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# PATENT SPECIFICATION

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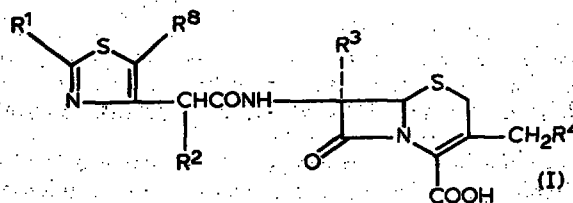
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## (54) CEPHEM COMPOUNDS

(71) We, TAKEDA YAKUHI KOGYO KABUSHIKI KAISHA, also known as TAKEDA CHEMICAL INDUSTRIES, LTD., of 27, Doshomachi, 2-chome, Higashi-ku, Osaka, Japan, a joint stock company organised under the laws of Japan, 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 cephem compounds, and more particularly to thiazolyl-acetamido cephem compounds.

The invention provides a cephem compound of the formula (I):



wherein R<sup>1</sup> represents an amino or hydroxyl group which may be protected; R<sup>2</sup> represents an amino or a hydroxyl group which may be protected, or

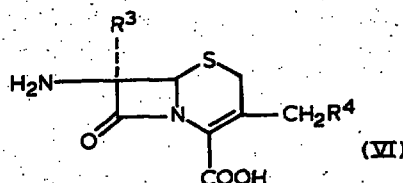


represents a group of the formula

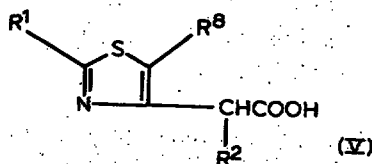


where R<sup>6</sup> is a hydroxyl group which may be protected; R<sup>3</sup> represents hydrogen or a methoxy group; R<sup>4</sup> represents hydrogen or a residue of a nucleophilic compound; and R<sup>5</sup> represents hydrogen or a halogen atom, or a pharmaceutically acceptable salt or ester thereof.

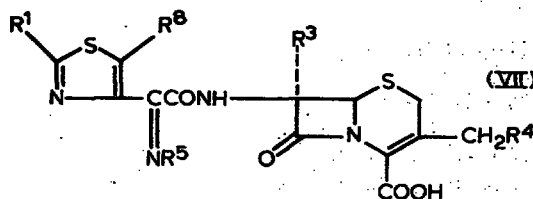
After extensive research we have succeeded in synthesizing a novel cephem compound (I), or a pharmaceutically acceptable salt or ester thereof, by reacting a compound of the formula:



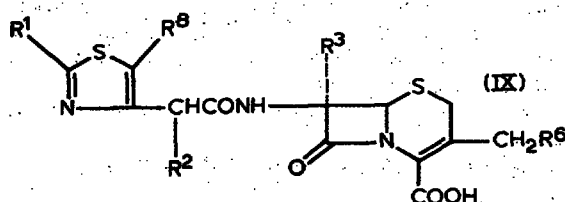
wherein  $R^3$  and  $R^4$  are as defined above, or a salt or ester thereof, with a compound of the formula:



wherein  $R^1$ ,  $R^2$  and  $R^3$  are as defined above, or a salt or a reactive derivative thereof, followed by, if necessary, removal of the protective group; or, when  $R^2$  is amino, reducing a compound of the formula:



wherein each of the symbols has the same meaning as defined above, or a salt or ester thereof, followed by, if necessary, removal of the protective group; or when  $R^4$  is other than hydrogen, reacting a compound of the formula:



wherein  $R^4$  represents an acyloxy or carbamoyloxy group or a halogen and each of the other symbols has the same meaning as defined above, or a salt or ester thereof, with a nucleophilic compound, followed by, if necessary, removal of the protective group.

We have found that the cephem compounds (I) obtained by the foregoing method have strong antibiotic properties against a wide variety of microorganisms including gram-positive bacteria as well as gram-negative ones, especially by oral administration, and that the cephem compounds (I) can be used as therapeutic agents for various bacterial infections of animals including human beings.

The present invention is therefore directed to:

- (1) A cephem compound (I),
- (2) A process for preparing a cephem compound (I), which comprises reacting a compound (VI) or a salt or ester thereof with a compound (V) or a reactive derivative thereof,
- (3) A process for preparing a cephem compound (I), wherein  $R^2$  is amino, which comprises reducing a compound (VII) or a salt or ester thereof,
- (4) A process for preparing a cephem compound (I), wherein  $R^4$  is other than hydrogen which comprises reacting a compound (IX) or a salt or ester thereof with a nucleophilic compound,
- (5) A pharmaceutical composition containing compound (I).

In the cephem compounds (I), the symbol  $R^1$  represents amino or a hydroxyl group, or a protected amino or hydroxyl group. The term "protected amino group" means an amino group protected with such easily removable protective groups of amino groups as are usually employed in peptide chemistry, which are exemplified by alkanoyl groups such as formyl, acetyl and propionyl, an alkoxycarbonyl group such as t-butoxycarbonyl, an alkoxyalkylcarbonyl group such as methoxyacetyl and methoxypropionyl, a substituted alkoxycarbonyl group such as trichloroethoxycarbonyl, a substituted alkylcarbonyl group such as monochloromethylcarbonyl, monochloroethylcarbonyl, dichloromethylcarbonyl, dichloroethylcarbonyl, trichloromethylcarbonyl, trichloroethylcarbonyl or trichloropropylcarbonyl, an aralkyloxycarbonyl group such as

benzyloxycarbonyl, a substituted aralkyloxycarbonyl group such as *p*-nitrobenzyloxycarbonyl, or an amino group protected with a proton. The term "protected hydroxyl group" means a hydroxyl group protected with an easily removable protective group. Such easily removable protective groups for hydroxyl groups may be exemplified by, e.g., an acyl group such as formyl, acetyl, chloroacetyl, trifluoroacetyl, methoxyacetyl, phenoxyacetyl, benzoyl, benzoylformyl, *p*-nitrobenzoyl, ethoxycarbonyl,  $\beta,\beta,\beta$ -trichloro-ethoxycarbonyl,  $\beta,\beta,\beta$ -tribromoethoxycarbonyl and *p*-nitrophenoxycarbonyl, an easily removable protective group under relatively mild conditions such as tetrahydropyranyl, tetrahydrothiofuranyl and methoxytetrahydropyranyl. The symbol  $R^2$  represents an amino or a hydroxyl group, or a protected amino or hydroxy group or, in the general formula (I), the group



is replaced by the group



wherein  $R^5$  is a hydroxyl group which may be protected. Examples of protected amino and hydroxyl groups are the same as those of the protected amino or hydroxyl group as mentioned for the symbol  $R^1$ .

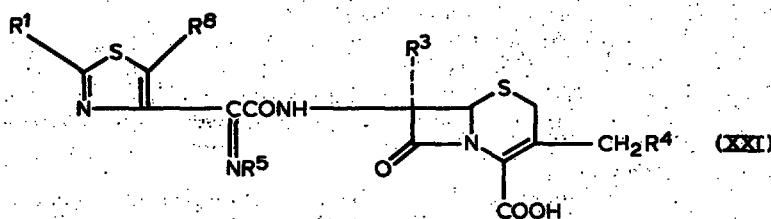
Where the group



is replaced by the group



the cephem compounds are shown by the formula:



wherein the symbols have the same meaning as defined above. The symbol  $R^2$  means hydrogen or a methoxy group.  $R^5$  represents hydroxyl or a protected hydroxyl group, e.g.  $C_1$  or  $C_2$  alkoxy. The symbol  $R^3$  represents hydrogen, or a halogen such as chlorine or bromine. Therefore, the substituents are represented by the formula:  $\text{---CH}_2R^4$ . The residue of the nucleophilic compound shown by the symbol  $R^4$  may be cyano, azido, amino, *N*-alkylamino (e.g. *N*-methylamino, *N,N*-dimethylamino, *N*-ethylamino, *N,N*-diethylamino, *N*-propylamino, etc.), hydroxy phenyl, a tertiary ammonium group or a group of the formula;



wherein *W* represents an oxygen or sulfur atom and *R* represents hydrogen, carbamoyl, *N*-alkylcarbamoyl, thiocarbamoyl, *N*-alkylthiocarbamoyl, acyl, sulfamoyl, alkylsulfonyl or a hetero ring. Typical residues of nucleophilic compounds may be exemplified by hydroxyl, mercapto, carbamoyloxy, carbamoylthio or thiocarbamoyloxy group, or those substituted with alkyl (e.g. methyl, ethyl or propyl), acyloxy (e.g. acetyloxy, propionyl-oxy, butyryloxy, benzoyloxy, *p*-chlorobenzoyloxy or *p*-methylbenzoyloxy), or a tertiary ammonium group, or hydroxyphenyl, sulfamoyloxy, alkylsulfonyloxy or (cis-1,2-epoxypropyl)phosphono. Furthermore, the symbol  $R^4$  may preferably represent a mercapto group substituted with a hetero ring which may be 5- or 6-membered one containing one to four hetero atoms selected from oxygen, sulfur and nitrogen. The hetero ring is exemplified by a 6-membered nitrogen-containing hetero ring, e.g. pyridyl, *N*-oxido-pyridyl, pyrimidyl, pyridazinyl or *N*-oxido-pyridazinyl, a 5-membered nitrogen-con-

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taining hetero ring, e.g. pyrazolyl, imidazolyl, thiazolyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1H-tetrazolyl or 2H-tetrazolyl. Each of these hetero rings may be further substituted and, as the substituents, there may be mentioned, for example, lower alkyl such as methyl, ethyl or propyl, lower alkoxy such as methoxy or ethoxy, halogen such as chlorine or bromine, halogen-substituted alkyl such as trifluoromethyl or trichloroethyl, amino, mercapto, hydroxyl, carbamoyl or carboxyl. The tertiary ammonium group represented by  $R^4$  may be exemplified by, e.g., pyridinium, 3-methylpyridinium, 4-methylpyridinium, 3-chloropyridinium, 3-bromopyridinium, 3-iodopyridinium, 4-carbamoylpyridinium, 4-(N-hydroxymethylcarbamoyl)pyridinium, 4-(N-carbomethoxycarbamoyl)pyridinium, 4-(N-cyanocarbamoyl)pyridinium, 4-(carboxymethyl)pyridinium, 4-(hydroxymethyl)pyridinium, 4-(trifluoromethyl)pyridinium, quinolinium, picolinium and lutidinium. The carboxyl group at 4-position of the cephem ring may be free or in the form of a salt with a non-toxic cation such as sodium or potassium; a basic amino acid such as arginine, ornithine, lysine or histidine; or a polyhydroxyalkylamine such as N-methylglucamine, diethanolamine, triethanolamine or trishydroxymethylaminomethane. Alternatively, the carboxyl group may be in the form of a biologically active ester (i.e. an ester in which the ester group is easily splittable by body enzymes), said ester being conductive to, for instance, an increased blood level or/and a longer duration of activity. As the ester residues of use for this purpose, there may be mentioned, for example, alkoxymethyl and  $\alpha$ -alkoxyethyl and other  $\alpha$ -alkoxy- $\alpha$ -substituted methyl groups, e.g. methoxymethyl, ethoxymethyl, isopropoxymethyl,  $\alpha$ -methoxyethyl and  $\alpha$ -ethoxyethyl; alkylthiomethyl groups, e.g. methylthiomethyl, ethylthiomethyl and isopropylthiomethyl; and acyloxymethyl and  $\alpha$ -acyloxy- $\alpha$ -substituted methyl groups, e.g. pivaloyloxymethyl and  $\alpha$ -acetoxybutyl.

Thus, the preferable examples of the present cephem compounds (I) may be the compounds where the group  $-\text{CH}_2\text{R}^4$  means methyl, acetoxyethyl, (1-methyl-1H-tetrazol-5-yl)thiomethyl, (1,2,3-triazol-5-yl)thiomethyl or (2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl.

According to another embodiment of the invention, in the cephem compound (I)  $R^4$  represents hydrogen or an acetoxy, thiadiazolylthio, triazolylthio or tetrazolylthio group which may be substituted with a  $\text{C}_1-\text{C}_6$  alkyl group.

Specific examples of cephem compounds (I) are

- (i)  $7\beta$  - [ $\alpha$  - Hydroxy -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.
- (ii)  $7\beta$  - [ $\alpha$  - (2 - Aminothiazol - 4 - yl)glycylamido]cephalosporanic acid.
- (iii)  $7\beta$  - [ $\alpha$  - (2 - Hydroxythiazol - 4 - yl)glycylamido]cephalosporanic acid.
- (iv)  $7\alpha$  - Methoxy -  $7\beta$  - [ $\alpha$  - hydroxy -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - desacetoxycephalosporanic acid.
- (v)  $7\beta$  - [ $\alpha$  - (2 - Aminothiazol - 4 - yl) -  $\alpha$  - methoxyiminoacetamido] - 3' - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

Like the known cephalosporins or penicillins, the contemplated compounds (I) of this invention can each be administered in the forms of injectable preparations, capsules, tablets, granules, solutions or suspensions, if necessary, with a physiologically acceptable carrier or excipient, in accordance with the established pharmaceutical procedure.

Thus the invention also provides a pharmaceutical composition which contains a medicinally effective amount of a cephem compound (I) or a pharmaceutically acceptable salt or ester thereof together with at least one pharmaceutically acceptable carrier therefor. Preferably the composition is provided in a unit dosage form. The contemplated compounds of this invention, such as sodium  $7\alpha$ -methoxy- $7\beta$ -(2-aminothiazol - 4 - ylglycylamido) - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, may be injected intramuscularly at a daily dose level of about 5 to 20 mg./kg. human body weight in 3 to 4 divided doses per day and are effective against respiratory-organ infections and urinary-tract infections. And, the compounds (I) show strong antibiotic properties against a wide variety of microorganisms including gram-positive or -negative bacteria, even by oral administration.

