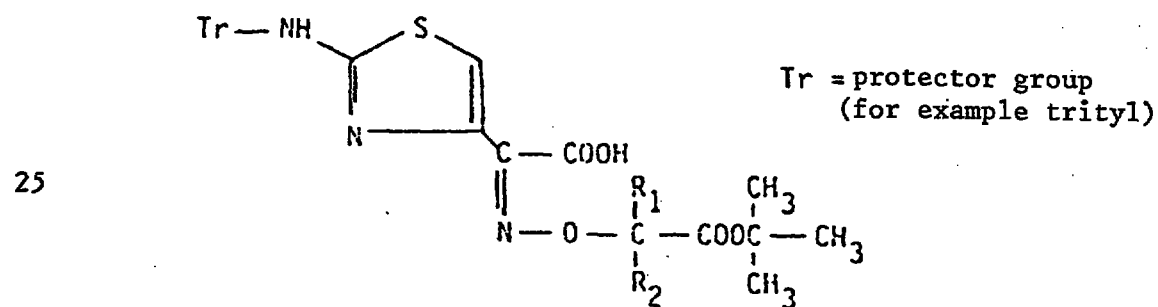
Tr = protecteur group (for example trityl)
 Z = mineral anion

The first step consists in causing a pyridine thione (VII) to act on the 7-formylamino cephalosporanic acid (IX) or preferably an alkaline salt thereof. Operation is then carried out in aqueous solution in the presence of sodium iodide at a temperature of between 40 and 80°C. The product (X) is then isolated in the form of pyridinium iodide and possibly of alkaline salt.

For the following step, the carboxylic acid function is released by action of an acid such as hydrochloric acid then, preferably, for reasons of stability, the pyridinium iodide is converted into chloride by passage over an ion exchanger column in the form of hydrochloride.

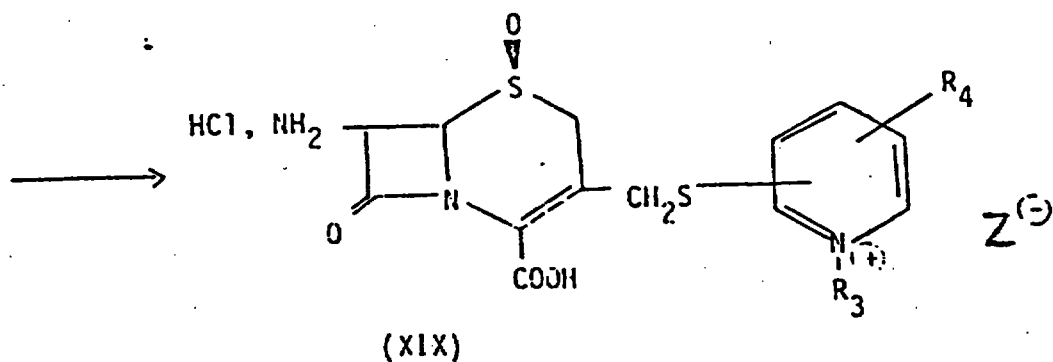
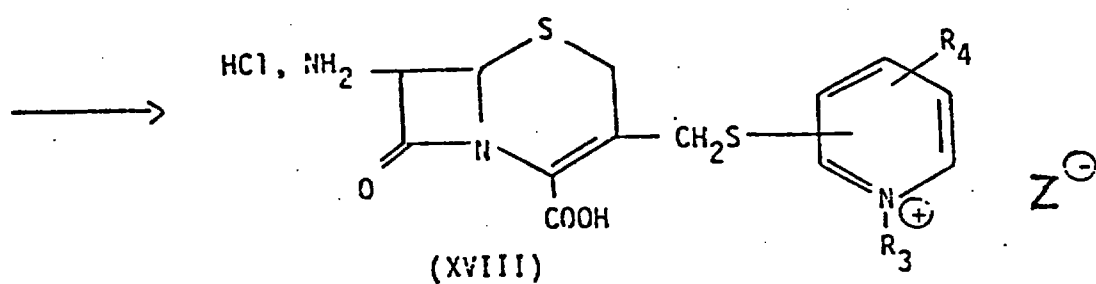
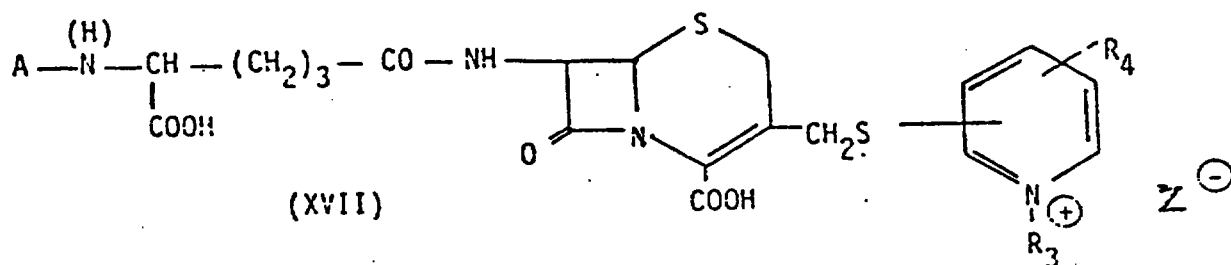
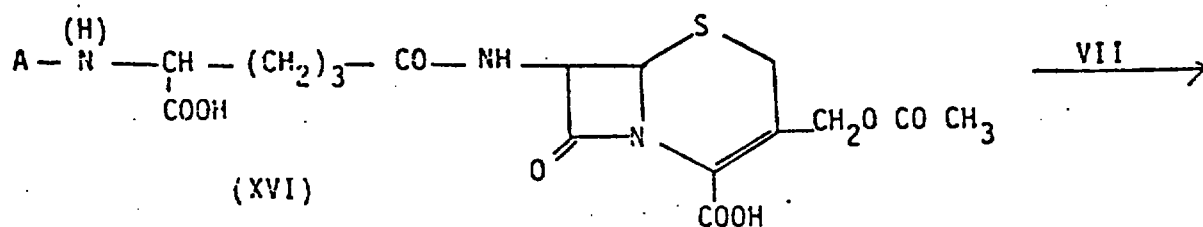
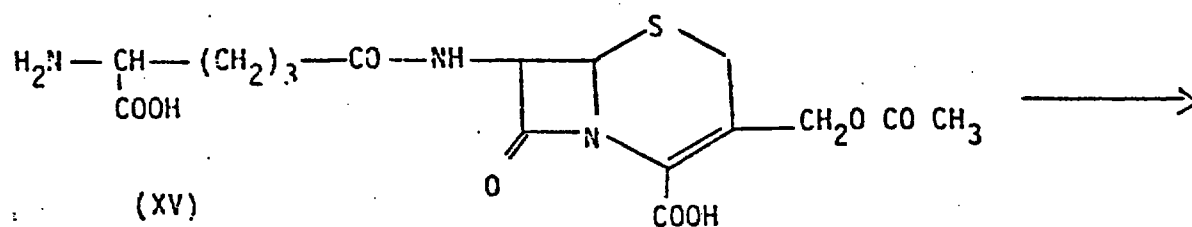
The product (X) is then converted into corresponding sulfoxide (XI) by action of hydrogen peroxide or of a peracid such as metachloroperoxybenzoic acid. The sulfoxide (XI) is then converted into ester (XII) in which the group Y represents a group which will subsequently be easy to eliminate such as the diphenylmethyl, tertibutyl or trimethylsilyl group.

