

PATENT SPECIFICATION

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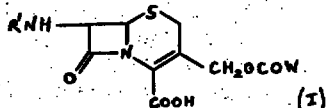


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(54) 3-ACYLOXYMETHYL-CEPHEM COMPOUNDS

(71) We, TAKEDA YAKUHIN KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES LIMITED, of 27, Doshomachi 2-chome, Higashi-ku, Osaka, Japan, a Corporation 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:—

The present invention relates to novel 3 - acyloxymethyl - cephem compounds and preparations thereof. More particularly, this invention relates to compounds of the formula (I):



in which R¹ is hydrogen or an acyl group, and W is acetonyl or a group represented by —X—COOH or —X—OH (X is an organic residue), or pharmaceutically acceptable salts thereof,

and also relates to processes for producing them.

Cephalosporin derivatives with a 3 - hydroxymethyl moiety have only been obtainable by enzymatic cleavage of the 3 - acetyl group in 3 - acetoxymethyl - cephalosporins, or by separation from a cephalosporin C fermentation by-product. Recently, it has become possible to produce 7 - (D - 5 -

amino - 5 - carboxyvaleramido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid (desacetylcephalosporin C, DCPC) in high titer by fermentation (U.S. Patent No. 3,926,726, Nature New Biology, 246, 154 (1963)) and, in parallel with cephalosporin C, this substance has been attracting attention as a starting material for cephalosporin compounds which could be more potent antibiotic activity.

There has, however, been a teaching in the art that the 3 - hydroxymethyl group of the 3 - hydroxymethyl compound (cephalosporadetic acid) could only be acylated with difficulty. For example, Heyningen [Van Heyningen: J. Med. Chem., 8 22 (1965), Advan. Drug. Res., 4 28 (1968)] reported that the O - acylation of cephalosporadetic acid was feasible only with the use of a large excess of aroyl chloride (yield 32%—57%); and that the use of ketene, aliphatic acid chloride or acetic anhydride did not cause O - acylation, or induced lactonization. Kukolha [J. Med. Chem. 13, 1114 (1970)] reported a roundabout process for the synthesis of O - acyloxymethylcephalosporins which comprised O - acylating a 3 - hydroxymethyl - 2 - cephem compound and then causing the latter to become isomerized to the 3 - cephem compound. U.S. Patent No. 3,532,694 and Japanese Patent Publication No. 33080/1975 disclosed a process in which, to prevent the lactonization reaction, the 4 - carboxyl group of cephalosporadetic acid is first protected, for example, by esterification, and the O - acylation is then carried out. A process is

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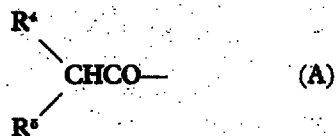
proposed in Japanese Patent Application (As-Laid-Open) No. 42792/1972 which comprises O - acylating cephalosporadetic acid with azolide. However, these processes are not commercially practicable because, for example, they provide only low yields, or involve troublesome and time-consuming procedures, or require expensive reagents. Thus, for example, the esterification reaction of cephalosporadetic acid cannot be accomplished by an ordinary esterification process in which the rearrangement of the double bond or the lactonization predominates. While it is possible to introduce such limited groups as, e.g., methyl, ethyl, diphenylmethyl or benzyl, by means of diazo compounds such as diazomethane, diazoethane, diphenyldiazomethane or phenyldiazomethane, for example, it is difficult, after 3 - acylation, to deesterify the compound without the accompaniment of some side-reaction such as a fission of the β - lactam ring or a shift of the double bond.

On the other hand, the reaction by which the 3 - acetoxymethyl group of a cephalosporin compound is substituted with a nucleophilic reagent entails a concurrent decomposition of the starting material, intermediate and product in its course, and a protracted reaction time, and therefore, results in lower yields [A. B. Taylor, J. Chem. Soc., 7020 (1965)]. Thus, it has been desired to have available a derivative possessing a group which will lend itself more readily to substitution than the acetoxy group.

We have now found that the use as an acylating agent of diketene or of compound [III] or [IV] (hereinafter described) allows cephalosporadetic acid to be O - acylated in a high yield and that the resulting O - acylated cephalosporin undergoes with ease the desired substitution with a nucleophilic compound. This invention has been developed on the basis of the latter findings.