The cephem compounds (I) of the present invention can be prepared by, e.g., the following processes:

(a) Reaction of compounds (V) and (VI).

The carboxyl group of the starting compounds (VI) of this reaction may be used in salt form with alkali metals or organic amines such as sodium, potassium or triethylamine, or in ester form which can be easily converted into the free carboxyl

group by mild treatment with an acid or alkali, or by reduction, the ester residue being exemplified by, e.g.,  $\beta$ -methylsulfonyl, trimethylsilyl, dimethylsilyl, benzhydryl,  $\beta,\beta,\beta$ -trichloroethyl, phenacyl, *p*-methoxybenzyl, *p*-nitrobenzyl and methoxymethyl. As the reactive derivatives of the other starting compound (V), there may be employed acid halides, acid anhydrides, mixed acid anhydrides, cyclic carboxyanhydrides, active amides and esters thereof.

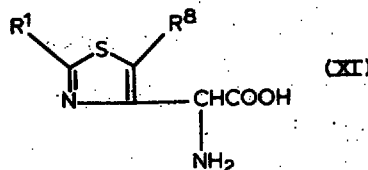
This reaction can be conducted advantageously and smoothly in the presence of a solvent. As said solvent, use may be made of the common solvents and their mixtures provided that such solvents do not interfere with the present reaction. There may be mentioned, therefore, such solvents as water, acetone, tetrahydrofuran, dioxane, acetonitrile, chloroform, dichloromethane, 1,1 or 1,2 dichloroethylene, dimethylformamide, dimethylacetamide and dimethylsulfoxide. This reaction may proceed more advantageously when to the solvent there is added a base such as pyridine, triethylamine, *N,N*-dimethylaniline or sodium bicarbonate. The quantity of the base to be added is 100–300%, desirably 100–200%, of that of the starting compound (V). While the reaction temperature is not initial, the reaction usually is carried out under cooling or at room temperature. The reaction is generally completed within several minutes to several hours.

If necessary, the thus obtained compounds are subjected to removal of the protective group and/or conversion of the convertible group. The removal of the protective group of the amino group may be conducted, for example, by acid treatment for *t*-butoxycarbonyl, by a treatment with zinc and an acid for  $\beta,\beta,\beta$ -trichloroethoxycarbonyl, or by catalytic reduction for *p*-nitrobenzyloxycarbonyl. The protective groups of the hydroxyl group are removed, for example, by potassium bicarbonate in aqueous methanol for formyl or trifluoroacetyl, by dilute hydrochloric acid treatment for tetrahydropyranyl, by zinc and an acid treatment for  $\beta,\beta,\beta$ -trichloroethoxycarbonyl. The ester residues of the carboxylic acids are removed, for example, by acid treatment for benzhydryl or *p*-methoxybenzyl, by alkali treatment for  $\beta$ -methylsulfonyl, by aqueous treatment for trimethylsilyl or dimethylsilyl, by zinc-an acid treatment for  $\beta,\beta,\beta$ -trichloroethyl, or by reduction for *p*-nitrobenzyl. The methylthio or methylseleno group may be converted into a methoxy group by, e.g., reacting with methanol in the presence of metal compounds including silver, mercury, lead and thallium. The removal of the protective groups and the conversion of the groups may be performed at the same time if possible, or any of them may be removed or converted in accordance with the reaction conditions of the next step.

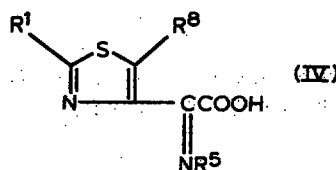
While the starting carboxylic acids (V) may occur as D- and L-isomers with respect to the  $\alpha$ -carbon, either of these isomers, as well as their mixture, can be successfully employed for the purposes of this invention. It is known that generally, of cephalosporins or penicillins having a center of asymmetry at  $\alpha$ -position, D-isomers are more antibiotic than the L-isomers.

The compounds (V) employed as the starting materials in this reaction may be prepared, e.g., as follows:

(a) A process for preparing a compound of the formula:

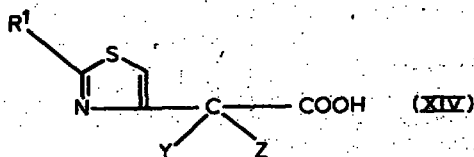


wherein the symbols have the same meanings as defined above, or a salt or ester thereof, which comprises reducing a compound of the formula:

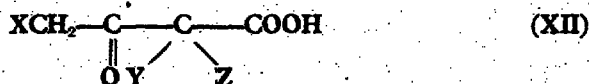


wherein  $\text{R}^5$  represents hydroxyl group which may be protected and each of other symbols has the same meaning as defined above, or a salt or ester thereof, followed by, if necessary, removal of the protective group.

(b) A process for preparing a compound of the formula;



wherein  $\text{R}^1$  represents amino or hydroxyl which may be protected and other symbols have the same meanings as defined below, or a salt or ester thereof, which comprises reacting a compound of the formula;



wherein X means a halogen and, when Y is hydrogen, Z means amino group which may be protected or Y and Z together represent a group of the formula;

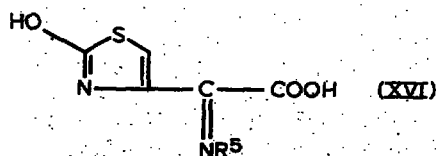


( $\text{R}^5$  is hydroxyl group which may be protected) or a salt or ester thereof and a compound of the formula;

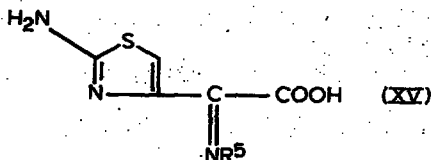


wherein  $\text{R}^{12}$  represents a lower alkoxy group or amino group which may be protected, followed by, if necessary, removal of the protective group,

(c) A process for preparing a compound of the formula;

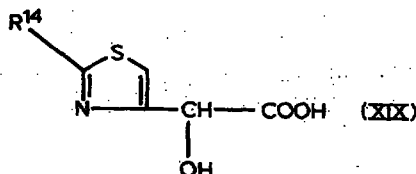


wherein the symbol has the same meaning as defined above or a salt or ester thereof, which comprises reacting a compound of the formula;



wherein the symbol  $\text{R}^5$  has the same meaning as defined above, or a salt or ester thereof, with a diazotizing reagent,

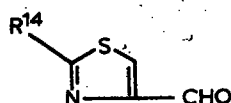
(d) A process for preparing a compound of the formula;



wherein the symbol has the same meaning as defined below, or a salt or ester thereof, which comprises reacting a compound of the formula;



wherein  $\text{R}^{14}$  represents an amino group which may be protected, with a trihalogenoacetone in the presence of a base to obtain a compound of the formula;



(XVIII)

wherein the symbol has the same meaning as defined above, subjecting the compound (XVIII) to an addition reaction with hydrogen cyanide and hydrolyzing the thus obtained product, followed by, if necessary, removal of the protective group.

These intermediates and their preparation are described and claimed in our copending Application No. 51582/75, (Serial No. 1536282).

Similar conditions to those used above are applied also to the reaction between the compounds (VI) and the compounds (IV).

#### (b) Reduction of the compounds (VII)

In the starting compounds (VII), the symbols other than  $R^5$  have the same meanings as defined above, and symbol  $R^5$  means a hydroxyl group which may be protected. As the protective groups of hydroxyl groups, any of the conventional ones may be used so far as they do not disturb the reaction of this invention and there may be generally employed a lower alkyl group such as methyl or ethyl, an aryl group such as phenyl or thienyl or an acyl group such as acetyl or benzoyl.

The reduction conditions employed in the present reaction may be selected from the known reduction methods so far as the compounds (VII) can be reduced into the compounds (I), and preferable ones are the catalytic reduction employing catalysts such as Raney nickel, platinum oxide, palladium-carbon, ruthenium-carbon, rhodium-carbon or copper-chromium oxide, reduction means employing nascent hydrogen obtained by the co-existence of metals such as sodium, sodium amalgam or aluminium amalgam and water or alcohols, reduction means employing metallic hydride complexes such as lithium aluminium hydride, diethyl aluminium hydride, sodium aluminium hydride or sodium borohydride, reduction means employing metals such as zinc or iron, in solvents such as acetic anhydride, formic acid, or aqueous mixtures thereof, or electroreduction. The reaction conditions such as reaction temperature, pressure, nature of solvent, reaction time, are selected suitably according to the sort of starting materials and reduction means. After the reduction reaction, the reaction mixture may be, if necessary, subjected to removal reaction of the protective group directly or after separation of the desired compounds (I). The removal of the protective group is conducted in accordance with the conventional removing methods employed for the removal of each protective group.

#### (c) Reaction of the compounds (IX) with nucleophilic compounds.

In the compounds (IX), the symbol  $R^6$  represents carbamoyloxy, acyloxy such as acetyloxy, propionyloxy, 3-oxobutyryloxy, 3-carboxypropionyloxy, 2-carboxybenzoyloxy, 4-carboxybutyryloxy, mandelyloxy, 2-(carboethoxycarbamoyl)-benzoyloxy, 2-(carboethoxysulfamoyl)benzoyloxy or 3-ethoxycarbamoylpropionyloxy or a halogen such as bromine or chlorine and among them the acyloxy groups are generally employed. The nucleophilic compounds correspond to the compounds having the symbol  $R^4$  of the compounds (I) mentioned above. Among the nucleophilic compounds, the mercapto compounds are each reacted in such forms as the free compound or a salt with an alkali metal such as sodium or potassium. This reaction is desirably conducted in a solvent. For example, use can be made of water, heavy water or organic solvents readily miscible with water and inert to the starting compounds, such as dimethylformamide, dimethylacetamide, dioxane, acetone, alcohol, acetonitrile, dimethylsulfoxide or tetrahydrofuran. The reaction temperature and time depend, among other factors, upon the starting compounds and solvent to be employed but generally the reaction is carried out at a selected temperature within the range of 0 to 100°C for a selected time of a few hours to several days. The reaction is desirably conducted in the neighborhood of neutrality or between pH 2 and 8 and, for better results, between pH 5 and 8. The reaction often proceeds more smoothly by the addition of a quaternary ammonium salt having a surface active effect (e.g. trimethylbenzylammonium bromide, triethylbenzylammonium bromide or triethylbenzylammonium hydroxide) to the reaction system. To prevent oxidation of the mercapto compounds, it is advantageous to carry out the reaction in an inert gaseous atmosphere, e.g. nitrogen gas.

The cephem compounds (I), obtained by the processes illustrated hereinbefore, can be purified by such procedures as column chromatography, extraction, precipita-

tion and recrystallization. The compounds (I), if desired, are further converted to a contemplated salt or ester, by a *per se*-known process.

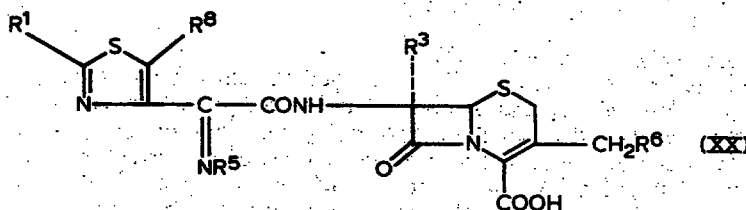
When



represents a group of the formula



the compound (IX) may be represented by the formula;



which may be reacted with nucleophilic compounds under similar conditions to those mentioned above.

The starting compound employed in the above mentioned processes for preparing the cephem compounds (I) can be prepared, for example, by the following processes.

(d) Reduction of the compounds (IV)

Similar reduction conditions as mentioned above in (b) are employed in this reaction. The carboxyl group of the starting compounds (IV) may be protected with a protective group removable under mild conditions which will not interfere with the thiazole ring, for example acid or alkaline conditions or reduction. Therefore, such protective groups may be selected from those of carboxyl groups generally employed in peptide synthesis and are exemplified by alkali metals such as sodium or potassium, alkyl such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl or tert-butyl, substituted alkyl such as  $\beta$ -methylsulfonylethyl, trichloroethyl or diphenylmethyl, aryl such as phenyl or tolyl, substituted aryl such as *p*-tert-butylphenyl or *p*-nitrophenyl, aralkyl such as benzyl, phenethyl or toluenyl (i.e. *o*-, *m*- or *p*-methylbenzyl), or substituted aralkyl such as *p*-methoxybenzyl or *p*-nitrobenzyl. In the present reaction, it seems that the starting compound (IV), wherein R<sup>5</sup> is protected hydroxyl, gives a particularly good result. The contemplated compounds (V) may be purified by a *per se* known purification method such as solvent extraction, pH adjustment, crystallization, recrystallization, distillation, chromatography or ion-exchange chromatography. The isolated compound (V) is a DL-mixture and may be resolved into D-form and L-form by introducing it to a suitable optically-active crystal-forming salt, e.g. a salt with tartaric acid, mandelic acid, malic acid or camphor-sulfonic acid.

(e) Reaction of the compounds (XII) with (XIII)

In the starting compounds (XII), the symbol X means halogen such as chlorine, bromine, iodine or fluorine. When Y represents hydrogen, Z means amino which may be protected, such group being exemplified above. Alternatively, Y and Z together represent a group of the formula; =NR<sup>5</sup>, such group being also illustrated above. The carboxyl group of the compounds (XII) may be protected in a similar manner to that mentioned in respect of the compounds (IV). In the present reaction, it is desirable to react substantially equimolar amounts of the starting compounds (XII) and (XIII). The reaction is generally conducted in a solvent and such solvent may be selected from the organic solvents which will not disturb the contemplated reaction. Thus, methanol, ethanol, propanol and tetrahydrofuran, for example, are suitably employed. The reaction is carried out smoothly at room temperature or under reflux conditions. The reaction is generally completed within one to several hours. The reaction may be carried out more smoothly by adding a base such as N,N-dimethylaniline or triethylamine to the reaction system. After the reaction is completed, the removal of the protecting group may be conducted directly from the reaction mixture or after isolation of the compounds (XIV), if desired. The purification of the compounds (XIV) may be carried out by similar means as mentioned above (d). When the starting

compounds (XIII) wherein  $R^{12}$  represents a lower alkoxy group are employed, there is obtained a contemplated compound (XIV) wherein  $R^1$  is hydroxyl according to the present reaction.