The compound (XII) is deformylated on amino nitrogen, for example by action of the thionyl chloride within the methanol. The 7-amino compound is isolated in the form of hydrochloride (XIII). The latter is acylated on nitrogen, using the chloride of the acid:

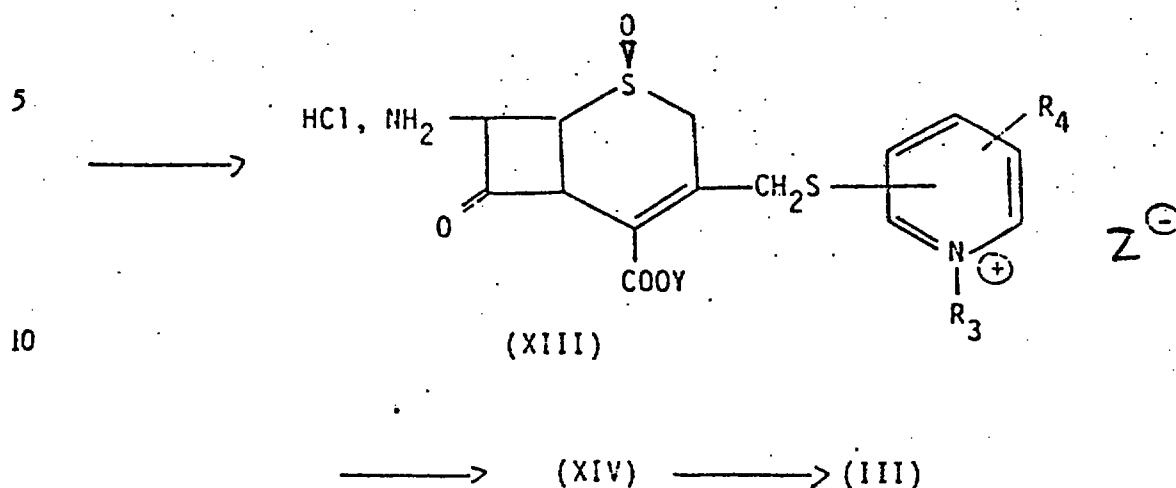


The reaction of acylation is effected in a solvent such as methylene chloride in the presence of dimethylaniline. In this way is obtained the protected cephalosporin (XIV) which, by treatment in strong acid medium, leads to the compounds (III) according to the invention. In particular, the hydrochloric acid-formic acid mixture may be used for effecting deprotection, or trifluoroacetic acid.

Compounds (III) may also be prepared from the Cephalosporin C according to the following reaction diagram:



-II-



The first step consists in blocking the primary amine function of the cephalosporin C by a protector group A, according to a known process.

Among the protector groups which may be used, mention may be made of the phthalidyl group or ethoxycarbonyl group. From compound (XVI), the quaternary ammonium compound (XVII) is obtained by action of a pyridine thione (VII). Operation is carried out in aqueous solution in the presence of sodium iodide and sodium bicarbonate intended to salify the carboxyl functions of the starting product.

The acyl chain is then cut from compound (XVII). After having blocked the carboxylic functions of compound (XVII), for example by formation of trimethylsilyl ester), the cut of the acyl chain is effected by action of phosphorus pentachloride of an alcohol such as methanol or a diol such as 2,3-butanediol. In this way the compound (XVIII) is isolated in the form of hydrochloride of the primary amine and of pyridinium chloride ($X^- = Cl^-$).

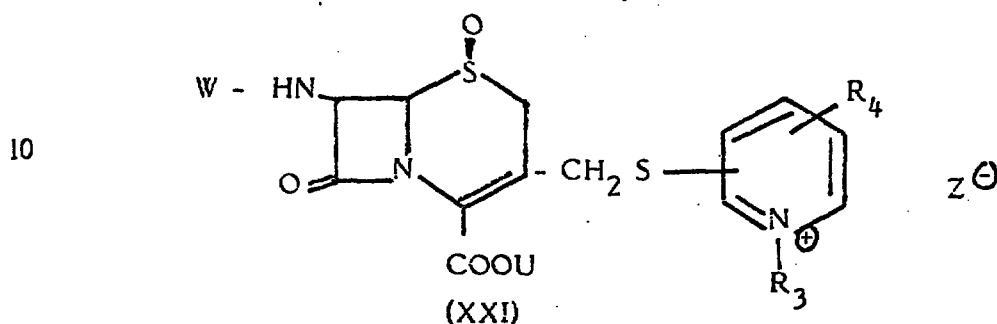
Compound (XVIII) is converted into corresponding sulfoxide derivative (XIX) by action of an organic peracid such as metachloropero- benzoic acid in acid medium.

The acid (XIX) is esterified by a labile group such as the diphenylmethyl or tertibutyl group to lead to the ester (XIII) or protec-

ted by a trimethylsilyl group.

From compound (XIII), compounds (III) are arrived at in 2 steps as indicated hereinabove.

Compounds XII, XIII, and XIX are new and constitute the key intermediaries of the process of the present invention. They may be grouped in the following general formula



15 where R_3 , R_4 and Z are such as defined hereinabove, U represents hydrogen or a group easy to eliminate by hydrolysis or hydrogenolysis and W represents hydrogen or a formyl group. Compounds XXI hereinabove as well as their acid addition or possible mineral salts represent a subsequent aspect of the present invention.

The compounds of formula XXI where U , R_4 and W are hydrogen, R_3 is allyl and Z is chlorine, and its hydrochloride as well as compound XXI where U and R_4 are hydrogen, W is formyl, R_3 is allyl and Z is chlorine, are the preferred intermediate compounds.

25 The compounds (III) of the invention, in which A is other than H , are obtained from the compounds (III) in which A is H by reactions known per se.

In this way, the mineral salts are obtained by action on compounds (III) in which $A = H$ of a mineral base such as sodium or potassium hydroxide or sodium bicarbonate, in equimolecular quantity. Reaction is effected in a solvent such as water or ethanol and the salt obtained is isolated by evaporation of the solution. The salts of organic bases are obtained by action, on a solution of the acid (III $A = H$) in a solvent or a mixture of suitable solvents, of an equimolecular quantity of the organic base. The salt is isolated by precipitation

35

with ether.

The esters are obtained by the known processes of esterification; for example, the action of a halogen derivative on a salt such as the sodium salt of the acid will advantageously be used. Reaction will preferably be carried out in a solvent capable of dissolving the starting acid derivative, for example in dimethylformamide.

The isomers of syn and anti form are obtained by suitably selecting the reagents.

The following examples will enable the scope of the invention to be more readily understood.

As is usual in this family of compounds, the products according to the invention do not present a clear melting point, but only points of decomposition which do not enable them to be characterized.

The products will therefore be characterized by their nuclear magnetic resonance spectrum recorded at 60 MHz, the inner standard being hexamethyldisiloxan.

The following abbreviations will be used:

- S : singlet
- D : doublet
- 20 - T : triplet
- Q : quadruplet
- D of D: doublet of doublet
- S.e. : enlarged singlet
- M : multiplet
- 25 - AB : system AB
- J : represents the coupling constant.

Moreover, elementary microanalyses have been made in each case and are in agreement with the formulae indicated.

EXAMPLE 1

30 Trifluoroacetate of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic S-oxide-1 acid, syn isomer (CM 40 874)

(III) $R_1 = R_2 = CH_3$; $R_3 = -CH_2CH = CH_2$; $R_4 = H$; $A = H$; $X = CF_3COO^-$

a) N-allylpyridine 2-thione

35 The mixture of 5 g of 2-bromo pyridine and 4.2 g of allyl

bromide is heated to 70°C for 2 hrs. 30 mins. 10 ml of acetone are added and the crystals of N-allyl 2-bromo pyridinium bromide are drained and washed with acetone then with ether and dried.

A solution of potassium hydrosulfide is prepared by bubbling
 5 a stream of sulfuretted hydrogen in a solution of 2.6 g of potassium hydroxide in 40 ml of water up to decoloration of the phenolphthaleine. 2 g of the product obtained hereinabove are then added and the mixture is stirred at 20°C for 15 mins., and extracted three times with 50 ml of methylene chloride. The solution is dried over magnesium sulfate
 10 and the solvent is evaporated to dryness.