The above compounds [I] include compounds in which the substituent R^1 in the 7 position is hydrogen, phenylacetyl, phenoxyacetyl or 5 - amino - 5 - carboxyvaleryl (whose amino or/and carboxyl groups may optionally be protected), or any of the groups found in the 6 - or 7 - positions of naturally occurring or synthetic penicillin or cephalosporin derivatives, as the case may be. Thus, for example, the acyl group R^1 may be chosen from among aliphatic acyl groups such as formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, cyclopentylcarbonyl, cyclohexylcarbonyl, cycloheptylcarbonyl, cyclopentyl acetyl or cyclohexadienyl acetyl; aromatic acyl groups such as benzoyl, *p* - nitrobenzoyl, toluoyl or naphthoyl; mono-substituted aliphatic acyl groups such as 2 - thienylacetyl, cyanoacetyl, acetoacetyl, 4 - chloro - 3 - oxobutyryl, 4 - bromo - 3 - oxobutyryl, chloroacetyl, bromoacetyl, 4 - methylthio - 3 - oxobutyryl, 4 - carbamoylmethylthio - 3 - oxo-

butyryl, α - phenoxypropionyl, α - phenoxybutyryl, tetrazolylthioacetyl, tetrazolylacetyl, *p* - nitrophenylacetyl, trifluoromethylthioacetyl, trifluoromethylsulphonylacetyl, trifluoromethylsulphonylacetyl, cyanomethylthioacetyl, thiadiazolylthioacetyl, *p* - nitrophenylacetyl, (2 - pyridyloxy) - acetyl, (2 - oxo - 4 - thiazolin - 4 - yl) - acetyl, (2 - imino - 4 - thiazolin - 4 - yl) - acetyl, (2 - thioxo - 4 - thiazolin - 4 - yl) - acetyl, 4 - pyridylthioacetyl, (3 - sydnone) - acetyl, 1 - pyrazolylacetyl, 2 - furylacetyl, (2 - oxo - 3 - methylpyridazinyl) - thioacetyl, (2 - aminomethylphenyl) - acetyl or (2 - aminomethylcyclohexenyl) - acetyl; di-substituted aliphatic acyl groups such as α - carboxyphenylacetyl, mandelyl, α - sulphophenylacetyl, α - sulpho - (*p* - aminophenyl) acetyl, phenylglycyl, (4 - hydroxyphenyl glycyl, (4 - methylthiophenyl) - glycyl, (4 - methoxyphenyl) - glycyl, (4 - methane - sulphinylphenyl) - glycyl, (3 - methanesulphonamidophenyl) - glycyl, - 1 - cyclohexenylglycyl, thienylglycyl, furylglycyl, cyclohexadienylglycyl or (3,4 - dihydroxyphenyl) - glycyl; 5 - methyl - 3 - phenyl - 4 - isoxazolylcarbonyl; and 3 - (2,6 - dichlorophenyl) - 5 - methyl - 4 - isoxazolylcarbonyl. It should be understood that the above-mentioned groups are only illustrative of the acyl groups that are of use for the purposes of this invention, but preferred acyl groups may be represented by the formula (A):

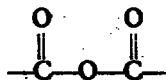


in which R^4 represents a group such as, for example, acetyl, halogenoacetyl, phenyl, *p* - hydroxyphenyl, thienyl, 2 - imino - 4 - thiazolin - 4 - yl, 2 - oxo - 4 - thiazolin - 4 - yl, tetrazolyl, phenoxy or 3 - amino - 3 - carboxypropyl; and R^5 represents a substituent such as, for example, hydrogen, sulpho, amino or hydroxy.

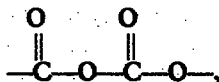
It should also be understood that any functional groups, e.g. amino or/and carboxyl, in such acyl groups may be suitably protected. Suitable protective groups for the amino groups include, for example, aromatic acyl groups such as phthaloyl, benzoyl, *o* - carboxybenzoyl, *p* - nitrobenzoyl, toluoyl, naphthoyl, *p* - tert - butylbenzoyl, *p* - tert - butylbenzenesulphonyl, phenylacetyl, benzenesulphonyl, phenoxyacetyl, toluenesulphonyl or chlorobenzoyl; aliphatic acyl groups such as acetyl, valeryl, capryl, *n* - decanoyl, acryloyl, pivaloyl, camphorsulphonyl, methanesulphonyl or chloroacetyl; esterified carboxyl groups such as tert - butoxycarbonyl, ethoxycarbonyl, isobornyloxycarbonyl, phenyloxy carbonyl, trichloroethoxycarbonyl, benzyloxycarbonyl or β - methylsulphonylethoxycarbonyl; car-