(f) Diazotization of the compounds (XV)

The carboxylic group of the starting compounds (XV) employed in this reaction may be protected in a similar manner as mentioned for the above compounds (IV). The reaction is generally conducted in a solvent, for example, water or a mixture of water and organic solvents which are readily miscible with water and do not disturb the present reaction, such as alcohols, e.g. methanol or ethanol or ethers, e.g. tetrahydrofuran or dioxane. As the diazotization reagents, nitrous acid, alkyl nitrites, nitrogen dioxide and nitrosyl chloride are conveniently employed and, among them, sodium nitrite and amyl nitrite are generally used. The reaction is generally conducted in the presence of an acid at a selected temperature within the range of 0 to 50°C for a selected time of one to several hours. The acid employed in the reaction is exemplified by hydrochloric acid, sulfuric acid, phosphoric acid, formic acid or acetic acid.

While  $\alpha$ -oxymino-2-substituted-thiazol-4-ylacetic acid derivatives (XIV), (XV) and (XVI) may occur theoretically as syn- and anti-isomers with respect to the oxymino group, each or both of the isomers can be used, similarly for the present reaction.

(g) Reaction of the compounds (XVII) and trihalogenoacetone

The symbol  $R^{14}$  represents such amino group which may be protected as illustrated hereinbefore and, therefore, the compounds (XVII) are exemplified by N-(trichloroethoxycarbonyl)thiourea, N-(t-butoxycarbonyl)thiourea and N-(benzyloxycarbonyl)thiourea. As the trihalogenoacetone, there are generally used, for example, 1,1,3-trichloroacetone and 1,1,3-tribromoacetone.

The reaction may be carried out advantageously in a solvent. Any solvent which can dissolve both starting materials may be employed so far as it does not disturb the reaction, and more preferably one is exemplified by alcohols such as methanol, ethanol or propanol; ketones such as acetone or methyl ethyl ketone; ethers such as ether, tetrahydrofuran or dioxan, or mixtures thereof. The present reaction proceeds more smoothly in the presence of a base such as pyridine, picoline, quinoline, isoquinoline, triethylamine, tributylamine, N-methylpiperidine, N-methylmorpholine, N,N-dimethylaniline or N,N-diethylaniline. The reaction may proceed at room temperature and is accelerated by heating. Therefore, it is convenient to heat at about the boiling point of the solvent employed. When suitable conditions are selected in the present reaction, an intermediate, i.e. 4-bromomethylthiazole, may be obtained in some cases.

Thus obtained compounds (XVIII) are subjected to an addition reaction with hydrogen cyanide to give so-called cyanhydrin compounds. For this purpose, cyanides such as sodium cyanide or potassium cyanide, are generally reacted with the compounds (XVIII). The cyanhydrin compounds can be isolated as stable acyl derivatives by protecting with a suitable protective group, e.g. formyl or acetyl. An example of the desirable procedure is to react acetic anhydride with the cyanhydrin compounds in pyridine. The reaction to obtain the cyanhydrin compounds or their derivatives is preferably conducted in a solvent in the presence of a base. The solvent is desired to dissolve both of the starting compounds and base, and water or a mixture of water and an organic solvent miscible with water (e.g. methanol, ethanol, acetone or dimethylformamide) is generally employed. As the base employed, there may be conveniently used, for example, such a weak base as potassium dihydrogen phosphate, sodium hydrogen sulfite or triethylamine. The reaction is carried out advantageously under cooling or in the neighborhood of room temperature to avoid undesirable side reactions. The reaction to obtain the cyanhydrin compounds such as  $\alpha$ -acetoxy-acetonitrile derivatives is usually conducted by reacting acetic anhydride with the former compounds in a solvent in the presence of a base. In this reaction, any solvent so far as it does not disturb the reaction may be employed and there may be generally used, for example, an aprotic solvent such as chloroform, carbon tetrachloride, tetrahydrofuran, pyridine or dimethylformamide, or a mixture thereof. Any base so far as it does not disturb the present reaction can be employed and preferable ones are organic tertiary bases such as pyridine, quinoline, isoquinoline, triethylamine or N,N-dimethylaniline. Among them, pyridine is most preferable because it also works as a solvent. Acetic anhydride mentioned above is most preferable as the acylating agent, but other acylating agents including acetyl chloride may be also employed. The reaction proceeds smoothly under cooling, but, if desired, it may be conducted in the neighborhood of room temperature.

Thus obtained cyanhydrin compounds in the form of their acyl derivatives are subjected to hydrolysis to give the desired compounds (XIX). The hydrolysis is carried out in a solvent in the presence of acid or base. As the solvent, methanol or ethanol is generally used. The reaction is conducted advantageously under cooling or in the neighborhood of room temperature to avoid undesirable side reactions. In the reaction, an inorganic acid such as hydrochloric acid or sulfuric acid is preferably employed as the acid and sodium hydroxide or potassium hydroxide is used as the base.

While the thus obtained  $\alpha$ -hydroxyacetic acid derivative (XIX) is a racemic mixture, it can be resolved into optically active isomers i.e. the D-form and L-form by *per se* known processes, for example, by reacting them with a suitable diastereomer followed by separation.

The following are some examples of the preferable ones of the cephem compounds (I) of the present invention.

- 7 $\beta$ -[ $\alpha$ -Hydroxy- $\alpha$ -(2-aminothiazol-4-yl)acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid  
 7 $\beta$ -[ $\alpha$ -(2-Aminothiazol-4-yl)glycylamido]-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid  
 7 $\beta$ -[ $\alpha$ -(2-Aminothiazol-4-yl)glycylamido]-3-(1,2,3-triazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid  
 7 $\alpha$ -Methoxy-7 $\beta$ -[ $\alpha$ -(2-aminothiazol-4-yl)glycylamido]cephalosporanic acid  
 7 $\alpha$ -Methoxy-7 $\beta$ -[ $\alpha$ -(2-aminothiazol-4-yl)glycylamido]desacetoxycephalosporanic acid  
 7 $\beta$ -[ $\alpha$ -(2-Aminothiazol-4-yl)glycylamido]-3-(5-methyl-1,3,4-thiadiazol-3-ylthiomethyl)-3-cephem-4-carboxylic acid  
 7 $\beta$ -[ $\alpha$ -Hydroxy- $\alpha$ -(2-aminothiazol-4-yl)acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-cephem-4-carboxylic acid.

The invention is further illustrated by the following Examples in which Reference Examples 1 to 7 and some of the Examples illustrate the preparation of the starting materials.

#### Reference Example 1

(1) A solution of 19.3 g. of ethyl  $\alpha$ -oxyimino- $\beta$ -oxo- $\gamma$ -chlorobutyrate and 8.0 g. of thiourea in 200 ml. of ethanol is heated for 2 hours under reflux. The mixture is condensed under reduced pressure and the residue is dissolved in 10% aq. HCl. The solution is washed twice with ether to remove unreacted butyrate and is adjusted to pH 7.0—7.5 with NaHCO<sub>3</sub>. Chloroform extraction of the mixture gives ethyl  $\alpha$ -oxyimino-2-aminothiazol-4-ylacetate. 6.4 g. Melting point: 137—138°C (dec.).

Analysis:—

|                                                                           |                              |
|---------------------------------------------------------------------------|------------------------------|
| Calcd. for C <sub>7</sub> H <sub>9</sub> O <sub>3</sub> N <sub>3</sub> S: | C, 39.06; H, 4.21; N, 19.52. |
| Found:                                                                    | C, 39.64; H, 4.09; N, 19.62. |

IR (Nujol, cm<sup>-1</sup>): 3430 (C=NOH), 1710 (ester).

(2) Ethyl  $\alpha$ -oxyimino-2-aminothiazol-4-yl-acetate (2.15 g.) obtained in the foregoing part is dissolved in a mixture of 20 ml. of 50% aq. formic acid and 10 ml. of methanol. To this is added 1.5 g. of zinc dust and the mixture is stirred for 3 hours under ice-cooling. The filtered reaction mixture is condensed under reduced pressure and the concentrate is passed through a column packed with ion-exchange resin [Amberlite (Registered Trade Mark) IR-120(H)]. The column is washed with water to remove formic acid and then is eluted with 10% aq. ammonia to give 2-aminothiazol-4-yl-glycine. 1.49 g. Recrystallization from aq. ethanol gives pure sample; Melting point: 186—190°C (dec.).

Analysis:—

|                                                                                               |                              |
|-----------------------------------------------------------------------------------------------|------------------------------|
| Calcd. for C <sub>6</sub> H <sub>7</sub> O <sub>2</sub> N <sub>3</sub> S · ½H <sub>2</sub> O: | C, 32.96; H, 4.43; N, 23.06, |
| Found:                                                                                        | C, 32.94; H, 4.61; N, 22.22. |

NMR (ppm, 100 MHz, CF<sub>3</sub>COOD): 5.25 (1H, s, >CH—COOH), 6.75 (1H, s, 5-H).

Violet color with Ninhydrin reagent.

#### Reference Example 2

A solution of 19.3 g. of thiourea and 53.5 g. of ethyl  $\alpha$ -oxyimino- $\beta$ -oxo- $\gamma$ -chloro-

butyrate in 300 ml. of ethanol is stirred for 3 hours at room temperature, and condensed under reduced pressure. Water (200 ml) is added to the residue and the obtained aqueous solution is washed twice with ether. To this are added 130 ml. of 85% aq. formic acid and 150 ml. of ethanol. Under ice-cooling 37 g. of zinc dust is added gradually to this mixture and stirred for 3 hours at room temperature.

The filtered reaction mixture is passed through a column packed with ion-exchange resin (Amberlite IR-120(H)). The column is washed with water and eluted with 10% aq. ammonia to give purified 2-aminothiazol-4-ylglycine (27.5 g.) which is identical with the sample obtained in Reference Example 1 in all respects.

#### Reference Example 3

Ethyl  $\alpha$ -oxyimino-2-aminothiazol-4-ylacetate hydrochloride (503 mg.) is dissolved in 10 ml. of 50% aq. formic acid and 5 ml. of ethanol. Zinc dust (300 mg.) is added to this solution under ice-cooling and stirred for 3 hours. The reaction mixture is condensed under reduced pressure at below 30°C, and the residue is adjusted to pH 7.5 by adding 1N-NaOH. Ethyl acetate extraction gives 2-aminothiazol-4-ylglycine ethyl ester. 130 mg.

NMR (ppm, 60 MHz,  $\text{CF}_3\text{COOD}$ ): 1.04 (3H, t,  $-\text{CH}_2\text{CH}_3$ ), 4.18 (2H, q,  $-\text{CH}_2\text{CH}_3$ ), 5.35 (1H, s,  $>\text{CH}-\text{COOC}_2\text{H}_5$ ), 6.90 (1H, s, 5-H).

Mass: m/e 201,0549 (theoretical: 201,0571).

#### Reference Example 4

To a solution of 26.6 g. of ethyl  $\alpha$ -acetamido- $\beta$ -oxo- $\gamma$ -bromobutyrate in a mixture of 50 ml. of ethanol and 20 ml. of ether are added 9.14 g. of thiourea and 15 ml. of pyridine. The mixture is stirred for 1 hour at room temperature and then for 4 hours under reflux. The reaction mixture is condensed under reduced pressure and 50 ml. of ethyl acetate is added to the residue. The mixture is extracted with 3N-HCl. The water layer thus separated is adjusted to pH 10 by adding 1N-NaOH and extracted with ethyl acetate.

The ethyl acetate extract is washed, dried and condensed. To this concentrate is added a small quantity of chloroform to cause crystallization of ethyl  $\alpha$ -acetamido-2-aminothiazol-4-ylacetate. 7.0 g. Melting point: 161.1°C.

Analysis:—

|                                                                    |                              |
|--------------------------------------------------------------------|------------------------------|
| Calcd. for $\text{C}_8\text{H}_{13}\text{O}_5\text{N}_3\text{S}$ : | C, 44.43; H, 5.39; N, 17.27. |
| Found:                                                             | C, 44.46; H, 5.24; N, 16.99. |

#### Reference Example 5

To a solution of 34.6 g. of ethyl  $\alpha$ -acetamido- $\beta$ -oxo- $\gamma$ -bromobutyrate in a mixture of 50 ml. of ethanol and 20 ml. of ether are added 18.9 g. of N-acetylthiourea and 15 ml. of pyridine. The mixture is heated under reflux for 4 hours, and condensed under reduced pressure. The condensate is extracted with ethyl acetate and the extract is washed with 5% aq.  $\text{NaHCO}_3$  and then with water, and dried. The oily substance which is obtained from the extract by removal of the solvent is purified by silica gel chromatography to give ethyl  $\alpha$ -acetamido-2-acetamidothiazol-4-ylacetate. 4.46 g. Melting point: 148.9–150°C.