1 g of N-allylpyridine 2-thione is thus obtained in the form of a yellow oil.

b) 7-[2-(2-tritylamino 4-thiazoly!) 2-(2-t-butoxycarbonyl 2-propyl oxyimino) acetamido] 3-bromomethyl 3-cepheme carboxylate of 4-tertio-
 15 butyl S-oxide I, syn isomer

(VI) $R_1 = R_2 = CH_3$

To a solution of 5 g of hydrochloride of 7-amino 3-bromomethyl 3-cepheme carboxylate of 4-tertiobutyl S-oxide-I in 90 ml of methylene chloride are added 1.72 ml of triethylamine, 7.57 g of 2-(2-tritylamino
 20 4-thiazoly!) 2-(2-t-butoxycarbonyl 2-propyl oxyimino) acetic acid, 2.84 g of dicyclohexylcarbodiimide and 0.1 g of hydroxybenzotriazole. The mixture is stirred for 15 hours at ambient temperature, then the dicyclohexylurea formed is filtered.

After evaporation of the solvent, the residue is chromatographed over a silica gel column (250 g). By eluting with a hexane-ethyl acetate 50-50 (vol/vol) mixture, 4.3 g of the expected product are obtained.

NMR spectrum (in solution in deuterium dimethylsulfoxide)

1H at 8.70 ppm (NH-Trit, S) - 1H at 8.07 ppm (NH-CO, D, J=9 Hz)
 30 - 15 H at 7.25 ppm (H Trit, S) - 1 H at 6.72 ppm (H thiazole, S) -
 1 H at 5.88 ppm (H₇, D of D, J₁=9 Hz, J₂= 4 Hz) - 1 H at 4.96 ppm (H₆, D, J=4 Hz) - 2 H at 4.50 ppm CH₂Br, AB, J_{AB}=12 Hz) - 2 H at

3.77 ppm (CH₂ in 2, S.e. 9 H at 1.45 ppm ($-C \begin{array}{c} \text{CH}_3 \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_3 \end{array} - CH_3$, S) - 6 H at

1.37 ppm ($\text{-}\overset{\text{CH}_3}{\underset{|}{\text{C}}}\text{-}, \text{S}$) - 9 H at 1.27 ppm ($\text{-}\overset{\text{CH}_3}{\underset{|}{\text{C}}}\text{-CH}_3, \text{S}$).

c) Bromide of 7-[2-(2-tritylamino 4-thiazolyl) 2-(2-t-but oxycarbonyl 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme carboxylate of 4-t-butyl S-oxide-1, syn isomer

5 (VIII) $\text{R}_1 = \text{R}_2 = \text{CH}_3$; $\text{R}_3 = \text{CH}_2 - \text{CH} = \text{CH}_2$ $\text{R}_4 = \text{H}$

The mixture of 0.7 g of the brominated derivative obtained in the preceding paragraph and 0.14 g of N-allyl-pyridine 2-thione in 4 ml of N,N-dimethylacetamide is left for 3 hours at 20°C. The mixture is precipitated by addition of isopropyl ether and the solid
10 is drained and washed with isopropyl ether. The solid is dissolved in the minimum of methylene chloride and chromatographed over a column of 20 g of silica gel.

By eluting with the methylene chloride-methanol 90-10 (vol/vol) mixture, 0.65 g of the expected product is obtained.

15 d) CM 40 874

The solution of 0.57 g of the protected product obtained herein-above in 6 ml of trifluoroacetic acid is left for 45 mins. at 20°C. It is concentrated in vacuo to about 3 ml then precipitated by addition of ether. The solid is drained and dried.

20 0.39g of the expected product is thus obtained.

NMR spectrum

1H at 9.05 ppl (H_6 , pyridine, D, J = 5 Hz) - 1 H at 8.50 ppm (NHCO , D, J=9 Hz) - 1 H at 8.35 ppm (H_4 , pyridine, M) - 1 H at 8.20 ppm (H_3 , pyridine, D, J=7 Hz) - 1 H at 7.95 ppl (H_5 , pyridine, M) - 4 H between
25 7 and 10 ppm (2 COOH , NH_2) - 1 H at 6.82 ppm (H thiazole, S) - 2 H at 6.0 ppm (H_7 and CH= , M) - 5 H between 5.0 and 5.6 ppm (CH_2N^+ , $\text{CH}_2=$ and H_6 , M) - 1 H at 4.5 ppm (CH_2S , A of AB, $\text{J}_{\text{AB}}=13$ Hz) - 1 H at 4.32 ppm (CH_2S , B of AB, $\text{J}_{\text{AB}}=13$ Hz) - 1 H at 4.0 ppm ($\text{CH}_2\text{S} \rightarrow \text{O}$, A of AB, $\text{J}_{\text{AB}}=17$ Hz) - 1 H at 3.8 ppm ($\text{CH}_2\text{S} \rightarrow \text{O}$, B of AB, $\text{J}_{\text{AB}}=17$ Hz) - 6 H at 1.45 ppm ($\text{-}\overset{\text{CH}_3}{\underset{|}{\text{O}}}\text{-CH}_3, \text{S}$).

EXAMPLE 2

Hydrochloride, bromide of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl)

3-cepheme 4-carboxylic S-oxide-l acid, syn isomer
 (CM 40 874 b)

9.3 g of the protected compound obtained in Example 1 c) are dissolved in 55 ml of 99% formic acid then 3.3 ml of concentrated hydrochloric acid are added drop by drop and the mixture is left for 1 hour with stirring at 25°C. The solid formed is filtered and washed with 25 ml of 50% formic acid. The filtrate is evaporated to dryness in vacuo at ambient temperature. The residue is redissolved in 100 ml of absolute ethanol and again evaporated to dryness in vacuo at ambient temperature. The residue is again dissolved in 50 ml of methanol and the solution is poured, with stirring, in 300 ml of ether. The precipitate is drained and washed with ether.

6.5 g of the expected product are thus obtained, which is purified by dissolution in 50 ml of methanol, the solution being poured slowly with stirring in 300 ml of ether.

After draining and drying, 6.2 g of CM 40 874 b are finally obtained.

NMR spectrum

1H at 9.10 ppm (H_6 , pyridine, D, J=6 Hz) - 1 H at 8.80 ppm ($NHCO$, D, J=9 Hz) - 1 H at 8.35 ppm (H_4 , pyridine, T, J=8 Hz) - 1 H at 8.22 ppm (H_3 , pyridine, D, J=8 Hz) - 1 H at 7.94 ppm (H_5 , pyridine, T, J=6 Hz) - 1 H at 7.00 ppm (H thiazole, S) - 2 H at 6.00 ppm (H_7 and $-CH=$, M) - 5 H between 5 and 5.5 ppm (H_6 , CH_2N^+ , $CH_2=$, M) - 2 H at 4.45 ppm (CH_2S , M) - 2 H at 4.0 ppm ($CH_2 \rightarrow O$, M)

25 EXAMPLE 3

Inner salt of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme carboxylate S-oxide-l. acids syn isomer (CM 40 874 a)

To a solution of 0.3 g of CM 40 874 b obtained in Example 2 is added 0.7 g of ion exchanger resin Amberlite IRA 400 in the form of acetate and the mixture is stirred for 35 mins. at ambient temperature. The resin is filtered and washed with water. The solution is evaporated to dryness in vacuo at ambient temperature. The residue is taken up in 10 ml of ethanol and evaporated to dryness in vacuo at ordinary temperature. The residue is taken up in ether and the

solid is drained. After drying, 0.210 g of the expected inner salt is obtained.

NMR spectrum

1 H at 8.5 ppm (NHCO , D, $J=9$ Hz) - 1 H at 8.0 ppm (H_6 pyridine, D, $J=6$ Hz) - 1 H at 7.45 ppm (H_3 pyridine, D, $J=8$ Hz) - 1 H at 7.30 ppm (H_4 pyridine, M) - 2 H at 7.25 ppm (NH_2 ; S.e.) - 2 H at 6.80 ppm (H thiazole, + H_5 pyridine, M) - 2 H at 6.00 ppm (H_7 and $-\text{CH}_2=$, M) - 7 H at 5.10 ppm (H_6 , CH_2N^+ , $\text{CH}_2\text{S}-$, $\text{CH}_2=$, M) - 1 H at 4.25 ppm ($\text{CH}_2\text{S}-\text{O}$, A of AB, $J_{\text{AB}}=17$ Hz) - 1 H at 3.80 ppm ($\text{CH}_2\text{S}-\text{O}$, B of AB, $J_{\text{AB}}=17$ Hz) - 6 H at 1.45 ppm ($-\text{C}-\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$, S).