bamoyl groups such as methylcarbamoyl, phenylcarbamoyl or naphthylcarbamoyl; the corresponding thiocarbamoyl groups; and 2-methoxycarbonyl - 1 - methylvinyl. As protective groups for the carboxyl groups of said acyls R¹ and for the 4 - carboxyl group of the cephem ring, there may be mentioned, for example, methyl, ethyl, tert - butyl, tert - amyl, benzyl, *p* - nitrobenzyl, *p* - methoxybenzyl, benzhydryl, 1 - indanyl, phenacyl, phenyl, *p* - nitrophenyl, methoxymethyl ethoxymethyl, benzyloxymethyl, acetoxymethyl, pivaloyloxymethyl, β - methylsulphonylethyl, methylthiomethyl, trityl, β, β, β - trichloroethyl, and silyl groups such as trimethylsilyl or dimethylsilyl. These carboxyl groups may also be in the form of inorganic or organic salts with alkali metals such as lithium, sodium, potassium or magnesium; alkaline earth metals such as calcium; or various amines such as dicyclohexylamine, triethylamine, tributylamine, di - *n* - butylamine or di - *n* - propylamine.

The organic residue denoted by X in the above-noted compound (I) is usually a carbon chain which is able to form a five- or six-membered ring with the radical:



or



and which may include a double bond or one or more hetero atoms, e.g. oxygen, nitrogen or sulphur, preferably oxygen. Moreover, the carbon chain may be such that its carbon atoms carry suitable substituent groups. Among examples of such substituents on the carbon chain, there may be mentioned: carboxyl, halogen, nitro, alkyl (e.g. methyl, ethyl, propyl, methylene or ethylene), aralkyl (e.g. benzyl or phenethyl), aryl (e.g. phenyl or tolyl), and hydroxy or mercapto groups substituted thereby (e.g. methoxy or *p* - chlorophenylthio). Where two or more such substituents are present, they may form a ring with the carbon chain.

The following are examples of compounds (I) according to the invention:

7 β - [5 - amino - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy) - methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - amino - 3 - (3 - oxobutyryloxy) - methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (α - sulphophenylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (4 - chloro - 3 - oxobutylamido) -

3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (4 - bromo - 3 - oxobutylamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (2 - thienylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - [2 - (1H - tetrazol - 1 - yl)acetamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 α - [α - amino - α - (*p* - hydroxyphenyl)acetamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - (2 - thienylacetamido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (5 - amino - 5 - carboxyvaleramido) - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - (5 - amino - 5 - carboxyvaleramido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - (5 - amino - 5 - carboxyvaleramido) - 3 - (3 - carboxyacroyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - (5 - amino - 5 - carboxyvaleramido) - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - (5 - amino - 5 - carboxyvaleramido) - 3 - (2,4 (or 5) - dicarboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

7 β - amino - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - amino - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - amino - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (α - sulphophenylacetamido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (α - sulphophenylacetamido) - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - [2 - (1H - tetrazol - 1 - yl)acetamido] - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - amino - 3 - mandelyloxymethyl -

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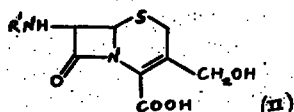
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3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (D - α - sulphophenylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

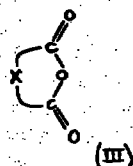
Of the compounds [I], those in which W is acetonyl can be produced by reacting with diketene a compound of the general formula [II]:



in which R¹ is as defined above.

The diketene may conveniently be used in the form of an adduct with acetone or acetophenone. The reaction just referred to is an equimolar reaction. However, to adjust for the decomposition of diketene which occurs in the presence of water or alcohol, an excess of diketene is normally employed. In general, the reaction can be successfully conducted by allowing a 3 - hydroxymethylcephalosporin [II] and diketene to interact in a suitable inert solvent at a temperature within the range of from -30°C. to 40°C., inclusive. Dichloromethane, chloroform, dichloroethane, dimethylformamide, dimethylacetamide, tetrahydrofuran, acetonitrile, ethyl acetate, acetone, dioxane or diethyl ether, and various mixtures thereof may be used, for example, as suitable inert solvents. The reaction of diketene with 3 - hydroxymethylcephalosporin [II] generally proceeds at a high rate but is, of course, dependent on the reaction temperature. In order to ensure completion, the reaction is usually conducted for a period of from 0.5 to 15 hours. If necessary, an amine such as triethylamine may be added to the reaction system, or, where the starting material [II] is an alkali metal salt, an equivalent of, for example, triethylamine hydrochloride may be added to effect a salt interchange before the desired reaction takes place.