Analysis:—

|                                                                                                   |                              |
|---------------------------------------------------------------------------------------------------|------------------------------|
| Calcd. for $\text{C}_{11}\text{H}_{18}\text{O}_4\text{N}_3\text{S} \cdot 1/4\text{H}_2\text{O}$ : | C, 45.59; H, 5.39; N, 14.50. |
| Found:                                                                                            | C, 45.73; H, 5.40; N, 14.21. |

#### Reference Example 6

To a solution of 2.51 g. of N-( $\beta,\beta,\beta$ -trichloroethoxycarbonyl)thiourea and 2.66 g. of ethyl  $\alpha$ -acetamido- $\beta$ -oxo- $\gamma$ -bromobutyrate in 50 ml. of ethanol is added 1.8 g. of N,N-dimethylaniline. The mixture is stirred for 24 hours at room temperature, and condensed under reduced pressure. The residue is dissolved in 30 ml. chloroform and the solution is washed with 3N-HCl, water, and dried. The solid matter which is obtained by removal of chloroform is purified by silica gel chromatography to give ethyl  $\alpha$ -acetamido-2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-ylacetate. 1.43 g. Melting point: 161.9°C.

Analysis:—

|                                                                                                       |                              |
|-------------------------------------------------------------------------------------------------------|------------------------------|
| Calcd. for $\text{C}_{12}\text{H}_{14}\text{O}_5\text{N}_3\text{SCl}_3 \cdot 1/2\text{H}_2\text{O}$ : | C, 33.70; H, 3.54; N, 9.82.  |
| Found:                                                                                                | C, 33.69; H, 3.64; N, 10.06. |

NMR (ppm, 100 MHz,  $d_6$ -DMSO): 1.15 (3H, t,  $-\text{CH}_2\text{CH}_3$ ), 4.09 (2H, q,  $-\text{CH}_2\text{CH}_3$ ), 1.88 (3H, s,  $\text{COCH}_3$ ), 4.96 (2H, s,  $\text{Cl}_2\text{CCH}_2$ ), 5.42 (1H, d,  $>\text{CHCOOC}_2\text{H}_5$ ), 7.13 (1H, s, 5-H).

#### Reference Example 7

To a suspension of 100 mg. of ethyl  $\alpha$ -acetamido-2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-ylacetate in 5 ml of water is added 2 ml. of 1N-NaOH and the mixture is stirred for 2 hours at room temperature. The reaction mixture is washed with ethyl acetate and the water layer is adjusted to pH 2.0 with 1N-HCl, and then extracted with ethyl acetate. The extract is washed, dried, and condensed to give N-acetyl-2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-ylglycine. 65 mg. Melting point: 158.0°C.

Analysis:—

Calcd. for  $\text{C}_{10}\text{H}_{10}\text{O}_6\text{N}_2\text{SCl}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$ : C, 30.05; H, 2.77; N, 10.51.  
Found: C, 30.15; H, 2.52; N, 10.23.

NMR (ppm, 100 MHz,  $d_6$ -DMSO): 1.89 (3H, s,  $\text{COCH}_3$ ), 4.97 (2H, s,  $\text{Cl}_2\text{CCH}_2$ ), 5.40 (1H, d,  $>\text{CHCOOH}$ ), 7.10 (1H, s, 5-H).

#### Example 1

A solution of 237 mg. of  $\alpha$ -( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid and 8 ml. of thionyl chloride is stirred at room temperature for 1.5 hours. The excess thionyl chloride is removed under reduced pressure. To the residue are added 183 mg. of 7-aminocephalosporanic acid and 5 ml. N,N-dimethylacetamide. The mixture is stirred for 4 hours and then 50 ml. of ethyl acetate and saturated aq. NaCl solution are added. The ethyl acetate layer is separated and dried over magnesium sulfate, followed by filtration. The filtrate is concentrated under reduced pressure to give 340 mg. of an oily residue. The residue is dissolved in excess 5% sodium bicarbonate aq. solution and subjected to column chromatography on polystyrene resin (Amberlite XAD-2; manufactured by Rohm & Haas Co., U.S.A.), followed by elution with water. The combined eluate is concentrated to obtain sodium 7 $\beta$ -( $\alpha$ -( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]-acetamido)cephalosporanate.

Analysis:—

Calcd. for  $\text{C}_{21}\text{H}_{19}\text{N}_5\text{O}_{10}\text{S}_2\text{Cl}_3\text{Na} \cdot \frac{1}{2}\text{H}_2\text{O}$ : C, 31.12; H, 2.48.  
Found: C, 30.96; H, 2.19.

NMR (ppm, 100 MHz,  $\text{CF}_3\text{CO}_2\text{D}$ ): 2.25 (3H, s,  $\text{CH}_3\text{CO}$ ), 3.70 (2H, q, 2- $\text{CH}_2$ ), 5.00 (2H, s,  $\text{Cl}_2\text{CCH}_2$ ), 8.12 (1H, s, thiazole ring proton).

#### Example 2

To a solution of 590 mg. of 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)cephalosporanic acid in 30 ml. of 90% aq. formic acid is gradually added 654 mg. of zinc dust under stirring at 0°C and the mixture is stirred for 1.5 hours at 0°C. The insoluble materials are filtered off and washed with 50% aq. formic acid. The filtrate and washings are concentrated under reduced pressure. The residue is dissolved in 20 ml. of water and hydrogen sulfide gas is bubbled through the solution for 20 minutes and insoluble material is filtered off. The filtrate is lyophilized to afford 380 mg. of the crude product, which is dissolved in 5% sodium bicarbonate aq. solution and purified with a column packed with Amberlite XAD-2 (manufactured by Rohm & Haas Co., U.S.A.) to obtain sodium 7 $\beta$ -(2-aminothiazol-4-ylglycylamido)cephalosporanate.

Analysis:—

Calcd. for  $\text{C}_{16}\text{H}_{16}\text{N}_5\text{O}_6\text{S}_2\text{Na} \cdot 3\text{H}_2\text{O}$ : C, 35.78; H, 4.40; N, 13.90.  
Found: C, 35.22; H, 4.03; N, 13.74.

NMR (ppm, 100 MHz,  $\text{D}_2\text{O}$ ): 2.25 (3H, s,  $\text{CH}_3\text{CO}$ ), 3.66 (2H, q, 2- $\text{CH}_2$ ), 5.26 (1H, d, 6-H), 5.30 (1H, s, CH), 5.75 (1H, d, 7-H), 6.88 (1H, s, thiazole ring proton).

#### Example 3

A solution of 1.40 g. of  $\alpha$ -( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid and 25 ml. of thionyl chloride

is stirred at room temperature for 2 hours. The excess thionyl chloride is removed under reduced pressure. To the residue are added 1.10 g. of 7-aminocephalosporanic acid and 25 ml. of N,N-dimethylacetamide and the mixture is stirred for 5 hours at room temperature. To the reaction mixture are added 250 ml. of ethyl acetate and saturated aq. NaCl solution. The ethyl acetate layer is separated, washed with water and dried over magnesium sulfate. The solvent is distilled off under reduced pressure and the oily residue is dissolved in 100 ml. of 90% formic acid. The solution is cooled to 0°C, followed by gradual addition of 5.0 g. of zinc dust and stirring for 3 hours at 0°C. The insoluble materials are filtered off and the filtrate is concentrated under reduced pressure. To the residue is added 30 ml. of water and hydrogen sulfide gas is passed through the aqueous solution for five minutes. The resulting insoluble materials are filtered off and the filtrate is concentrated under reduced pressure. The residue is dissolved in 5% sodium bicarbonate aq. solution and purified with a column packed with polystyrene resin (Amberlite XAD-2; manufactured by Rohm & Haas Co., USA) in the same manner as described in Example 1, to obtain sodium 7β-(2-aminothiazol-4-ylglycylamido)cephalosporanate.

Analysis: —

Calcd. for  $C_{18}H_{16}N_4O_7S_2Na \cdot 2H_2O$ : C, 37.11; H, 4.15.  
Found: C, 37.09; H, 3.93.

Example 4

To a solution of 4.0 g. of α-hydroxy-α-[2-(β,β,β-trichloroethoxycarbonylamino)-thiazol-4-yl]acetic acid in 40 ml. of tetrahydrofuran is introduced phosgene gas for 10 minutes at 0°C. Excess phosgene is removed by bubbling nitrogen gas at 40°C. The solvent is removed under reduced pressure to give 4.6 g. of powdered cyclic carboxyanhydride.

Then 500 mg. of 7-aminocephalosporanic acid is suspended in 18 ml. of N,N-dimethylacetamide and 690 mg. of the cyclic carboxyanhydride is added under stirring. After stirring for 1 hour, 100 ml. of ethyl acetate is added and the ethyl acetate layer is separated, washed with water and extracted with 5% sodium hydrogen carbonate aq. solution. The extract is acidified to pH 2.0 with 1N-hydrochloric acid. The acid solution is again extracted with ethyl acetate, and the ethyl acetate extract is washed with saturated aq. NaCl solution and dried over magnesium sulfate. The solvent is removed under reduced pressure to give 854 mg. of an oily residue, which is dissolved in 5% sodium bicarbonate aq. solution and purified with a column packed with polystyrene resin (Registered trade mark, Amberlite XAD-2; manufactured by Rohm & Haas Co., USA) in the same manner as described in Example 1, to obtain sodium 7β-(α-hydroxy-α-[2-(β,β,β-trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)cephalosporanate.

Analysis: —

Calcd. for  $C_{18}H_{16}N_4O_7S_2Cl_3Na \cdot 2\frac{1}{2}H_2O$ : C, 32.22; H, 3.16; N, 8.35.  
Found: C, 32.16; H, 3.06; N, 7.84.

NMR (ppm, 100 MHz,  $CF_3CO_2D$ ): 2.24 (3H, s,  $CH_3CO$ ), 3.70 (2H, q, 2- $CH_2$ ), 4.98 (2H, s,  $Cl_3CCH_2$ ), 5.22 (2H, q, 3- $CH_2$ ), 5.28 (1H, d, 6-H), 5.88 (1H, d, 7-H), 5.72 (1H, s, CH), 7.48 (1H, s, thiazole ring proton).

Example 5

To a solution of 745 mg. of 7β-(α-hydroxy-α-[2-(β,β,β-trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)cephalosporanic acid in 30 ml. of 90% formic acid is gradually added 800 mg. of zinc dust with stirring at 0°C and the mixture is stirred for 2 hours at 0°C. The insoluble materials are filtered off and washed with 10 ml. of 50% formic acid. The filtrate and washings are concentrated under reduced pressure. The residue is dissolved in 5% sodium bicarbonate aq. solution and the insoluble materials are filtered off. The filtrate is purified with a column packed with polystyrene resin (Amberlite XAD-2; manufactured by Rohm & Haas Co., USA) to obtain sodium 7β-(α-hydroxy-α-(2-aminothiazol-4-yl)acetamido)cephalosporanate.

Analysis: —

Calcd. for  $C_{18}H_{16}N_4O_7S_2Na \cdot 2H_2O$ : C, 37.04; H, 3.94; N, 11.52.  
Found: C, 36.70; H, 3.66; N, 11.86.

NMR (ppm, 100 MHz,  $CF_3CO_2D$ ): 2.24 (3H, s,  $CH_3CO$ ), 3.70 (2H, q, 2- $CH_2$ ), 5.23 (2H, q, 3- $CH_2$ ), 5.32 (1H, d, 6-H), 5.85 (1H, d, 7-H), 5.56 (1H, s, CH), 6.92 (1H, s, thiazole ring proton).

## Example 6

To a suspension of 1.17 g. of  $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid in 20 ml. of water is added 3 ml. of 1N-sodium hydroxide aq. solution. The resulting solution is lyophilized to give the sodium salt. To a suspension of the sodium salt in 30 ml. of benzene are added 889 mg. of oxalyl chloride and 1 drop of N,N-dimethylacetamide and the mixture is stirred for 1 hour at room temperature. The solvent is removed under reduced pressure and the residue is dissolved in 20 ml. of acetone.

The above solution of the acid chloride in acetone is dropwise added to a solution of 817 mg. of 7-aminocephalosporanic acid and 630 mg. of sodium hydrogen carbonate in 50 ml. of water and 25 ml. of acetone while stirring for 30 minutes at 0°C. The mixture is stirred for an additional 2 hours at room temperature. Acetone is distilled off under reduced pressure and the aqueous layer is washed with ethyl acetate and acidified to pH 2.0 with 3N-hydrochloric acid. The product is extracted with ethyl acetate and the combined extracts are washed with water and dried over anhydrous magnesium sulfate. Concentration of the extracts affords 970 mg. of an oily residue, 330 mg. of which is dissolved in 5% sodium bicarbonate solution, subjected to column chromatography on polystyrene resin (Amberlite XAD-2; manufactured by Rohm & Haas Co., USA) and elution is carried out with 50% aqueous ethanol. The fractions containing the contemplated compound are pooled and lyophilized to obtain 184 mg. of sodium 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)cephalosporanate.

## Analysis:—

Calcd. for  $C_{20}H_{19}N_5O_8S_2Cl_3Na \cdot H_2O$ : C, 35.07; H, 3.09; N, 10.23.  
Found: C, 35.24; H, 3.18; N, 10.33.

NMR (ppm, 100 MHz,  $CF_3CO_2D$ ): 1.50 (3H, t,  $CH_3CH_2$ ), 2.25 (3H, s,  $CH_3CO$ ), 3.37 (2H, q, 2- $CH_2$ ), 4.61 (2H, q,  $CH_2CH_3$ ), 4.99 (2H, s,  $Cl_3CCH_2$ ), 5.26 (2H, q, 3- $CH_2$ ), 5.34 (1H, d, 6-H), 6.06 (1H, d, 7-H), 7.95 (1H, s, thiazole ring proton).