EXAMPLES 4 to 9

Operation is as in Example 1 c) - from the brominated derivative of Example 1 b) - but varying the nature of the pyridine thione used.

By effecting thereafter the deprotection of the products thus obtained as indicated in Example 1 d), the different compounds III shown in Table I are obtained.

In this Table, in addition to the structure and the characteristics of the products III obtained, the experimental conditions (temperature and duration) of the reaction of substitution of the brominated derivative by the thione which vary according to the reagents used, have also been indicated.

EXAMPLE 10

Trifluoroacetate of 7-[2-(2-amino 4-thiazolyl) 2-(1-carboxy 1-cyclobutyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic S-oxide-1 acid, syn isomer (CM 40914)

(III) $\text{R}_1 + \text{R}_2 = (\text{CH}_2)_3$; $\text{R}_3 = \text{CH}_2 = \text{CH} - \text{CH}_2$; $\text{R}_4 = \text{H}$; $\text{A} = \text{H}$; $\text{X}^- = \text{CF}_3\text{COO}^-$

a) 7-[2-(2-tritylamino 4-thiazolyl) 2-(1-t-butoxycarbonyl 1-cyclobutyl oxyimino) acetamido] 3-bromomethyl 3-cepheme carboxylate of 4-t-butyl S-oxide-1 syn isomer

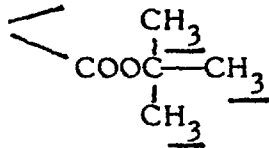
To a solution of 4.4 g of hydrochloride of 7-amino 3-bromomethyl 3-cepheme carboxylate of 4-t-butyl S-oxide-1 in 70 ml of anhydrous methylene chloride are added, in a nitrogen atmosphere,

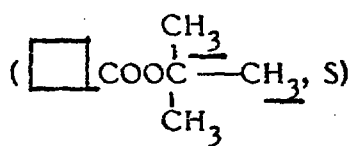
1.5 ml of triethylamine, 5.1 g of 2-(2-tritylamino 4-thiazolyl) 2-(1-t-butoxycarbonyl 1-cyclobutyl oxyimino) acetic acid, syn isomer, 2.4 g of dicyclohexylcarbodiimide and 0.1 g of 1-hydroxy benzotriazole. The solution is stirred for 1 hour at ambient temperature then the
 5 dicyclohexylurea formed is filtered and the solution is concentrated to 20 ml in vacuo. It is chromatographed over a column of silica gel (150 g).

By elution with the hexane-ethyl acetate 40-60 (vol/vol) mixture, 4.8 g of the expected product are obtained after evaporation
 10 of the solvent.

NMR spectrum

1 H at 7.90 ppm (NHCO , D, $J=9$ Hz) - 15 H at 7.26 ppm (aromatic H, S) - 1 H at 6.97 ppm (NH -trityl, S.e.) - 1 H at 6.65 ppm (H thiazole, S) - 1 H at 6.18 ppm (H_7 , D of D, $J_1=9$ Hz, $J_2=4.5$ Hz) - 2 H at 3.4
 15 ppm (CH_2S , S.e.) - 6 H between 1.5 and 2.6 ppm (cyclobutyl,

M) - 9 H at 1.46 ppm (= , S) - 9 H at 1.36 ppm



b) Bromide of 7-[2-(2-tritylamino 4-thiazolyl) 2-(1-t-butoxycarbonyl 1-cyclobutyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl)
 20 3-cepheme carboxylate of t.butyl, syn isomer

By using the brominated derivative of Example 9 a) and by operating as in Example 1 c), with N-allyl pyridine 2-thione, the expected compound is obtained in the form of a colourless solid.

c) CM 40 914

25 From the above compound, deprotection is effected as indicated in Example 1 d).

NMR spectrum

1 H at 9.05 ppm (H_6 pyridine, D, $J=6$ Hz) - 1 H at 8.20 ppm (NH - CO, D, $J=9$ Hz) - 3 H between 7.6 and 8.5 ppm (H_3 , H_4 , H_5 pyridine, 30 M) - 1 H at 6.85 ppm (H thiazole, S) - 2 H at 6.0 ppm (H_7 and = CH, M) - 5 H between 4.8 and 5.5 ppm (H_6 , CH_2 =, CH_2N^+ , M) - 2 H at

EXAMPLE 15

Trifluoroacetate of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxvimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic S-oxide-1 acid, syn isomer (CM 40 874)

- 5 a) Iodide of 3-(N-allyl 2-pyridinio thiomethyl) 7-formylamino 3-cepheme carboxylate of 4-potassium.

To the solution of 5.1 g of sodium iodide in 25 ml of water are added 17 g of the potassium salt of 7-formylamino cephalosporanic acid and 12.7 g of N-allylpyridine 2-thione.

- 10 The mixture is stirred for 4 hours at 60°C. After cooling, the solution is poured into 1.7 l of acetone and the precipitate is drained and rinsed with acetone then with ether. It is dried in vacuo.

b) Chloride of 3-(N-allyl 2-pyridinio thiomethyl) 7-formylamino 3-cepheme 4-carboxylic acid.

- 15 20 g of the product obtained hereinabove are dissolved in 100 ml of water and the solution is acidified by 2N hydrochloric acid up to pH = 1.5. The aqueous phase is separated from a slight precipitate and is poured over a column of ion exchanger resin Amberlite IRA 68[®] in the form of hydrochloride. It is eluted with water. The water
20 is evaporated to dryness in vacuo and the residue is taken up in absolute ethanol. It is evaporated again to dryness and the residue is taken up in ether. The solid is drained and dried in vacuo.

NMR spectrum

- 2 H at 9.10 ppm (H_6' pyridine, NH CO, M) - 1 H at 8.45 ppm (H_4' pyridine, TD) - 2 H at 3.10 ppm (H -CO-N, H_3' pyridine, M) - 1 H at 7.95 ppm (H_5' pyridine, TD) - 1 H at 6.00 ppm (CH =, M) - 1 H at 5.70 ppm (H_7 , M) - 5 H between 4.95 and 5.40 ppm (H_6 , CH_2N^+ and = CH_2 , M) - 2 H at 4.45 ppm (CH_2S in 3, AB, $J_{AB} = 13$ Hz) 2 H at 3.60 ppm (CH_2S cycle, AB, $J_{AB} = 17$ Hz).

- 30 c) Chloride of 3-(N-allyl 2-pyridinio thiomethyl) 7-formylamino 3-cepheme 4-carboxylic S-oxide-1 acid.

6 g of the product obtained in b) are dissolved in 30 ml of formic acid. 30 ml of methanol are added and the solution is cooled to 5°C. 2.7 g of metachloroperbenzoic acid are added in 5 minutes.

- 35 The temperature is allowed to rise to 20°C and the mixture is stirred

4.40 ppm (CH_2S , S.e.) - 2 H at 3.85 ppm ($\text{CH}_2\text{S}\rightarrow\text{O}$, S.e.) - 6 H between 1.5 and 2.6 ppm (, M).

EXAMPLES II, 12, 13

Operation is as in Example 10 b) from the brominated derivative of Example 10 a) but varying the nature of the pyridine thione used.

By effecting deprotection thereafter as indicated in Example 1 d), the compounds III shown in Table 2 are obtained.