Where W represents -X-COOH, the compound [I] can be produced by reacting a 3 - hydroxymethylcephalosporin [II] with a compound of the general formula [III]:



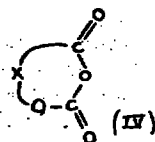
[where X has the same meaning as defined hereinbefore].

As specific examples of the compound [III], there may be mentioned, for instance,

maleic anhydride, succinic anhydride, phthalic anhydride, glutaric anhydride, diglycolic anhydride, thiodiglycolic anhydride, p - chlorophenylsuccinic anhydride, methylenesuccinic anhydride, 3 - nitrophthalic anhydride, trimellitic anhydride and isatoic anhydride.

Generally speaking, the reaction of the compound [II] with the compound [III] is expediently conducted in the presence of a suitable inert solvent like that used in the reaction described just above. The stoichiometric proportions of reactants, the reaction temperature and the other reaction conditions may also be similar to those used in the above-mentioned reaction of a compound [II] with diketene.

The compound [I] wherein W is -X-OH may be produced by reacting a 3 - hydroxymethylcephalosporin [II] with a compound of the general formula [IV]:

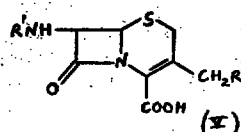


[wherein X has the same meaning as defined hereinbefore].

As examples of the compound [IV], there may be mentioned, for instance, O - carboxymandelic anhydride, O - carboxy - α - hydroxypropionic anhydride, O - carboxy - β - hydroxypropionic anhydride, O - carboxy - 3 - methylsalicylic anhydride, O - carboxy - (α - hydroxy - α - phenyl) - propionic anhydride and O - carboxy - (α - hydroxy - β - phenyl) - propionic anhydride.

The conditions of the reaction between the compound [II] and the compound [IV] are similar to those used for the reactions previously described. Where the starting material [II] has an unprotected amino group, the reaction thereof with diketene, the compound [III] or the compound [IV] may be conducted in such a way that both the 3 - hydroxy and the amino group may be simultaneously acylated by the same acyl groups.

The resulting compound [I] not only has antibiotic activity as such but is able to react with a nucleophilic compound in such a way that the residue of the nucleophilic compound is introduced into the 3 - methyl group of the cephalosporin, thereby yielding the compound represented by the formula [V]:



wherein R³ represents a residue of a nucleophilic compound and R¹ has the same meaning as defined above.

The nucleophilic compound used for this reaction may be any nucleophilic compound capable of replacing the 3 - acetoxy group of a cephalosporin. Furthermore, the reaction proceeds at a rate ranging from 4 to 16 times that of the 3 - acetoxy compound and almost quantitatively.