## Example 7

To a suspension of 390 mg. of  $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid in 5 ml. of benzene are added 300 mg. of oxalyl chloride and 1 drop of N,N-dimethylformamide and the mixture is stirred for 2 hours at room temperature. The solvent is removed under reduced pressure and the residue is dissolved in 10 ml. of acetone. The acetone solution is dropwise added while stirring for 30 minutes to a solution of 272 mg. of 7-aminocephalosporanic acid and 252 mg. of sodium hydrogen carbonate in 20 ml. of water and 10 ml. of acetone at 0°C. The mixture is stirred for an additional 2 hours at room temperature and the solvent is distilled off under reduced pressure. The residual aqueous solution is washed with ethyl acetate and acidified to pH 2.0 with 1N-hydrochloric acid. The solution is extracted with ethyl acetate and the combined extracts are washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent affords 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]-acetamido)cephalosporanic acid. This product is identical with the compound obtained in Example 6 in NMR spectrum (in  $CF_3CO_2D$ ).

## Example 8

To a suspension of 390 mg. of  $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid in 10 ml. of methylene chloride is added with stirring 312 mg. of phosphorus pentachloride.

The mixture become homogeneous while stirring within 10 seconds. After stirring for an additional hour at room temperature, the solvent is distilled off under reduced pressure and the residue is dissolved in 5 ml. of acetone. The acetone solution is dropwise added for 30 minutes to a solution of 272 mg. of 7-aminocephalosporanic acid and 840 mg. of sodium bicarbonate in 10 ml. of water and 5 ml. of acetone at 0°C. The mixture is stirred for an additional 2 hours at room temperature and acetone is removed under reduced pressure. The residual aqueous solution is washed with ethyl acetate and acidified to pH 2.0 with 1N hydrochloric acid. The solution is extracted with ethyl acetate and the extracts are washed with water and dried over anhydrous magnesium sulfate. Removal of the solvent gives 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)cephalosporanic acid. This product is identical with the compound obtained in Example 6 in NMR spectrum (in  $CF_3CO_2D$ ).

## Example 9

To a suspension of 347 mg. of  $\alpha$ -oxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid in 20 ml. of water is added 2 ml. of 1N-sodium hydroxide solution. The solution is lyophilized to give the sodium salt. To a suspension of the sodium salt in 10 ml. of benzene are added 300 mg. of oxalyl chloride and 1 drop of N,N-dimethylacetamide and the mixture is stirred for 1 hour at room temperature. The solvent is distilled off under reduced pressure and the residue is dissolved in 10 ml. of acetone. The acetone solution is dropwise added to a solution of 261 mg. of 7-amino-cephalosporanic acid and 200 mg. of sodium bicarbonate in 10 ml. of water under stirring at 0°C in the course of 10 minutes. The mixture is stirred for an additional 2 hours at room temperature. Acetone is distilled off under reduced pressure and the aqueous solution is washed with ethyl acetate and acidified to pH 2.0 with 1N-hydrochloric acid. The product is extracted with ethyl acetate and the combined extracts are washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent affords 7 $\beta$ -( $\alpha$ -oxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)cephalosporanic acid.

NMR (ppm, 100 MHz, CDCl<sub>3</sub> + d<sub>6</sub>-DMSO): 2.00 (3H, s, CH<sub>3</sub>CO), 3.46 (2H, q, 2-CH<sub>2</sub>), 4.85 (2H, s, Cl<sub>3</sub>CCH<sub>2</sub>), 4.96 (2H, q, 3-CH<sub>2</sub>), 5.06 (1H, d, 6-CH), 5.89 (1H, q, 7-CH), 7.39 (1H, s, thiazole ring proton), 9.26 (1H, d, 7-NH).

## Example 10

A solution of 900 mg. of  $\alpha$ -( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid and 15 ml. of thionyl chloride is stirred at room temperature for 1.5 hours. The excess thionyl chloride is removed under reduced pressure. To the oily residue are added 700 mg. of 7-aminocephalosporanic acid and 15 ml. of N,N-dimethylacetamide and the mixture is stirred for 4 hours at room temperature. The reaction mixture is added to 100 ml. of ethyl acetate and then is washed with saturated aq. NaCl solution. The ethyl acetate layer is separated and dried over magnesium sulfate. The solvent is removed under reduced pressure to give an oily residue, which is dissolved in 50 ml. of 90% formic acid. The solution is cooled to 0°C and 2.0 g. of zinc dust is gradually added. The mixture is stirred for 1.5 hours at 0°C. The insoluble materials are filtered off and the filtrate is concentrated under reduced pressure. To the residue is added 20 ml. of water and then hydrogen sulfide gas is passed through the aqueous solution. The resulting insoluble materials are filtered off and the filtrate is concentrated under reduced pressure. The residue is dissolved in 5% sodium bicarbonate aq. solution and purified with a column packed with polystyrene resin (Amberlite XAD-2; manufactured by Rohm & Haas Co., USA) in the same manner described in Example 1, to obtain sodium 7 $\beta$ -(2-aminothiazol-4-ylglycylamido)-cephalosporanate. This product is identical with the compound obtained in Example 2 in all respects.

## Example 11

To a suspension of 1.563 g. of  $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid in 20 ml. of methylene chloride is added 1.250 g. of phosphorus pentachloride while stirring. The mixture is stirred for 1 hour at room temperature and concentrated under reduced pressure. The residue is dissolved in 20 ml. of acetone. The acetone solution thus obtained is dropwise added to a solution of 857 mg. of 7-amino-desacetoxy cephalosporanic acid and 1.68 g. of sodium hydrogen carbonate in 40 ml. of water and 20 ml. of acetone at 0°C under stirring in the course of 30 minutes. The mixture is stirred for 2 hours at room temperature and acetone is distilled off under reduced pressure. The residual aqueous solution is washed with ethyl acetate, acidified to pH 2.0 with 1N-hydrochloric acid and extracted with ethyl acetate. The combined extracts are washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent affords 2.04 g. 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)desacetoxycephalosporanic acid. Yield 86.9%.

NMR (ppm, 100 MHz, CDCl<sub>3</sub> + d<sub>6</sub>-DMSO): 1.26 (3H, t, CH<sub>3</sub>CH<sub>2</sub>), 2.13 (3H, s, 3-CH<sub>2</sub>), 3.40 (2H, q, 2-CH<sub>2</sub>), 4.23 (2H, q, CH<sub>2</sub>CH<sub>2</sub>), 4.86 (2H, s, Cl<sub>3</sub>CCH<sub>2</sub>), 5.06 (1H, d, 6-H), 5.80 (1H, q, 7-H), 7.26 and 7.83 (1H, two s, thiazole ring proton).

573 mg. of the acid obtained in the above method is dissolved in 5% sodium bicarbonate aq. solution, subjected to column chromatography on polystyrene resin,

Amberlite XAD-2 (manufactured by Rohm & Haas Co., USA) and eluted with 50% aqueous ethanol. The fractions containing the contemplated compound are pooled and lyophilized to obtain 233 mg. of sodium 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta$ , $\beta$ , $\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)desacetoxycephalosporanate. Yield 39.2%.

## 5 Analysis: —

Calcd. for  $C_{18}H_{17}N_5O_7S_2Cl_3Na \cdot H_2O$ : C, 34.49; H, 3.06; N, 11.17. 5

Found: C, 34.96; H, 3.43; N, 11.17.

## Example 12

To a solution of 1.467 g. of 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -[2-( $\beta$ , $\beta$ , $\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido)desacetoxycephalosporanic acid in 80 ml. of 90% aq. formic acid is gradually added 1.634 g. of zinc dust at 0°C and the mixture is stirred for 1.5 hours at 0°C. The insoluble material is filtered off and washed with 50% aq. formic acid. The filtrate and washings are concentrated under reduced pressure and 200 ml. of water is added to the residue. Hydrogen sulfide gas is bubbled through the aqueous solution and insoluble material is filtered off. The filtrate is lyophilized to yield 1.15 g. of the formate of 7 $\beta$ -(2-aminothiazol-4-ylglycylamido)desacetoxycephalosporanic acid. Yield 100%. The formate is dissolved in 5% sodium bicarbonate aq. solution and purified with a column packed with Amberlite XAD-2 (manufactured by Rohmn & Haas Co., (USA) to afford 614 mg. of sodium 7 $\beta$ -(2-aminothiazol-4-ylglycylamido)desacetoxycephalosporanate. Yield 62.7%.

## Analysis: —

Calcd. for  $C_{18}H_{14}N_6O_7S_2Na \cdot 1\frac{1}{2}H_2O$ : C, 37.31; H, 4.10; N, 16.74.

Found: C, 37.81; H, 4.24; N, 16.69.

NMR (ppm, 100 MHz,  $D_2O$ ): 2.05 (3H, s, 3-CH<sub>3</sub>), 3.48 (2H, q, 2-CH<sub>2</sub>), 5.13 (1H, s, CH), 5.18 (1H, d, 6-H), 5.79 (1H, d, 7-H), 6.99 (1H, s, thiazole ring proton).

## Example 13

A mixture of 650 mg. of  $\alpha$ -ethoxyimino- $\alpha$ -(2-hydroxythiazol-4-yl)acetic acid and 750 mg. of phosphorus pentachloride in 50 ml. of dry ether is stirred for 2 hours at room temperature. The acid chloride solution is added to a suspension of 1.10 g. of 7-aminocephalosporanic acid in 30 ml. of N,N-dimethylacetamide and the mixture is stirred for 3.5 hours at room temperature. To the mixture is added 200 ml. of ethyl acetate and the organic solution is washed 7 times with water. The ethyl acetate layer is concentrated under reduced pressure and the residue is dissolved in 5% sodium bicarbonate aq. solution and purified with a column packed with Amberlite XAD-2 to afford sodium 7 $\beta$ -( $\alpha$ -ethoxyimino- $\alpha$ -(2-hydroxythiazol-4-yl)acetamido)-cephalosporanate.

## Analysis: —

Calcd. for  $C_{17}H_{17}N_4O_8S_2Na \cdot 3H_2O$ : C, 37.36; H, 4.24; N, 10.25.

Found: C, 37.37; H, 3.90; N, 9.86.

NMR (ppm, 100 MHz,  $CF_3CO_2D$ ): 1.44 (3H, t, CH<sub>3</sub>CH<sub>2</sub>), 2.21 (3H, s, CH<sub>3</sub>CO), 3.70 (2H, q, 2-CH<sub>2</sub>), 4.48 (2H, q, CH<sub>2</sub>CH<sub>3</sub>), 5.25 (2H, q, 3-CH<sub>2</sub>), 5.30 (1H, d, 6-H), 6.05 (1H, d, 7-H), 7.13 (1H, s, thiazole ring proton).

## Example 14

To a suspension of 1.08 g. of  $\alpha$ -ethoxyimino- $\alpha$ -(2-hydroxythiazol-4-yl)acetic acid in 20 ml. of absolute ether is added 1.25 g. of phosphorus pentachloride and the mixture is stirred for 2 hours at room temperature. The acid chloride solution is dropwise added to a suspension of 1.80 g. of 7-aminocephalosporanic acid in 50 ml. of N,N-dimethylacetamide and the mixture is stirred for 4 hours at room temperature. The mixture is extracted 3 times with portions of 120 ml. of ethyl acetate and the combined extracts are washed with water and dried over anhydrous magnesium sulfate. The solvent is removed and the residue is dissolved in 60 ml. of 90% aq. formic acid. To the acidic solution is added 4.30 g. of zinc dust at 0°C and the mixture is stirred for 2 hours at this temperature.

Insoluble material is filtered off and the filtrate is concentrated under reduced pressure. To the residue is added 20 ml. of water and hydrogen sulfide gas is bubbled through the aqueous solution for 5 minutes. Insoluble material is filtered off and the filtrate is again concentrated. The residue is dissolved in 5% sodium bicarbonate aq.

solution and purified with a column packed with Amberlite XAD-2. The fractions obtained by elution with 5% aqueous ethanol are lyophilized to afford sodium 7 $\beta$ -(2-hydroxythiazol-4-ylglycylamido)-cephalosporanate.

Analysis: —

5 Calcd. for  $C_{15}H_{18}N_4O_7S_2Na \cdot H_2O$ : C, 38.46; H, 3.66; N, 11.96.  
Found: C, 38.35; H, 4.04; N, 12.28. 5

EXAMPLE 15

10 To a suspension of 2.4 g. of 7-amino-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid in 20 ml. of dimethylacetamide is added 2.4 g. of  $\alpha$ -hydroxy-2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-ylacetic acid cyclic carboxy anhydride (obtained by the procedure described in Example 4) and the mixture is stirred for 15 hours at room temperature. Ethyl acetate (100 ml) is added to the reaction mixture and the resulting mixture is filtered. The filtrate is extracted several times with 5% aq.  $NaHCO_3$ . The combined extract is adjusted to pH 3.0 with 10% aq. HCl and 15 extracted with ethyl acetate. The ethyl acetate layer is washed with saturated aq. NaCl and dried. Evaporation of the solvent gives 7 $\beta$ -[ $\alpha$ -hydroxy-2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido]-3-(1-methyl-1H-tetrazol-5-ylthio-20 methyl)-3-cephem-4-carboxylic acid (1.8 g.) as a jelly which is then dissolved in 100 ml of 90% aq. formic acid under ice-cooling. To this solution is added 1.8 g. of zinc dust and the resulting mixture is stirred for 1 hour under ice-cooling. 20

The reaction mixture is filtered and condensed under reduced pressure. Water (100 ml.) is added to the condensate and  $H_2S$  gas is bubbled into the aqueous solution to precipitate zinc sulfide. Zinc sulfide is filtered off and the filtrate is condensed under reduced pressure and the concentrate is dissolved in 5% aq.  $NaHCO_3$  solution and the solution is passed through a column packed with polystyrene resin (Amberlite XAD-2) to give purified 7 $\beta$ -[ $\alpha$ -hydroxy- $\alpha$ -(2-aminothiazol-4-yl)acetamido]-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid sodium salt. 25

Analysis: —

30 Calcd. for  $C_{16}H_{18}O_8N_5S_3Na \cdot 3H_2O$ : C, 32.14; H, 3.78; N, 19.99.  
Found: C, 32.53; H, 3.77; N, 19.50. 30

NMR (ppm, 100 MHz,  $D_2O$ ): 3.71 (2H, q, 2- $CH_2$ ), 4.14 (3H, s, N- $CH_3$ ), 5.28 (1H, s,  $>CH-CONH$ ), 5.22 (1H, d, 6-H), 5.75 (1H, d, 7-H), 6.84 (1H, s, 5-H).