EXAMPLE 14

Trifluoroacetate of 7-[2-(2-amino 4-thiazolyl) 2-(1-carboxy 1-ethyl oxyimino)- acetamido] 3-(2-N-methylpyridinio thiomethyl) 3-cepheme 4-carboxylic S-oxide-1 acid, syn isomer (CM 40 800)

(III) $\text{R}_1 = \text{H}$; $\text{R}_2 = \text{CH}_3$; $\text{R}_3 = \text{CH}_3$; $\text{R}_4 = \text{H}$; $\text{A} = \text{H}$; $\text{X}^- = \text{CF}_3\text{COO}^-$

Operation is as in Example 1, but replacing in step b) the 2-(2-tritylamino 4-thiazolyl) 2-(2-t-butoxycarbonyl 2-propyl oxyimino) acetic acid, syn isomer, by 2-(2-tritylamino 4-thiazolyl) 2-(1-t-butoxycarbonyl 1-ethyl oxyimino) acetic acid, syn isomer.

Steps c and d are carried out identically and lead to the expected compound CM 40800 .

NMR spectrum

1 H at 9.0 ppm (H_6 pyridine, M) - 0.4 H at 8.65 ppm (NHCO , D, J=9 Hz) - 0.6 H at 8.60 ppm (NHCO , D, J=9 Hz) - 1 H at 8.32 ppm (H_4 pyridine, M) - 1 H at 8.10 ppm (H_3 pyridine, D, J=6 Hz) - 1 H at 7.8 ppm (H_5 pyridine, M) - 4 H at 7.20 ppm (NH_2 , 2 COOH , S.e.) - 0.6 H at 6.82 ppm (H thiazole, S) - 0.4 H at 6.80 ppm (H thiazole, S)
 - 1 H at 5.92 ppm (H_7 , D of D, $\text{J}_1=9$ Hz), $\text{J}_2=4$ Hz) - 1 H at 5.0 ppm (H_6 , M) - 1 H at 4.60 ppm ($\frac{\text{CH}}{\text{CH}}$ M) - 1 H at 4.50 ppm (CH_2S , D, J=12 Hz) - 1 H at 4.35 ppm (CH_2S , D, J=12 Hz) - 3 H at 4.20 ppm (CH_3N^+ , S) - 1 H at 4.0 ppm ($\text{CH}_2\text{S}\rightarrow\text{O}$, D, J=17 Hz) - 1 H at 3.87 ppm ($\text{CH}_2\text{S}\rightarrow\text{O}$, D, J=17 Hz) - 3 H at 1.4 ppm (CH , M)
 CH_3

The division of the signals of the protons due to the - NH - CO group and to the thiazole indicates that CM 40 800 exists in the form of a mixture of 2 diastereoisomers due to the existence of an asymmetric carbon in the substituent of the oxime.

at this temperature for 30 minutes.

An insoluble is filtered and the solution obtained is poured into 600 ml of ether. The solid is drained, rinsed with ether and dried in vacuo.

5 NMR spectrum

1 H at 8.96 ppm (H_6' pyridine, D, $J = 6$ Hz) - 2 H at 8.30 ppm (H_4' pyridine, NH CO, M) - 2 H at 8.10 ppm (H_3' pyridine, H -CO-N-, M) - 1 H at 7.80 ppm (H_5' pyridine, TD, $J = 6$ Hz) - 1 H at 6.0 ppm ($CH=$, M) - 1 H at 5.90 ppm (H_7 , M) - 5 H between 5.0 and 5.50 ppm (CH_2 N⁺, $CH_2=$, H_6 , M) - 2 H at 4.45 ppm (CH_2S , AB, $J_{AB} = 13$ Hz) - 2 H at 3.95 ppm ($CH_2S \rightarrow O$, AB, $J_{AB} = 17$ Hz).

d) Chloride of 3-(N-allyl 2-pyridinio thiomethyl) 7-formylamino 3-cepheme carboxylate of 4-diphenylmethyl S-oxide-I

4.5 g of the product obtained in c) are dissolved in 45 ml of water and 130 ml of solution of diphenyldiazomethane in methylene chloride are added. The mixture is stirred vigorously and 90 ml of absolute ethanol are added and the pH is maintained at 2 by addition of concentrated hydrochloric acid.

After 45 minutes, the solution is decolorized. The organic layer is decanted and the aqueous phase is reextracted with methylene chloride. The organic extracts are combined and concentrated to dryness. The residue is taken up in absolute ethanol and evaporated to dryness again. The residue is taken up in ether, the solid is drained and dried in vacuo.

25 NMR spectrum

1 H at 9.10 ppm (H_6' pyridine, D, $J = 5$ Hz) - 1 H at 8.45 ppm (CO NH , D, $J = 9$ Hz) 1 H at 8.20 ppm (H_4' pyridine, T, $J = 7$ Hz) - 1 H at 8.10 ppm (H CO-, S) - 1 H at 8.0 ppm (H_3' pyridine, D, $J = 7$ Hz) - 1 H at 7.85 ppm (H_5' pyridine, deformed T) - 10 H at 7.30 ppm (aromatic H, M) - 1 H at 6.85 ppm (COO CH <, S) - 2 H at 6.00 ppm (H_7 + $CH=$, M) - 5 H between 5 and 5.5 ppm (H_6 , CH_2N^+ , $=CH_2$, M) - 2 H at 4.45 ppm (CH_2S , AB, $J_{AB} = 13$ Hz) 2 H at 4.0 ppm ($CH_2 \rightarrow O$, AB, $J_{AB} = 17$ Hz).

e) Hydrochloride of the chloride of 3-(N-allyl 2-pyridinio thiomethyl)

35 7-amino 3-cepheme carboxylate of diphenylmethyl, S-oxide-I

3 g of the product obtained hereinabove are dissolved in 10 ml of methanol in an inert atmosphere. The solution is cooled to 10°C and 0.8 ml of thionyl chloride is added in 5 minutes, the temperature being maintained lower than 20°C.

- 5 The mixture is then stirred for 30 minutes at 20°C and is poured into 300 ml of ether. The solid is drained and rinsed with ether. It is dried in vacuo over phosphoric anhydride.

- f) Chloride of 7-[2-(2-tritylamino 4-thiazolyl) 2-(2-t-butoxycarbonyl 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl)
 10 3-cepheme carboxylate of 4-diphenylmethyl S-oxide-1, syn isomer.
 - Chloride of 2-(2-tritylamino 4-thiazolyl) 2-(2-t-butoxycarbonyl 2-propyl oxyimino) acetic acid, syn isomer.

- 3.4 g of 2-(2-tritylamino 4-thiazolyl) 2-(2-t-butoxycarbonyl 2-propyl oxyimino) acetic acid, syn isomer are suspended in an atmosphere of nitrogen in 20 ml of methylene chloride. 1.4 g of phosphorus pentachloride are added and the mixture is stirred for 30 minutes, the temperature being maintained lower than 0°C. The solution is poured into 200 ml of hexane. The solid is drained and dried in vacuo over phosphoric anhydride. The chloride of acid is used as such.

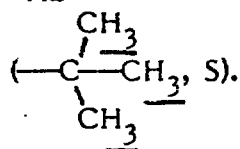
- 20 3.3 g of the derivative obtained in paragraph e) are suspended in an atmosphere of nitrogen in 30 ml of methylene chloride. The mixture is cooled to 5°C and 1.7 ml of dimethylaniline is added, then the chloride of acid obtained hereinabove, the temperature being allowed to rise to 20°C. After one hour of stirring, the solution is washed
 25 with 30 ml of a 0.5 N hydrochloric acid solution. The organic solution is dried and the solvents are concentrated in vacuo up to a volume of 10-15 ml. This solution is poured into 150 ml of isopropyl ether. The solid is drained, rinsed with isopropyl ether and dried in vacuo.

- The crude product thus obtained is chromatographed over
 30 a column of silica gel (120 g). By eluting with a methylene chloride-methanol 85-15 (vol/vol) mixture, the expected product is obtained.