Suitable nucleophilic compounds include nitrogen-containing heterocyclic thiols containing one or more nitrogen atoms which may optionally be in the form of oxides or/and which contain such atoms as oxygen or/and sulphur in addition to the nitrogen atom, with or without nuclear substitution. Common examples of the nitrogen-containing heterocyclic group of such a thiol compound, include, for instance, pyridyl, N - oxidopyridyl, pyrimidyl, pyridazinyl, N - oxidopyridazinyl, pyrazolyl, diazolyl, 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 and 2H - tetrazolyl. As the substituents on such nitrogen-containing heterocyclic groups, there may be mentioned such monovalent groups as hydroxy, mercapto, amino, carboxyl, carbamoyl, substituted or unsubstituted C₁₋₄ alkyl (for example, methyl, ethyl, trifluoromethyl, propyl, isopropyl, butyl or isobutyl), C₁₋₄ alkoxy (for example, methoxy, ethoxy, propoxy, isopropoxy or butoxy), halogens (e.g. chlorine or bromine), and various substituent groups attached through C₁₋₄ alkylene groups, —S—, —N— or other polyvalent groups. When such polyvalent groups are C₁₋₄ alkylene groups, the substituents may, for example, be hydroxy, mercapto, amino, morpholino, carboxyl, sulphy, carbamoyl, alkoxycarbonyl, C₁₋₄ alkylcarbamoyl, alkoxy, alkylthio, alkylsulphonyl, acyloxy and morpholinocarbonyl. When such a polyvalent group is —S— or —N—, the substituents may be C₁₋₄ alkyl or C₁₋₄ alkylene groups having the aforementioned substituents. When the polyvalent group is —N—, such substituents as carboxyl, alkoxycarbonyl, acyl, carbamoyl or C₁₋₄ alkylcarbamoyl may be directly attached. More particularly, there may be mentioned, for example, substituted alkyl groups such as carboxymethyl, carbamoylmethyl N - C₁₋₄ alkylcarbamoylmethyl (e.g. N,N - dimethylcarbamoylmethyl), hydroxy C₁₋₄ alkyl (e.g. hydroxymethyl or 2 - hydroxyethyl), acyloxy C₁₋₄ alkyl (e.g. acetoxymethyl or 2 - acetoxyethyl), alkoxycarbonylmethyl (e.g. methoxycarbonylmethyl, hexyloxycarbonylmethyl or octyloxycarbonylmethyl), methylthiomethyl, methylsulphonylmethyl, N - C₁₋₄ alkylamino C₁₋₄ alkyl (e.g. N,N - dimethylaminomethyl, N,N - dimethylaminoethyl or N,N,N - trimethylammoniummethyl), morpholinomethyl; substituted amino groups such as C₁₋₄ alkylamino (e.g. methylamino), sulphy - C₁₋₄

alkylamino (e.g. 2 - sulphyethylamino), hydroxy - C₁₋₄ alkylamino (e.g. hydroxyethylamino), C₁₋₄ alkylamino - C₁₋₄ alkylamino (e.g. 2 - dimethylaminoethylamino or 2 - trimethylammoniummethylamino), acylamino (e.g. acetylamino, 2 - dimethylaminoacetylamino or 2 - trimethylammoniumacetylamino) or C₁₋₄ alkoxycarbonylamino (e.g. methoxycarbonylamino); and substituted thio (mercapto) groups such as methylthio, 2 - hydroxyethylthio, 2 - acyloxyethylthio (e.g. 2 - acetoxyethylthio, 2-phenylacetoxyethylthio or 2 - capropoxyethylthio), carboxymethylthio, alkoxycarbonylmethylthio (e.g. methoxycarbonylthio or hexyloxycarbonyl methylthio), carbamoylmethylthio, N - lower alkylcarbamoylmethylthio (e.g. N,N - dimethylcarbamoylmethylthio) acetylmethylthio, N - C₁₋₄ alkylamino - C₁₋₄ alkylthio (e.g. 2 - N,N - dimethylaminoethylthio or 2 - N,N,N - trimethylammoniumethylthio), morpholinocarbonylmethylthio and 2 - sulphyethylthio. More particularly, there may be mentioned various heterocyclic thiols such as tetrazolethiol, methyltetrazolethiol, phenyltetrazolethiol (2 - N,N - dimethylaminoethyl) - tetrazolethiol, methylthiadiazolethiol, hydroxyethylthiothiadiazolethiol, methylthiothiadiazolethiol, thiadiazolethiol, carbamoylaminothiadiazolethiol, carbamoylmethylthiothiadiazolethiol, thiazolethiol, methylthiazolethiol, carboxymethylthiazolethiol, triazolethiol, dimethyltriazolethiol, pyrazolethiol, ethoxycarbonylmethyltriazolethiol, imidazolethiol, methyloxadiazolethiol, pyridinethiol, pyrimidinethiol, methylpyridazinethiol and triazinethiol. In addition, use may also be made of such nitrogen-containing heterocyclic compounds as aliphatic or aromatic thiols, e.g. methanethiol, ethanethiol or thiophenol; thio-urea and its derivatives such as N - methylthiourea or N - methyl - N' - pyridylthiourea; thioamide derivatives such as thiosemicarbazide, thioacetamide, or thiobenzamide; sodium thiosulphate, sodium sulphite, potassium thiocyanate or sodium azide; pyridine and pyridine derivatives such as quinoline, picoline, nicotinic acid, nicotinamide, isonicotinamide, isonicotinic acid hydrazide, m - bromopyridine, pyridinesulphonic acid, pyridine - m - carbinol (3 - hydroxymethylpyridine), pyridinaldehyde, quinoline or isoquinoline; and such other nitrogen-containing heterocyclic compounds as pyrazine, pyrazinamide (2 - carbamoylpyrazine), pyridazine, pyrimidine, imidazole, 1 - methylimidazole and pyrazol. It is also possible to employ carbon nucleophilic agents toward which the 3-position is known to be refractory. As examples of such carbon nucleophilic reagents, there may be mentioned cyanides, pyrrole, substituted pyrrole, indole, acetylene, active methylene compounds, e.g. acetylacetone, acetoacetic acid esters, malonic acid esters, cyclohexane - 1,3 - dione, triacetylmethane or enamine compounds. Alcohols such as for ex-