Example 16

35 To a solution of 100.8 mg. of  $NaHCO_3$  and 127.6 mg. of 5-mercapto-1-methyl-1H-tetrazole in 10 ml. of water is added 486 mg. of sodium 7 $\beta$ -[ $\alpha$ -hydroxy-(2-aminothiazol-4-yl)acetamido]cephalosporanate. The mixture is stirred at 55°C for 20 hours. After cooling the mixture is passed through a column packed with polystyrene resin (Amberlite XAD-2) to give purified sodium 7 $\beta$ -[ $\alpha$ -hydroxy- $\alpha$ -(2-aminothiazol-4-yl)-40 acetamido]-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylate, which is identical with the sample obtained in Example 15 in all respects. 40

Example 17

45 To a suspension of 781 mg. of  $\alpha$ -ethoxyimino-2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetic acid in 15 ml. of  $CH_2Cl_2$  is added 625 mg. of phosphorus pentachloride. The mixture is homogenized by stirring for 10 seconds. After stirring at room temperature for 1 hour, the solvent is removed under reduced pressure and the residue is dissolved in 10 ml. of acetone. On the other hand 7-amino-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (657 mg.) and  $NaHCO_3$  (1.68 g.) are dissolved in a mixture of 20 ml. of water and 10 ml. of acetone. To this solution while ice-cooling, the foregoing acetone solution is added dropwise in a period of 30 minutes. After the dropping, the mixture is stirred at room temperature for 2 hours, and condensed under reduced pressure to remove acetone. The concentrate is washed with ethyl acetate, adjusted to pH 2.0 with 1N-HCl and extracted with ethyl acetate. The ethyl acetate extract is washed with water, dried over anhydrous magnesium sulfate and condensed to give 7 $\beta$ -[ $\alpha$ -ethoxyimino-50 2-( $\beta,\beta,\beta$ -trichloroethoxycarbonylamino)thiazol-4-yl]acetamido]-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid. 925 mg. Yield 660%. 55

NMR (ppm, 100 MHz  $\text{CF}_3\text{COOD}$ ): 1.50 (3H, t,  $\text{CH}_3\text{CH}_2$ —), 4.60 (2H, q, — $\text{CH}_2\text{CH}_3$ ), 3.85 (2H, q, 2- $\text{CH}_2$ ), 4.12 (3H, s, N- $\text{CH}_3$ ), 4.98 (2H, s,  $\text{Cl}_2\text{CCH}_2$ —), 5.38 (1H, d, 6-H), 6.02 (1H, q, 7-H), 7.91 (1H, s, 5-H).

#### Example 18

Sodium 7 $\beta$  - { $\alpha$  - ethoxyimino - [2 - ( $\beta,\beta,\beta$  - trichloroethoxycarbonylamino)-thiazol - 4 - yl]acetamido}cephalosporanate (667 mg.)  $\text{NaHCO}_3$  (90 mg.) and 5-mercapto-1-methyl-1H-tetrazole (120 mg.) are dissolved to 20 ml. of water. The mixture is stirred at 60°C for 8 hours and filtered. The filtrate is adjusted to pH 2.0 with 1N-HCl and the solid is separated. The separated solid is filtered, washed with water and dried over phosphorus pentachloride under reduced pressure to give 7 $\beta$  - { $\alpha$  - ethoxyimino - [2 - ( $\beta,\beta,\beta$  - trichloroethoxycarbonylamino)thiazol - 4 - yl]-acetamido} - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid, (238 mg.) which is identical with the sample obtained in Example 17 in all respects.

#### Example 19

To a suspension of 2.92 g. of  $\alpha$  - ethoxyimino - [2 - (chloroacetamido)thiazol - 4 - yl]acetic acid in 50 ml. of methylene chloride is added 2.08 g. of phosphorus pentachloride. The mixture is stirred for 2 hours at room temperature, after which time methylene chloride is distilled off and the residue is dissolved in 30 ml. of acetone. The acetone solution is added dropwise under ice-cooling in a period of 15 minutes to a solution of 3.29 g. of 7 - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid and 8.4 g. of sodium bicarbonate in a mixture of 100 ml. of water and 50 ml. of acetone. After the addition has been completed, the obtained solution is stirred at room temperature for 2 hours and concentrated under reduced pressure to remove acetone. The concentrate is washed with ethyl acetate and adjusted to pH 2.0 with 3N hydrochloric acid under ice-cooling, followed by extracting with ethyl acetate. The ethyl acetate layer is washed with water and dried over anhydrous magnesium sulfate, followed by distillation of the solvent to give 3.80 g. of 7 $\beta$  - { $\alpha$  - ethoxyimino - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido} - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. Yield 63.1%.

NMR (ppm, 60 MHz,  $\text{d}_6$ -DMSO): 1.34 (3H, t,  $\text{CH}_3\text{CH}_2$ —), 3.56 (2H, broad s, 2- $\text{CH}_2$ ), 3.95 (3H, s, N- $\text{CH}_3$ ), 4.05—4.50 (4H, m,  $\text{CH}_3\text{CH}_2$ — and 3- $\text{CH}_2$ ), and 4.24 (2H, s,  $\text{ClCH}_2\text{CO}$ ), 5.04 (1H, d, 6-H), 5.70 (1H, d, 7-H), 7.82 (1H, s, thiazole ring proton).

IR(KBr,  $\text{cm}^{-1}$ ): 1760 ( $\beta$ -lactam), 1035 ( $=\text{N}-\text{O}-\text{C}$ ).

#### Example 20

To a solution of 3.90 g. of sodium 7 $\beta$  - { $\alpha$  - ethoxyimino - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido} - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate in 50 ml. of water is added 1.52 g. of thiourea. The mixture is stirred at room temperature for 3 hours and adjusted to pH 2.0 with 1N hydrochloric acid. The resulting precipitates are filtered off washed with water and dried to obtain 2.40 g. of 7 $\beta$  - [ $\alpha$  - ethoxyimino - (2 - aminothiazol - 4 - yl)-acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. Yield 73.1%.

NMR (ppm, 60 MHz,  $\text{d}_6$ -DMSO): 1.26 (3H, t,  $\text{CH}_3\text{CH}_2$ —), 3.84 (3H, s, N- $\text{CH}_3$ ), 3.90—4.40 (4H, m,  $\text{CH}_3\text{CH}_2$ — and 3- $\text{CH}_2$ ), 5.02 (1H, d, 6-H), 5.70 (1H, d, 7-H), 7.94 (1H, s, thiazole ring proton).

IR (KBr,  $\text{cm}^{-1}$ ): 1770 ( $\beta$ -lactam), 1030 ( $=\text{N}-\text{O}-\text{C}$ ).

#### Example 21

2.0 g. of 7 $\beta$  - [ $\alpha$  - ethoxyimino - (2 - aminothiazol - 4 - yl)acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid is dissolved in 50 ml. of 90% formic acid. To the solution is added 2.48 g. of zinc dust under stirring and cooling at the temperature of -10°C, and the mixture is further stirred for 15 minutes. After the reaction has been completed, the insoluble materials are filtered off. Into the filtrate is poured ether to precipitate a white solid, which is centrifugally separated and washed three times with ether and dried (yield; 1.66 g.). This white solid is dissolved in an aq. solution of sodium bicarbonate. The solution is subjected to purification using a column of Amberlite XAD-2 and the fractions eluted with water are lyophilized to obtain sodium 7 $\beta$  - [(2 - aminothiazol -

4 - yl)glycylamido) - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate.

NMR (ppm, 100 MHz, D<sub>2</sub>O): 3.94 (3H, S, N-CH<sub>3</sub>), 6.74 (1H, S, thiazole ring proton).

IR (KBr, cm<sup>-1</sup>): 1760 (β-lactam).

UV (H<sub>2</sub>O, nm): 260 (cephem).

#### Example 22

(1) To a suspension of 6.25 g. of phosphorus pentachloride in 45 ml. of anhydrous methylene chloride, cooled at -10°C, is added 20 ml. of anhydrous methylene chloride solution containing 2.4 g. of pyridine and the mixed solution is stirred for 30 minutes. To the solution is dropwise added a solution of 9.3 g. of 7β-(2-thienylacetamido)-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid benzhydryl ester in 30 ml. of anhydrous methylene chloride during 30 minutes under stirring and keeping at -20°C to -10°C. The mixture is further stirred for 2 hours at the same temperature and then cooled to -30°C to -20°C, followed by adding dropwise 56 ml. of methanol. Thus obtained solution is stirred for 30 minutes, keeping the temperature of the solution between -5°C and 5°C, and 60 ml. of water is added dropwise thereto, followed by further stirring for 30 minutes. The methylene chloride layer is washed with saturated aq. NaCl solution and concentrated. To the concentrate is added a mixture of water and ethyl acetate under stirring to give 4.746 g. of 7β - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid benzhydryl ester as crystals. Yield 64.0%.

(2) To a suspension of 2.05 g of α - ethoxyimino - α - [2 - (chloroacetamido)-thiazol - 4 - yl]acetic acid in 50 ml. of methylene chloride is added 1.50 g. of phosphorus pentachloride under cooling. The mixture is stirred for 2 hours at room temperature and subjected to distillation of methylene chloride. The residue is dissolved in 20 ml. of tetrahydrofuran. The tetrahydrofuran solution is dropwise added while stirring to a solution of 2.92 g. of 7β - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid benzhydryl ester, dissolved in 50 ml. of absolute methylene chloride, followed by the addition of 2.87 g. of pyridine under ice-cooling. After the addition has been completed and the temperature of the reaction solution becomes the same as room temperature, the solution is stirred for 2 hours and then concentrated. The residue is dissolved in a mixture of 100 ml. of ethyl acetate and a little amount of tetrahydrofuran. Thus obtained solution is washed with dilute hydrochloric acid, sodium bicarbonate aq. solution and water in this order, dried over anhydrous magnesium sulfate and concentrated. The concentrate is purified by means of a column of silica gel. The fractions eluted with the mixture of ethyl acetate and chloroform (1:1) are concentrated to obtain 2.36 g. of 7β - {α - ethoxyimino - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido} - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid benzhydryl ester. Yield 50%.

NMR (ppm, 60 MHz, CDCl<sub>3</sub>): 1.34 (3H, t, CH<sub>3</sub>CH<sub>2</sub>-), 3.68 (2H, broad S, 2-CH<sub>2</sub>), 3.80 (3H, S, N-CH<sub>3</sub>), 4.26 (2H, S, ClCH<sub>2</sub>CO), 5.04 (1H, d, 6-H), 5.92 (1H, q, 7-H), 6.94 (1H, S, -CHPh<sub>2</sub>), 7.20-7.60 (10H, m, Ph<sub>2</sub>), 7.90 (1H, S, thiazole ring proton), 8.45 (1H, d, 7-NH).

#### Example 23

To a solution of 2.2 g. of 7β - {α - ethoxyimino - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido} - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid benzhydryl ester in 20 ml. of tetrahydrofuran is added a solution of 550 mg. of thiourea in 20 ml of ethanol, followed by addition of 50 mg. of triethylbenzylammonium bromide. The mixture is stirred for 20 hours at room temperature and concentrated. The residue is dissolved in a mixture of ethyl acetate and tetrahydrofuran and the solution is washed with aq. sodium bicarbonate solution and then water, followed by drying over anhydrous magnesium sulfate. The dried solution is subjected to distillation of the solvent to give a powder which is washed with chloroform and ether and dried. The procedure yields 1.047 g. of 7β - [α - ethoxyimino - (2 - aminothiazol - 4 - yl)acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid benzhydryl ester. Yield 52.9%.

NMR (ppm, 60 MHz, CDCl<sub>3</sub>+d<sub>6</sub>-DMSO (3:1)): 1.38 (3H, t, CH<sub>3</sub>CH<sub>2</sub>-), 3.78 (2H, broad S, 2-CH<sub>2</sub>), 3.90 (3H, S, N-CH<sub>3</sub>), 4.30 (2H, broad S,

3-CH<sub>3</sub>), 4.38 (2H, q, CH<sub>2</sub>CH<sub>3</sub>), 5.14 (1H, d, 6-H), 6.00 (1H, d, 7-H), 6.88 (1H, s, CHPh<sub>2</sub>), 7.20—7.60 (10H, m, Ph<sub>2</sub>), 7.48 (1H, s, thiazole ring proton).

## Example 24

To a solution of 750 mg of 7 $\beta$  - [ $\alpha$  - ethoxyimino - (2 - aminothiazol - 4 - yl)-acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid benzhydryl ester in 10 ml. of 90% formic acid is added 654 mg. of zinc dust, with cooling to -10°C. After the mixture is stirred for 20 minutes at -10°C and further for 10 minutes at room temperature, the insoluble material is filtered off. The filtrate is neutralized with aq. sodium bicarbonate solution and extracted with ethyl acetate. The ethyl acetate layer is washed with water and dried over anhydrous magnesium sulfate, after which time the solvent is distilled off to give 465 mg. of foamy materials. All of the foamy materials are dissolved in a mixture of 2 ml. of trifluoroacetic acid and 0.5 ml. of anisole and stirred for 30 minutes under ice-cooling and then for 5 minutes at room temperature. The thus obtained solution is concentrated, and onto the residue is poured ether to precipitate a solid. The solid is washed twice with ether and dried to obtain 320 mg. of solid product. This solid product is dissolved in an excess amount of aq. sodium bicarbonate solution and purified with a column of Amberlite XAD-2. The fractions eluted with water are lyophilized to obtain sodium 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate. This product is identical with the compound obtained in Example 21 in all respects.