NMR spectrum

- 1 H at 9.05 ppm (H_6' pyridine, D, J=6 Hz) - 1 H at 8.85 ppm (NH Trit, s.e.) - 2 H at 8.25 ppm (H_4' pyridine, NH CO, M) - 1 H at 8.0 ppm
 35 (H_3' pyridine, D, J = 7 Hz) - 1 H at 7.80 ppm (H_5' pyridine, TD) -

25 H at 7.27 ppm (aromatic H, M) - 1 H at 6.85 ppm (H thiazole, S) - 1 H at 6.75 ppm (COOCH₃, S) - 2 H at 5.95 ppm (H₇ + CH₂=, M) - 5 H between 5.0 and 5.5 ppm (CH₂N⁺, CH₂=, H₆, M) - 2 H at 4.45 ppm (CH₂S, AB, J_{AB} = 13 Hz) - 2 H at 4.0 ppm (CH₂S-O, AB, J_{AB} = 17 Hz) - 6 H at 1.40 ppm [(CH₃)₂-C-, S] - 9 H at 1.30 ppm



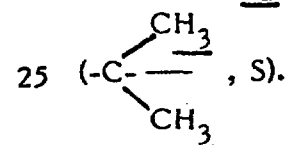
g) CM 40874

1 g of the protected product obtained in f) is dissolved in 2 ml of anisole and cooled to 5°C then 10 ml of trifluoroacetic acid are added. The temperature is allowed to rise to 20°C and the mixture is left at this temperature for 2 hours.

The trifluoroacetic acid is evaporated in vacuo and the product is precipitated by addition of ether. The product is drained, washed with ether and dried.

15 NMR spectrum

1 H at 9.05 ppm (H₆' pyridine, D, J = 5 Hz) - 1 H at 8.50 ppm (NH CO, D, J = 9 Hz) - 1 H at 8.35 ppm (H₄' pyridine, M) - 1 H at 8.20 ppm (H₃' pyridine, D, J = 7 Hz) - 1 H at 7.95 ppm (H₅' pyridine, M) - 4 H between 7 and 10 ppm (2 COOH, NH₂) - 1 H at 6.82 ppm (H thiazole, S) - 2 H at 6.0 ppm (H₇ and CH₂=, M) - 5 H between 5.0 and 5.6 ppm (CH₂N⁺, CH₂= and H₆, M) - 1 H at 4.5 ppm (CH₂S, A of AB, J_{AB} = 13 Hz) - 1 H at 4.32 ppm (CH₂S, B of AB, J_{AB} = 13 Hz) - 1 H at 4.0 ppm (CH₂S-O, A of AB, J_{AB} = 17 Hz) - 1 H at 3.8 ppm (CH₂S-O, B of AB, J_{AB} = 17 Hz) - 6 H at 1.45 ppm



This product is identical in all points with the product obtained in Example 1.

EXAMPLE 16

30 Trifluoroacetate of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme

4-carboxylic S-oxide-I acid, syn isomer (CM 40874)

a) Iodide of 7-(5-phthalamino 5-carboxy valeramido) 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic acid

To the solution of 213 g of sodium iodide in 90 ml of water
5 is added the mixture of 71 g of N-phthalyl cephalosporin C and 32.2 g of sodium bicarbonate. The mixture is heated to 60°C and left with stirring for 1 hour 45 minutes at this temperature.

After cooling to 10°C, the mixture is poured into 5 litres
10 of acetone with vigorous stirring. The solid is drained, rinsed with acetone and with ether.

The product is dried and dissolved in 500 ml of water. The
solution is cooled to 5°C and acidified with stirring by the addition
of 2 N hydrochloric acid up to pH = 2.5. The solid is drained, rinsed
with a little iced water and dried in vacuo in the presence of phosphoric
15 anhydride.

b) Chloride, hydrochloride of 7-amino 3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic acid.

15 g of the product obtained in paragraph a) hereinabove
are suspended, in an atmosphere of nitrogen, in 150 ml of methylene
20 chloride, then 13 ml of dimethylaniline and 12 ml of chloro trimethylsilane are added. The temperature is raised to 32°C and the solution is stirred at this temperature for 1 hour. The solution is cooled to -50°C and 10 ml of dimethylaniline then 16 g of phosphorus pentachloride are added. The mixture is stirred at -50°C for 1 hour then at -30°C
25 for 2 hours 30 minutes.

The mixture is poured onto a solution of 30 ml of 2,3-butane-
diol in 200 ml of methylene chloride cooled to -20°C. It is left with
stirring until the temperature of the mixture has reached about 20°C
then the solid is drained. The mixture is washed with methylene chloride
30 then with ether and is dried in vacuo over phosphoric anhydride.

NMR spectrum

3 H at 9.70 ppm (CH_3^+ , s.e.) - 1 H at 9.10 ppm (H_6' pyridine, D, J
= 5 Hz) - 1 H at 8.50 ppm (H_4' pyridine, D, J = 7 Hz) - 1 H at 8.10
ppm (H_3' pyridine, D, J = 7 Hz) - 1 H at 7.35 ppm (H_5' pyridine, T)
35 - 1 H at 6.05 ppm ($\text{CH}=\text{}$, M) - 6 H between 5 and 5.5 ppm (H_6' , H_7' ,

$\text{CH}_2\text{N}^{\oplus}$, $\text{CH}_2=$, M) - 2 H at 4.50 ppm (CH_2S in 3, AB, $J_{\text{AB}} = 13$ Hz) - 2 H at 3.80 ppm (CH_2S cycle, S).

c) Chloride, hydrochloride of 7-amino-3-(N-allyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic S-oxide-1 acid

5 4 g of the product obtained hereinabove are dissolved in 15 ml of formic acid, then 20 ml of methanol are added.

The solution is cooled to 0°C and 1.85 g of metachloroperbenzoic acid are added in 10 minutes. The solution is then stirred for 10 minutes at 10°C, then it is poured into 800 ml of ether. The precipitate is drained, washed with ether then dried in vacuo over phosphoric anhydride.

NMR spectrum

1 H at 9.65 ppm (H_6' pyridine, D, $J = 6$ Hz) - 1 H at 8.40 ppm (H_4' pyridine, M) - 1 H at 8.10 ppm (H_3' pyridine, M) - 1 H at 7.90 ppm (H_5' pyridine, M) - 1 H at 6.00 ppm ($=\text{CH}_2$, M) - 6 H between 4.70 and 5.50 ppm (H_6 , H_7 , $\text{CH}_2\text{N}^{\oplus}$, $=\text{CH}_2$, M) - 2 H at 4.40 ppm (CH_2S , AB, $J_{\text{AB}} = 13$ Hz) - 2 H at 3.90 ppm ($\text{CH}_2\text{S}-\text{O}$, AB, $J_{\text{AB}} = 17$ Hz).

d) Hydrochloride of the chloride of 3-N-allyl 2-pyridinio thiomethyl) 7-amino 3-cepheme 4-carboxylate of diphenylmethyl S-oxide-1

20 To the solution of 3.5 g of the product obtained in paragraph c) in 30 ml of methanol are added 30 ml of a solution of diphenyl diazomethane, at 20°C. The solution is left for 30 minutes at this temperature then 40 ml of diphenyl diazomethane solution are added and the mixture is left for 1 hour at 20°C.

25 It is evaporated to dryness and taken up in a small quantity of methylene chloride and the solution is poured into ether. The solid is drained, rinsed with ether and dried in vacuo.

This product is identical to that obtained in Example 15 e).

e) CM 40874

30 Using the above product, operation is as indicated in Example 15, paragraphs f) and g), to obtain the product CM 40874.

EXAMPLE 17

Hydrochloride, chloride of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyimino) acetamido] 3-(N-allyl 2-pyridinio thiomethyl)

35 3-cepheme 4-carboxylic S-oxide-1 acid, syn isomer.

Operation is as in Example 15 up to paragraph f) inclusive, then the protected compound thus obtained is treated with concentrated hydrochloric acid in formic acid as described in Example 2.