ample, methanol, ethanol or propanol may also be employed in this reaction.

The substitution reaction between such a nucleophilic compound and a compound of the formula [I] is normally conducted in a solvent. While the commonest solvent is water, the hydrophilic organic solvents inert to the reaction, e.g. acetone, tetrahydrofuran, dimethylformamide, methanol ethanol or dimethylsulphoxide, and aqueous solvents such as mixtures of water and such reaction-inert, polar solvents as are mentioned above are employed in preference.

While the compound [I] may be a free compound, it is more advantageous to subject the compound [I] to the reaction in the form of an alkali metal salt, e.g. the sodium, potassium or other salt, or an organic amine salt, e.g. the triethylamine, trimethylamine or other amine salt. The nucleophilic agent is also reacted in its free form or as an alkali metal, organic amine or other salt.

The proportion of the nucleophilic compound to be used in this reaction is preferably one equivalent or more with respect to the compound [I]. While the optimum pH depends upon the particular nucleophilic compound and the compound [I], the reaction is generally carried out under weakly acid to weakly alkaline conditions. The reaction temperature is preferably somewhere between 40°C and 70°C, although there are no particular limits. The reaction time cannot be specified in general terms, either, for it depends upon the reaction temperature, pH, type of nucleophilic reagent and other factors. Roughly speaking, however, the reaction goes to completion in from 30 minutes to 2 hours when the reaction temperature is 60°C. The reaction may also be carried out in the presence of, as an addition to the reaction system, an inorganic salt, e.g. the chloride, bromide, iodide, thiocyanide or nitrate of lithium, sodium, potassium or ammonium.

The compound [I] wherein R¹ is an acyl group can be converted to the corresponding compound wherein R¹ is hydrogen by splitting off the 7 - acyl group in a manner conventional *per se* (e.g. any of the procedures set forth in Japanese Patent Publication No. 13862/1966 and No. 40899/1970, Japanese Patent Application (As-Laid-Open) No. 34387/1972, No. 95292/1975 and No. 96591/1975, Japanese Patent Publication No. 35079/1975 or United States Patent No. 3,632,578).

Into this compound there may be introduced a compound which has previously been mentioned by way of example for R¹ as the 6- or 7-substituents of penicillin or cephalosporin compounds, by previous activation in a known manner. Thus, for example, a compound in which R¹ is 4 - halogeno - 3 - oxobutyryl may be obtained by reacting the said compound [I] in which R¹ is hydrogen with

a 4 - halogeno - 3 - oxobutyryl halide which, in turn, may be reacted with thiourea to produce a desired 7 - [2 - (2 - imino - 4 - thiazolin - 4 - yl) - acetamido] compound. While the activity depends to some extent on the type of 3-substituent present, these compounds invariably display excellent antibiotic activity. For example, the compound with 1 - methyltetrazol - 5 - ylthiomethyl in the 3-position is particularly useful; approximately the same effect is accomplished with this compound at a dose which is about one-fifth that of cephalosin.

The present invention is illustrated in further detail below with reference to a number of specific examples, but it is to be understood that the examples are solely for the purpose of illustration and are not to be construed as limitations of the invention, and also that many variations may be resorted to without departing from the scope of the invention. In this specification, "g", "mg", "ml", "cm", "Hz", "DMSO", and "decomp." are abbreviations of "gram", "milligram", "milliliter", "centimeter", "Herz", "dimethylsulphoxide", and "decomposed", respectively. The resins referred to as "Amberlite" (trade mark) are products manufactured by Rohm & Haas Co. in the U.S.A. "Celite" (trade mark) and "Sephadex" (trade mark) are marketed by Johns-Manville Sales Corp. and Pharmacia A.B., respectively. All the temperatures are uncorrected and the percentages are all on a weight basis except where otherwise specifically stated. The NMR spectra given therein were measured using a Varian (trade mark) Model HA 100 (100 MHz) or T60 (60 MHz) spectrometer with tetramethylsilane as the internal reference and all δ values as in ppm. The symbol *s* represents a singlet, *d* a doublet, *t* a triplet, *q* a quartet, *m* a multiplet, and *J* a coupling constant.