## Example 25

To a suspension of 6.0 g. of 7 $\alpha$  - methoxy - 7 $\beta$  - amino - desacetoxycephalosporanic acid *t*-butyl ester in 180 ml. of N,N-dimethylacetamide is added under stirring 8.25 g. of powdered 2 - ( $\beta,\beta,\beta$  - trichloroethoxycarbonylamino)thiazol - 4 - yl -  $\alpha$  - hydroxyacetic acid cyclic carboxyanhydride and then the resulting mixture is further stirred for one hour. The reaction mixture is shaken well with 500 ml. of ethyl acetate and the organic layer is washed with water, 5% sodium bicarbonate aq. solution and saturated aq. NaCl solution in this order, followed by drying over anhydrous magnesium sulfate. The ethyl acetate layer is subjected to distillation of ethyl acetate to give 10.2 g. of crude 7 $\alpha$  - methoxy - 7 $\beta$  - [2 - ( $\beta,\beta,\beta$  - trichloroethoxycarbonylamino)thiazol - 4 - yl -  $\alpha$  - hydroxyacetamido] - desacetoxycephalosporanic acid *t*-butyl ester as an oil. To a solution of this oily product in 400 ml. of 90% formic acid is added 10 g. of zinc dust under cooling and stirring and the mixture is reacted for 2 hours. After insoluble materials are filtered off, the filtrate is added to 200 ml. of saturated NaCl aq. solution, and the resultant solution is extracted with ethyl acetate. The ethyl acetate layer is washed with water and dried, followed by distillation of ethyl acetate to obtain 5.3 g. of crude 7 $\alpha$  - methoxy - 7 $\beta$  - [(2 - aminothiazol - 4 - yl) -  $\alpha$  - hydroxyacetamido] - desacetoxycephalosporanic acid *t*-butyl ester as an oil.

This oily product is added to a mixture of 25 ml. of trifluoroacetic acid and 25 ml. of anisole under ice-cooling, and the mixture is stirred for 30 minutes under the same conditions. Onto the reaction mixture is poured 200 ml. of absolute ether, and resulting precipitates are collected by means of filtration. The precipitates are dissolved in 30 ml. of 5% sodium bicarbonate aq. solution, and the thus obtained solution is passed through a column of Amberlite XAD-2, followed by elution with water. The procedure yields 525 mg. of sodium 7 $\alpha$  - methoxy - 7 $\beta$  - [(2 - aminothiazol - 4 - yl) -  $\alpha$  - hydroxyacetamido] - desacetoxycephalosporanate as a powder.

## Analysis:—

Calcd. for C<sub>14</sub>H<sub>15</sub>O<sub>6</sub>N<sub>4</sub>S<sub>2</sub>Na · 2H<sub>2</sub>O: C, 36.67; H, 4.17; N, 12.22.  
Found: C, 36.23; H, 4.38; N, 11.97.

NMR (ppm, 100 MHz, CF<sub>3</sub>COOD): 2.15 (3H, s, 3-CH<sub>3</sub>), 3.45 (3H, s, 7-OCH<sub>3</sub>), 6.25 (1H, s, thiazole ring proton).

## Example 26

To a suspension of 555.4 mg. of  $\alpha$  - methoxyimino -  $\alpha$  - [2 - (chloroacetamido)-thiazol - 4 - yl]acetic acid in 5 ml. of methylene chloride is added 416.3 mg. of phosphorus pentachloride under ice-cooling. The resultant solution is stirred for 30 minutes, and *n*-hexane is added thereto to precipitate 620 mg. of  $\alpha$  - methoxyimino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetyl chloride hydrochloric acid salt.

Analysis: —

Calcd. for  $C_8H_7N_3O_3SCl_2 \cdot HCl$ : C, 28.89; H, 2.42; N, 12.63.

Found: C, 28.35; H, 2.81; N, 12.00.

5.26 g. of the above-mentioned salt is added under ice-cooling to a solution of 2.66 g. of pyridine and 4 g. of 7 - aminocephalosporanic acid *t*-butyl ester in 60 ml. of methylene chloride. After stirring for 1 hour at room temperature, 60 ml. of chloroform is added to the reaction mixture and the resultant solution is washed twice with 0.5N hydrochloric acid and then with water. After drying, the solution is subjected to distillation to obtain 5 g. of a white powder of 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido] - cephalosporanic acid *t*-butyl ester. Melting point: 126—127°C.

Analysis: —

Calcd. for  $C_{22}H_{26}N_6O_5S_2Cl$ : C, 44.93; H, 4.46; N, 11.91.

Found: C, 44.74; H, 4.64; N, 11.61.

NMR (ppm, 60 MHz,  $CDCl_3$ ): 1.50 (9H, S,  $t-C_4H_9$ ), 2.10 (3H, S,  $CH_3CO$ ), 4.10 (3H, S,  $OCH_3$ ), 4.28 (2H, S,  $ClCH_2CO$ ), 7.84 (1H, S, thiazole ring proton).

Example 27

5 g. of 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido]cephalosporanic acid *t*-butyl ester, obtained in Example 26, 970.5 mg. of thiourea and 250 mg. of triethylbenzylammonium bromide are dissolved in a mixture of 25 ml. of ethanol and 500 ml. of tetrahydrofuran. The solution is stirred at room temperature overnight. The reaction mixture is poured into 100 ml. of 10% sodium bicarbonate aq. solution and extracted with ethyl acetate. The ethyl acetate layer is separated and concentrated. The oily concentrate is subjected to column chromatography in a column packed with silica gel to obtain 2.23 g. of 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido]cephalosporanic acid *t*-butyl ester as a powder.

NMR (ppm, 60 MHz,  $CDCl_3$ ): 1.54 (9H, S,  $t-C_4H_9$ ), 2.08 (3H, S,  $CH_3CO$ ), 4.12 (3H, S,  $OCH_3$ ), 7.45 (1H, S, thiazole ring proton).

This powder is dissolved in a mixture of 1.6 ml. of anisole and 16 ml. of trifluoroacetic acid and the solution is stirred for 2 hours at room temperature, followed by the addition of 200 ml. of a mixture of ether and hexane (10:1) to precipitate 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido]cephalosporanic acid trifluoroacetic acid salt which is collected by filtration and washed with ether. Yield 1.45 g.

NMR (ppm, 60 MHz,  $CF_3COOH$ ): 1.85 (3H, S,  $CH_3CO$ ), 4.00 (3H, S,  $OCH_3$ ), 7.74 (1H, S, thiazole ring proton).

Example 28

A solution of 450 mg. of 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido]cephalosporanic acid trifluoroacetic acid salt and 170 mg. of sodium bicarbonate in 5 ml. of water is passed through a column of Amberlite XAD-2 and eluted with water. The procedure yields 141 mg. of sodium 7 $\beta$  - [ $\alpha$  - methoxy -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido]cephalosporanate. Melting point: 162—163°C (dec.).

Analysis: —

Calcd. for  $C_{16}H_{16}N_6O_7S_2Na \cdot 2H_2O$ : C, 37.43; H, 3.93; N, 13.64

Found: C, 37.10; H, 4.13; N, 13.34

NMR (ppm, 100 MHz,  $D_2O$ ): 2.17 (3H, S,  $CH_3CO$ ), 4.13 (3H, S,  $OCH_3$ ), 7.58 (1H, S, thiazole ring proton).

Example 29

7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido]cephalosporanic acid trifluoroacetic acid salt is dissolved in a solution of 272 mg. of 1-methyl-5-mercapto-1H-tetrazole, 555 mg. of sodium bicarbonate and 68 mg. of triethylbenzyl-ammonium bromide in 10 ml. of water. The solution is heated at 60°C in a

nitrogen atmosphere for 6 hours. After cooling, the reaction solution is passed through a column of Amberlite XAD-2 and eluted with water and then with 2.5% ethanol. The procedure yields sodium 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate. Melting point: 174—175°C (dec.).

Analysis: —

Calcd. for  $C_{16}H_{16}N_6O_5S_2Na \cdot 2H_2O$ : C, 33.74; H, 3.54; N, 22.13.  
Found: C, 34.25; H, 3.81; N, 21.69.

NMR (ppm, 100 MHz,  $D_2O$ ): 4.10 (3H, S, N-CH<sub>3</sub>), 4.14 (3H, S, OCH<sub>3</sub>), 7.58 (1H, S, thiazole ring proton).

The above-mentioned procedure gives a small amount of sodium 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - 3 - desacetylcephalosporanate as a by-product.

Melting point: 195—196°C (dec.).

Analysis: —

Calcd. for  $C_{14}H_{14}N_6O_5S_2Na \cdot 3H_2O$ : C, 34.35; H, 4.11; N, 14.30.  
Found: C, 34.43; H, 4.13; N, 13.14.

NMR (ppm, 60 MHz,  $D_2O$ ): 4.04 (3H, S, OCH<sub>3</sub>), 7.46 (1H, S, thiazole ring proton), 3.52 (2H, q, 2-CH<sub>2</sub>).

Example 30

To a suspension of 3.4 g. of 7 - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid in 25 ml. of dimethylacetamide is added 2.0 g. of  $\alpha$  - methoxyimino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetyl chloride hydrochloric acid salt under stirring. After further stirring for 12 hours at room temperature, the reaction mixture is poured into water and extracted with ethyl acetate. The ethyl acetate extract is washed with water and dried and subjected to distillation of the solvent. To the residue is added ethyl acetate and insoluble materials are filtered off. The filtrate is concentrated under reduced pressure to obtain as crude product 1.114 g. of 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid as an oil. The oily product is dissolved in 20 ml. of a mixture of ethanol and tetrahydrofuran (1:1) and to the solution is added 430 mg. of thiourea, followed by stirring for 15 hours at room temperature. The reaction solution is concentrated to dryness under reduced pressure and to the residue is added 10 ml. of water, followed by stirring to precipitate insoluble materials. The insoluble material is collected by filtration and dissolved in 10% sodium bicarbonate aq. solution. The solution is passed through a column of Amberlite XAD-2, and eluted with water and then with 2.5 ethanol to obtain sodium 7 $\beta$  - [ $\alpha$  - methoxyimino -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate. This product is identical with the compound obtained in Example 29 in all respects.

Example 31

To a suspension of 349 mg. of  $\alpha$  - t - butyloxycarbonylamino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetic acid in 5 ml. of methylene chloride is added 249 mg. of phosphorus pentachloride, and the mixture is stirred at room temperature. The mixture is added dropwise under stirring to a solution of 494 mg. of 7 $\beta$  - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid diphenylmethyl ester in 5 ml. of methylene chloride, followed by the addition of 474 mg. of pyridine. After that, stirring is continued for a further hour at room temperature. The reaction solution is washed with 0.5N hydrochloric acid and then with water, and dried. The oily product obtained by distillation of the solvent is purified by column chromatography in a column packed with silica gel. The procedure gives 513 mg. of 7 $\beta$  - [ $\alpha$  - t - butyloxycarbonylamino -  $\alpha$  - [2 - (chloroacetamido)thiazol - 4 - yl]acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid diphenylmethyl ester.

To a solution of 407 mg. of this product in 40 ml. of a mixture of tetrahydrofuran and ethanol (1:1) is added 152 mg. of thiourea, and the mixture is stirred for 15 hours at room temperature. The reaction solution is condensed under reduced pressure and the residue is dissolved in ethyl acetate. The ethyl acetate solution is

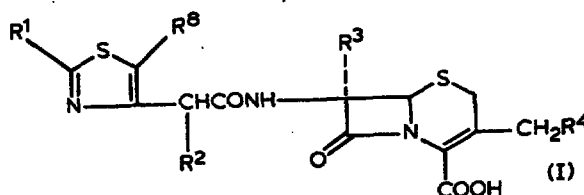
washed with water and then dried. The oily product obtained by the distillation of the solvent is dissolved in 5 ml. of a mixture of trifluoroacetic acid and anisole (10:1), and the solution is stirred for 2 hours at room temperature, followed by pouring into 50 ml. of ether to precipitate a crystalline product. The crystalline product is collected by filtration to give 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid trifluoroacetic acid salt. This product is dissolved in 5% sodium bicarbonate aq. solution and the solution is passed through a column of Amberlite XAD-2, followed by the elution with water. The procedure gives 103 mg. of sodium 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate. This product is identical with the compound obtained in Example 21 in all respects.

#### Example 32

To a suspension of 752 mg. of  $\alpha$  - (2 - chloroacetamidothiazol - 4 - yl) - N - chloroacetylglycine in 10 ml. of methylene chloride is added 499 mg. of phosphorus pentachloride. The mixture is homogenized under stirring at room temperature. The homogenized mixture is added to a suspension of 600 mg. of 7 $\beta$  - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid in 10 ml. of dimethylacetamide and the resultant mixture is stirred for 5 hours at room temperature. The reaction mixture is poured into ice-water and the mixture is made acidic with 10% hydrochloric acid, followed by extraction with ethyl acetate. After being washed with water and dried, the extract is subjected to distillation to give crude 7 $\beta$  - [(2 - chloroacetamidothiazol - 4 - yl) - N - chloroacetylglycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid as an oil. The crude product is dissolved in 100 ml. of ethanol and to the solution is added 456 mg. of thiourea, followed by stirring for 15 hours at room temperature. Ethanol is distilled off under reduced pressure and the residue is dissolved in 5% sodium bicarbonate aq. solution, which is passed through a column of Amberlite XAD-2, followed by elution with water to obtain 113 mg. of sodium 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. This product is identical with the compound obtained in Example 21 in all respects.