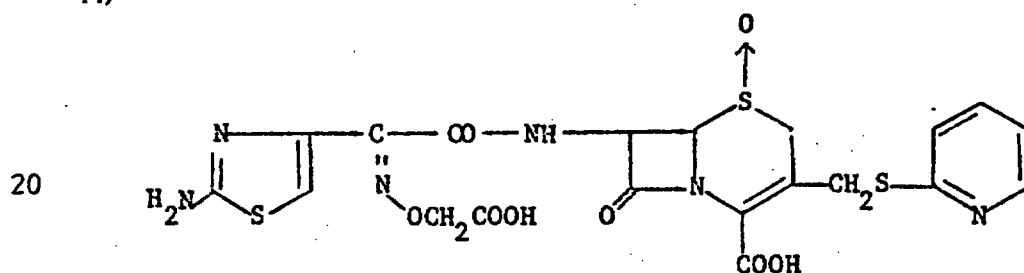
The compound is isolated in the same way.

5 Products (III) were studied as far as their pharmacological properties, and more especially their bacteriostatic action, are concerned.

In vitro bacteriostatic action was determined in a solid medium by the dilutions method. The results obtained are expressed in minimum inhibitory concentrations (MIC - $\mu\text{g/ml}$) and concern different strains of Enterobacteria and of Pseudomonas.

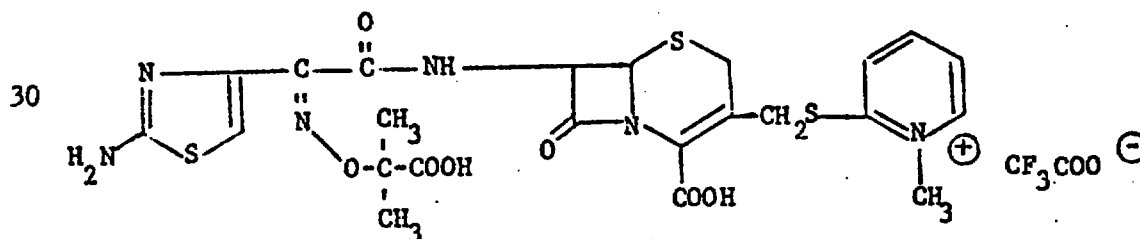
By way of comparison, the results obtained with 2 similar products described in the prior art have been added, namely:

15 - 7-[2-(2-amino thiazolyl) 2-carboxymethoxyimino acetamido] 3-(2-pyridyl thiomethyl) 3-cepheme 4-carboxylic S-oxide-l acid, syn isomer (compound A)



(Belgian Patent No. 886 038)

25 - The trifluoroacetate of 7-[2-(2-amino 4-thiazolyl) 2-(2-carboxy 2-propyl oxyimino) acetamido] 3-(N-methyl 2-pyridinio thiomethyl) 3-cepheme 4-carboxylic acid, syn isomer (compound B)



35

(German Patent Application No. 2 921 332)

The results obtained are shown in Table 3.

These results show a particularly interesting activity of the products according to the invention on strains which are usually hardly sensitive to the antibiotics of the cephalosporin family, namely the
5 Enterobacteria and the Pseudomonas.

Compared with reference product A, products (III) show a surprising activity on the following strains: Citrobacter, Enterobacter, Serratia and Pseudomonas, whilst conserving an activity at least equal to that of the reference product on Klebsiella and Proteus.

10 Compared with reference product B, products III show a clearly greater activity on Citrobacter, Proteus and Enterobacter, whilst conserving on the other strains an activity of the same order of magnitude and sometimes even greater.

Furthermore, the tests carried out on animals have demonstrated that there is no toxicity in the products according to the invention,
15 their toxicity being comparable to that of the compounds of the cephalosporin family.

The products of the invention may therefore be used as antibiotics in human or veterinary medicine. They may be used in all sensitive-
20 germ bacterial infections.

Pharmaceutical compositions are made from compounds (III) in their acid form or, when their solubility is insufficient, in the form of a salt.

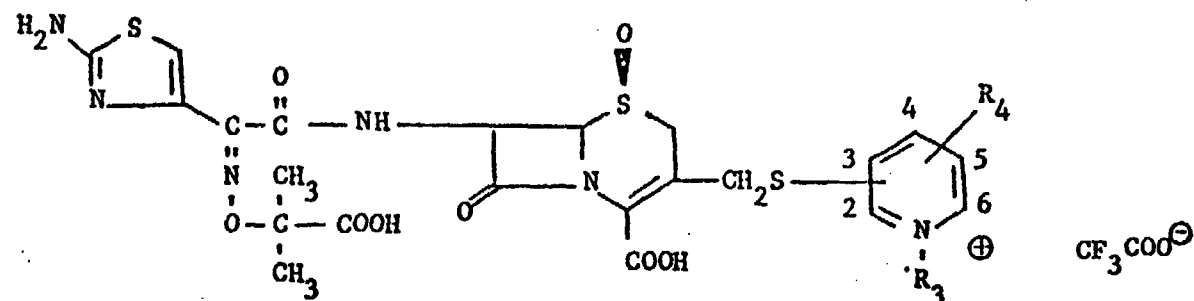
The pharmaceutical compositions may be solid or liquid and
25 be, for example, in the form of tablets, capsules, granules, ointments, creams, gels or injectable preparations.

Dosage may vary to considerable proportions, and depends in particular on the type and seriousness of the infection to be treated and on the mode of administration. In the adult by the injectable route,
30 it is most often between 0.250 g and 4 g per day.

By way of example of a pharmaceutical composition containing a product of the invention, injectable ampoules containing:

- CM 40 874 b 1 g
 - Water for injectable preparation 5 ml
 - 35 Sodium carbonate q.s.p. pH = 6.3
- may be prepared.

TABLE 1



Example No.	Code No. Product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C - duration	NMR spectrum
4	40763	CH ₃	H	2	20-24 hrs.	1 H at 9.00 ppm (H₆ pyridine, D, J=6 Hz) - 4 H between 7.60 and 8.60 ppm (NHCO, H₃, H₄, H₅ pyridine, M) - 1 H at 6.83 ppm (H thiazole, S) - 1 H at 5.97 ppm (H₇, M) - 1 H at 5.00 ppm (H₆, D, J=4 Hz) - 2 H at 4.40 ppm (CH₂S, S.e.) - 3 H at 4.20 ppm (CH₃-N⁺, S) - 2 H at 3.80 ppm (CH₂S→O, S.e.) - 6 H at 1.46 ppm (-C(CH₃)₂, S).

TABLE 1 (cont. 1)

Example No.	Code No. Product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C - duration	NMR spectrum
5	40876	CH ₃	H	4	20-3 hrs.	2 H at 8.64 ppm (H₂, H₆ pyridine, D, J=7 Hz) - 1 H at 8.40 ppm (NH CO, D, J=9 Hz) - 2 H at 7.95 ppm (H₃, H₅ pyridine, D, J=7 Hz) - 4 H at 7.50 ppm (NH₂, 2 COOH, S. e.) - 1 H at 6.80 ppm (H thiazole, S) - 1 H at 5.95 ppm (H₇, D of D, J₁=9 Hz, J₂=4 Hz) - 1 H at 5.05 ppm (H₆, D, J=4 Hz) - 1 H at 4.40 ppm (CH₂S, A of AB, J_{AB}=13 Hz) - 1 H at 4.35 ppm (CH₂S, B of AB J_{AB} = 13 Hz) - 3 H at 4.16 ppm (CH₃N⁺, S) - 1 H at 3.89 ppm (CH₂S → O, A of AB, J_{AB}=17 Hz) - 1 H at 3.76 ppm (CH₂S → O, B of AB, J_{AB}=17 Hz) - 6 H at 1.43 ppm (-C(CH₃)₂, 2 D).