#### WHAT WE CLAIM IS:—

1. A cephem compound of the formula (I):



wherein R<sup>1</sup> represents an amino or hydroxyl group which may be protected; R<sup>2</sup> represents an amino or a hydroxyl group which may be protected, or



represents a group of the formula



where R<sup>3</sup> is a hydroxyl group which may be protected; R<sup>3</sup> represents hydrogen or a methoxy group; R<sup>4</sup> represents hydrogen or a residue of a nucleophilic compound; and R<sup>8</sup> represents hydrogen or a halogen atom, or a pharmaceutically acceptable salt or ester thereof.

2. A cephem compound of the formula:



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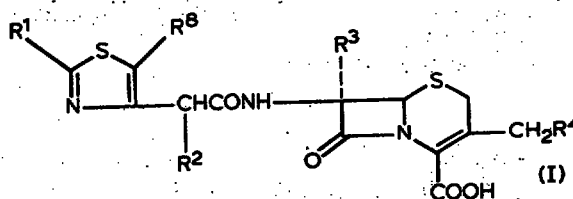
washed with water and then dried. The oily product obtained by the distillation of the solvent is dissolved in 5 ml. of a mixture of trifluoroacetic acid and anisole (10:1), and the solution is stirred for 2 hours at room temperature, followed by pouring into 50 ml. of ether to precipitate a crystalline product. The crystalline product is collected by filtration to give 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid trifluoroacetic acid salt. This product is dissolved in 5% sodium bicarbonate aq. solution and the solution is passed through a column of Amberlite XAD-2, followed by the elution with water. The procedure gives 103 mg. of sodium 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylate. This product is identical with the compound obtained in Example 21 in all respects.

#### Example 32

To a suspension of 752 mg. of  $\alpha$  - (2 - chloroacetamidothiazol - 4 - yl) - N - chloroacetyl glycine in 10 ml. of methylene chloride is added 499 mg. of phosphorus pentachloride. The mixture is homogenized under stirring at room temperature. The homogenized mixture is added to a suspension of 600 mg. of 7 $\beta$  - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid in 10 ml. of dimethylacetamide and the resultant mixture is stirred for 5 hours at room temperature. The reaction mixture is poured into ice-water and the mixture is made acidic with 10% hydrochloric acid, followed by extraction with ethyl acetate. After being washed with water and dried, the extract is subjected to distillation to give crude 7 $\beta$  - [(2 - chloroacetamidothiazol - 4 - yl) - N - chloroacetyl glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid as an oil. The crude product is dissolved in 100 ml. of ethanol and to the solution is added 456 mg. of thiourea, followed by stirring for 15 hours at room temperature. Ethanol is distilled off under reduced pressure and the residue is dissolved in 5% sodium bicarbonate aq. solution, which is passed through a column of Amberlite XAD-2, followed by elution with water to obtain 113 mg. of sodium 7 $\beta$  - [(2 - aminothiazol - 4 - yl)glycylamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid. This product is identical with the compound obtained in Example 21 in all respects.

#### WHAT WE CLAIM IS:—

1. A cephem compound of the formula (I):



wherein R<sup>1</sup> represents an amino or hydroxyl group which may be protected; R<sup>2</sup> represents an amino or a hydroxyl group which may be protected, or

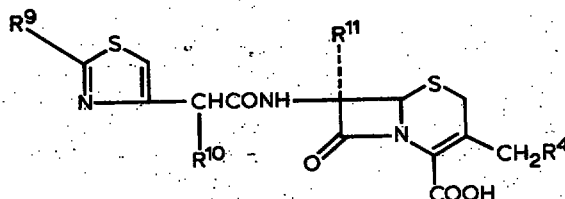


represents a group of the formula



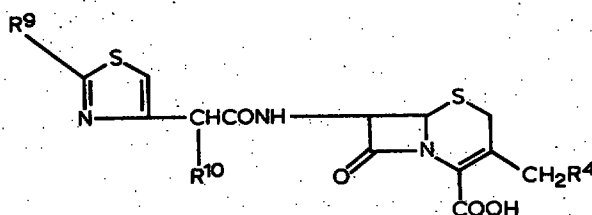
where R<sup>5</sup> is a hydroxyl group which may be protected; R<sup>3</sup> represents hydrogen or a methoxy group; R<sup>4</sup> represents hydrogen or a residue of a nucleophilic compound; and R<sup>8</sup> represents hydrogen or a halogen atom, or a pharmaceutically acceptable salt or ester thereof.

2. A cephem compound of the formula:



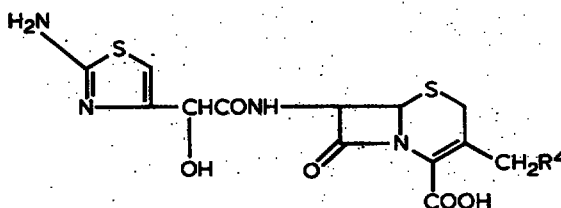
wherein  $\text{R}^4$  represents hydrogen or a residue of a nucleophilic compound, each of  $\text{R}^9$  and  $\text{R}^{10}$  represents an amino or a hydroxyl group, and  $\text{R}^{11}$  represents hydrogen or a methoxy group, or a pharmaceutically acceptable salt thereof.

3. A cephem compound of the formula:



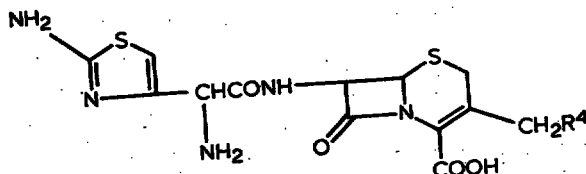
wherein  $\text{R}^4$  represents hydrogen or a residue of a nucleophilic compound and each of  $\text{R}^9$  and  $\text{R}^{10}$  represents an amino or hydroxy group, or a pharmaceutically acceptable salt thereof.

4. A cephem compound of the formula:



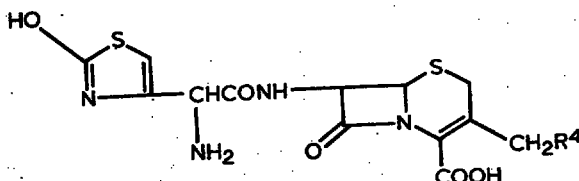
wherein  $\text{R}^4$  represents hydrogen or a residue of a nucleophilic compound, or a pharmaceutically acceptable salt thereof.

5. A cephem compound of the formula:



wherein  $\text{R}^4$  represents hydrogen or a residue of a nucleophilic compound, or a pharmaceutically acceptable salt thereof.

6. A cephem compound of the formula:



wherein  $\text{R}^4$  represents hydrogen or a residue of a nucleophilic compound, or a pharmaceutically acceptable salt thereof.

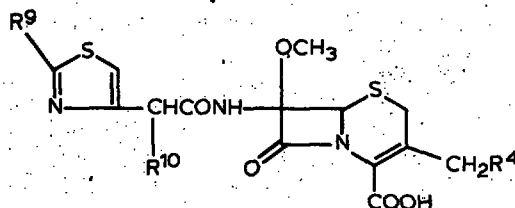
7. A cephem compound of the formula:



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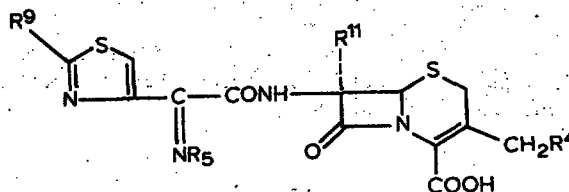
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wherein  $\text{R}_4$  represents hydrogen or a residue of a nucleophilic compound and each of  $\text{R}^9$  and  $\text{R}^{10}$  represents an amino or hydroxyl group, or a pharmaceutically acceptable salt thereof.

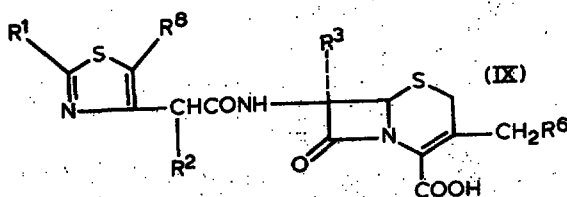
8. A cephem compound of the formula:



wherein  $\text{R}^4$  represents hydrogen or a residue of a nucleophilic compound,  $\text{R}^5$  represents hydroxyl or a  $\text{C}_1$ — $\text{C}_2$  alkoxy group,  $\text{R}^9$  represents an amino or hydroxyl group and  $\text{R}^{11}$  represents hydrogen or a methoxy group, or a pharmaceutically acceptable salt thereof.

9. A cephem compound as claimed in any of Claims 1 to 8, wherein  $\text{R}^4$  represents hydrogen or an acetoxy, thiadiazolylthio, triazolylthio or tetrazolylthio group which may be substituted with a  $\text{C}_1$ — $\text{C}_3$  alkyl group.

10. A cephem compound of the formula:



wherein  $\text{R}^1$  represents an amino or hydroxyl group which may be protected,  $\text{R}^2$  represents an amino or hydroxyl group which may be protected, or



represents a group of the formula



wherein  $\text{R}^5$  is a hydroxyl group which may be protected,  $\text{R}^3$  represents hydrogen or a methoxy group  $\text{R}^4$  represents an acyloxy or carbamoyloxy group, and  $\text{R}^6$  represents hydrogen or a halogen, or a pharmaceutically acceptable salt or ester thereof.

11. A cephem compound as claimed in claim 10, wherein  $\text{R}^1$  represents an amino group,



represents a group of the formula



wherein  $\text{R}^5$  is a hydroxyl group which may be protected, and  $\text{R}^3$  and  $\text{R}^6$  represent hydrogen.

12. A cephem compound as claimed in claim 11, wherein  $\text{R}^5$  represents a methoxy group.

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13. 7 $\beta$  - [ $\alpha$  - (2 - aminothiazol - 4 - yl) -  $\alpha$  - methoxyimino - acetamido] - 3 - desacetylcephalosporanic acid.

14. 7 $\beta$  - [ $\alpha$  - Hydroxy -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

15. 7 $\beta$  - [ $\alpha$  - (2 - Aminothiazol - 4 - yl)glycylamido]cephalosporanic acid.

16. 7 $\beta$  - [ $\alpha$  - (2 - Hydroxythiazol - 4 - yl)glycylamido] - cephalosporanic acid.

17. 7 $\alpha$  - Methoxy - 7 $\beta$  - [ $\alpha$  - hydroxy -  $\alpha$  - (2 - aminothiazol - 4 - yl)acetamido] - desacetoxycephalosporanic acid.

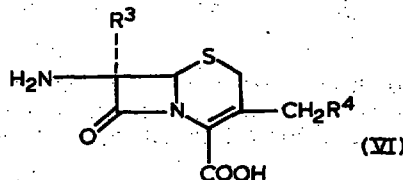
18. 7 $\beta$  - [ $\alpha$  - (2 - Aminothiazol - 4 - yl) -  $\alpha$  - methoxyiminoacetamido] - 3 - (1 - methyl - 1H - tetrazol - 5 - ylthiomethyl) - 3 - cephem - 4 - carboxylic acid.

19. 7 $\beta$  - [ $\alpha$  - (2 - aminothiazol - 4 - yl) -  $\alpha$  - methoxyiminoacetamido]cephalosporanic acid.

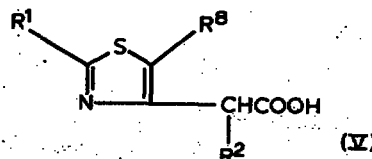
20. A cephem compound as claimed in Claim 1, substantially as hereinbefore described with reference to any of the Examples.

21. A process for preparing a cephem compound of the formula (I) as defined in claim 1 or a pharmaceutically acceptable salt or ester thereof, which comprises

(1) reacting a compound of the formula:

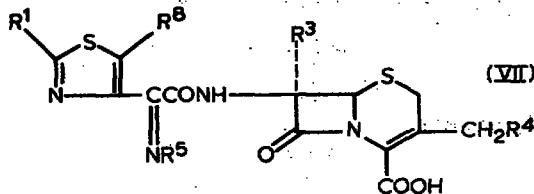


wherein R<sup>3</sup> and R<sup>4</sup> are as defined in claim 1, or a salt or ester thereof, with a compound of the formula:



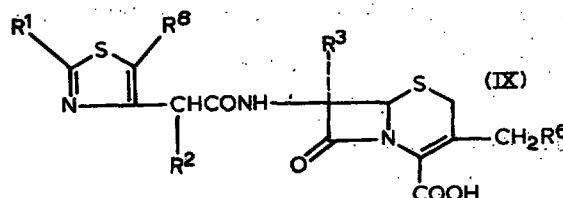
wherein R<sup>1</sup>, R<sup>2</sup> and R<sup>8</sup> are as defined in claim 1, or a salt or reactive derivative thereof, followed by, if necessary, removal of the protective group;

(2) when R<sup>2</sup> is amino, reducing a compound of the formula:



wherein R<sup>1</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>6</sup> are as defined in claim 1, or a salt or ester thereof, followed by, if necessary, removal of the protective group; or

(3) when R<sup>4</sup> is other than hydrogen, reacting a compound of the formula:



wherein R<sup>6</sup> represents acyloxy, carbamoyloxy or a halogen and R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>8</sup> are as defined in claim 1, or a salt or ester thereof, with a nucleophilic compound, followed by, if necessary, removal of the protective group.

22. A process for preparing a cephem compound as claimed in claim 21, substantially as herein described with reference to the specific Examples.



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23. A cephem compound when prepared by a process as claimed in claim 21 or 22.

24. A pharmaceutical composition which contains a medicinally effective amount of a cephem compound of the formula (I), as defined in claim 1, or a pharmaceutically acceptable salt or ester thereof, together with at least one pharmaceutically acceptable carrier therefor.

25. A pharmaceutical composition as claimed in claim 24, which is provided in a unit dosage form.

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