TABLE 1 (cont. 2)

Example No.	Code No. product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C -duration	NMR spectrum
6	40912	CH ₂ C≡CH	H	2	20-4 hrs.	5 H from 7.5 to 9.1 ppm (H₃, H₄, H₅ and H₆ pyridine and NHCO, M) - 1 H at 6.85 ppm (H thiazole, S) - 1 H at 6.0 ppm (H₇, M) - 2 H at 5.65 ppm (CH₂N⁺, M) - 3 H at 5.0 ppm (H₆ and CH₂S, M) - 2 H at 3.95 ppm (CH₂S→O, S.e.) - 6 H at 1.43 ppm (-C(CH₃)₂, S) - [≡CH masked by the dimethylsulfoxide]
7	40972	CH ₃	3-OH	2	20-24 hrs.	5 H between 6.5 and 10 ppm (H₂N, 2 COOH, OH, M) - 1 H at 8.60 ppm (H₆ pyridine, M) - 1 H at 8.45 ppm (NHCO, D, J=9 Hz) - 2 H at 7.9 ppm (H₄ and H₅ pyridine, M) - 1 H at 6.80 ppm (H thiazole, S) - 1 H at 6.0 ppm (H₇, M) - 1 H at 4.96 ppm (H₆, D, J=5 Hz) - 5 H at 4.30 ppm (CH₃N⁺ and CH₂S, M) - 2 H at 3.85 ppm (CH₂S→O, M) - 6 H at 1.47 ppm (-C(CH₃)₂, S).

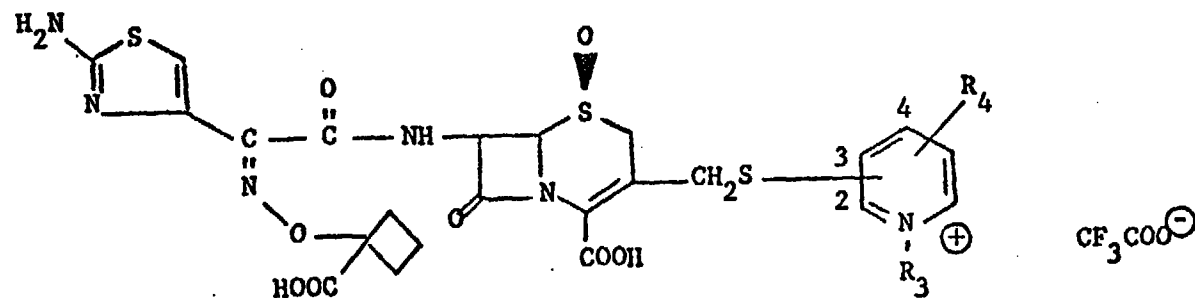
TABLE 1 (cont. 3)

Example No.	Code No. product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C - duration	NMR spectrum
8	41087	CH ₂ COOC ₂ H ₅	H	2	5-16 hrs. solvent tetrahydrofuran.	1 H at 9.2 ppm (NHCO, D, J=9 Hz) - 4 H between 7.5 and 8.8 ppm (H₃, H₄, H₅ and H₆ pyridine, M) - 1 H at 6.90 ppm (H thiazole, S) - 1 H at 6.0 ppm (H₇, M) - 2 H at 5.67 ppm (CH₂N⁺, S.e.) - 1 H at 5.0 ppm (H₆, D, J=5 Hz) - 2 H at 4.30 ppm (COOCH₂-, Q, J=7 Hz) - 2 H at 3.90 ppm (CH₂S→O, S.e.) - 6 H at 1.45 ppm (-C(CH₃)₂, S) - 3 H at 1.20 ppm (COOCH₂CH₃, T, J=7 Hz).

Table 1 (cont. 4)

Example No.	Code No. product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C - duration	NMR spectrum
9	41607	-CH ₂ -CH=CH ₂	H	4	20°C 2 hours	2 H at 8.70 ppm (H_α pyridine, D, J = 6 Hz) - 1 H at 8.45 ppm (NH-CO, D, J=9 Hz) - 2 H at 8.0 ppm ($\overline{H}_\beta$ pyridine, D, J = 6 Hz) - 1 H at 6.80 ppm (H thiazole, S) - 2 H at 6.0 ppm (H₇ + CH=, M) - 2 H at 5.40 ppm (=CH₂, M) - 3 H at 5.05 ppm (H₆ + CH₂N⁺, M) - 2 H at 4.45 ppm (CH₂S, M) - 2 H at 3.80 ppm (CH₂S → O, AB, J_{AB} = 16 Hz) - 6 H at 1.45 ppm (C - (CH₃)₂, 2 S).

TABLE 2



Example No.	Code No. product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C - duration	NMR spectrum
10	40882	CH ₃	H	2	20-5 hrs.	9 H between 7.5 and 9.5 ppm (NH₂, 2 COOH, NHCO, H₃, H₄, H₅ and H₆ pyridine, M) - 1 H at 6.88 ppm (H thiazole, S) - 1 H at 6.0 ppm (H₇, M) - 1 H at 5.07 ppm (H₆, D, J=4 Hz) - 2 H at 4.45 ppm (CH₂S-M) - 3 H at 4.25 ppm (CH₃N⁺, S) - 2 H at 3.93 ppm (CH₂S→O, M) - 6 H between 1.5 and 2.7 ppm , M).

TABLE 2 (cont.)

Example No.	Code No. product	R ₃	R ₄	Position substitution pyridine	Conditions temperature °C - duration	NMR spectrum
11	40954	CH ₃	H	4	20-16 hrs.	7 H between 8,3 and 9,0 ppm (NHCO, NH₂, 2 COOH, H₂ and H₆ pyridine, M) - 2 H at 7,90 ppm (H₃ and H₅ pyridine, D, J=6 Hz) - 1 H at 6,82 ppm (H thiazole, S) - 1 H at 6,00 ppm (H₇, D of D, J₁=9 Hz, J₂=5 Hz) - 1 H at 5,0 ppm (H₆, D, J=5 Hz) - 2 H at 4,40 ppm (CH₂S, S.e.) - 3 H at 4,20 ppm (CH₃-N⁺, S) - 2 H at 3,80 ppm (CH₂S→O, S.e.) - 6 H between 1,5 and 3 ppm (, M).

Table 2 (cont.)

Example No.	Code No. product	R ₃	R ₄	Position Substitution Pyridine	Conditions Temperature °C - duration	NMR spectrum
13	41647	-CH ₂ -CH=CH ₂	H	4	20°C 1 hour	1 H at 8.80 ppm (NH-CO, D, J = 9 Hz) 2 H at 8.70 ppm (H_αpyridine, D, J = 6 Hz) - 2 H at 8.0 ppm (H_βpyridine, D, J = 6 Hz) - 1 H at 6.80 ppm (H thiazole, S)- 2 H at 6.00 ppm (H₇ + CH=, M) - 2 H at 5.40 ppm (CH₂=, M) - 3H at 5.05 ppm (H₆ + CH₂N⁺, M) - 2H at 4.45 ppm (CH₂S, M) - 2H at 3.8 ppm (CH₂S-O, AB, J_{AB}=16 Hz) - 4H at 2.45 ppm (CH₂-COOH, M)-2 H at 1.9 ppm (CH₂-CH₂).

TABLE 3

Strain \ Product	40874	40914	40763	40876	40800	40882	40954	41087	A	B
Citrobacter 49	4	4	2	1	2	4	2	0,5	16	8
Proteus 1510	0,031	≤ 0,125	≤ 0,125	≤ 0,125	0,25	≤ 0,125	≤ 0,125	0,25	0,25	4
Serratia RL 72	0,5	0,25	0,25	0,25	2	0,25	0,25	2	32	2
Klebsiella RO 30	0,25	≤ 0,125	0,25	≤ 0,125	0,25	≤ 0,125	≤ 0,125	0,5	0,5	0,25
Enterobacter RO 46	0,5	0,25	0,5	0,25	0,25	0,25	≤ 0,125	1	8	64
Enterobacter P 99	0,5	0,5	1	0,5	1	0,5	0,5	2	16	256
Pseudomonas A 22 IP	8	8	4	2	16	2	2	-	> 256	8
Pseudomonas RL 112	8	8	4	4	8	4	4	-	256	16