Example 1

(1) 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid di-triethylamine salt (7.05 g) was dissolved in dichloromethane (50 ml), and this was followed by the addition of diketene (0.92 g). The reaction was carried out at room temperature for 3 hours. After the reaction had been completed, the solvent was distilled off under reduced pressure and the residue was diluted with water and adjusted to pH 6.0 with sodium bicarbonate. The aqueous solution was washed twice with ethyl acetate, brought down to pH 2.0 with 4N-HCl and extracted three times with ethyl acetate. The ethyl acetate solution was washed with a saturated aqueous solution of sodium chloride, treated with magnesium sulphate and filtered. The solvent was then distilled off under reduced pressure. Ether was added to the residue, and the resulting powder was collected by filtration,

washed with ether and dried. The above procedure provided 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.48 g) (yield: 93.2%).

IR(KBr): 3350, 1775, 1740, 1715, 1640, 1530 cm^{-1}

NMR (δ in d_6 - DMSO): 1.30—2.40 (6H, m), 2.17 (3H, s), 3.46 (2H, ABq, $J=18$ Hz), 3.60 (2H, s), 4.72 (1H, t), 4.90 (2H, ABq, $J=12$ Hz), 5.01 (1H, d, $J=5$ Hz), 5.62 (1H, dd, $J=5$ & 8 Hz), 7.88 (4H, s), 8.80 (1H, d, $J=8$ Hz)

(2) 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.88 g) was dissolved in water (50 ml), together with 5 - mercapto - 1 - methyl - 1H - tetrazole (1.50 g) and sodium hydrogen carbonate (2.10 g). Following the addition of sodium chloride (15.0 g), the solution was adjusted to pH 5.0 and, then, reacted at 60°C for 50 minutes. After cooling, a saturated aqueous solution of sodium chloride (50 ml) was added and the mixture was adjusted to pH 1.5 with 4N-HCl. The resulting solid precipitate was recovered by filtration, washed with a saturated aqueous solution of sodium chloride (20 ml) and dissolved in ethyl acetate (100 ml)-water (20 ml). After separation, the ethyl acetate solution was dried and, following the addition of toluene (50 ml), concentrated. The solid precipitate was recovered by filtration, washed with toluene - ether and dried. The procedure provided 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (5.75 g) (yield: 95.5%).

IR(KBr): 3325, 1780, 1730, 1715, 1650 cm^{-1}

NMR (δ in d_6 = DMSO): 1.40—1.76 (2H, m), 2.0—2.4 (4H, m), 3.64 (2H, ABq, $J=19$ Hz), 3.93 (3H, s), 4.30 (2H, ABq, $J=15$ Hz), 4.73 (1H, t, $J=8$ Hz), 5.01 (1H, d, $J=5$ Hz), 5.62 (1H, dd, $J=5$ & 9 Hz), 7.85 (4H, s), 8.80 (d, $J=9$ Hz).

(3) 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.88 g) was dissolved in water (60 ml) together with 2 - (2 - hydroxyethylthio) - 5 - mercapto - 1,3,4 - thiadiazole (2.90 g) and sodium bicarbonate (2.20 g). Following the addition of sodium bromide (30 g), the solution was adjusted to pH 5.5 and reacted at 60°C. for 50 minutes. After the reaction was complete, the reaction mixture was diluted with water (40 ml) and adjusted to pH 5.0. The aqueous solution was washed twice with

ethyl acetate, the pH was brought down to 2.0 with 4N-HCl, and the solution was extracted three times with a mixture of ethyl acetate and tetrahydrofuran (4:1). The organic layer was washed with a saturated aqueous solution of sodium chloride, dried over magnesium sulphate, filtered and distilled under reduced pressure to remove the solvent.

Ethyl acetate - diethylether was added to the residue and the resulting powder was recovered by filtration, washed with diethyl ether and dried. The procedure provided 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - [2 - (2 - hydroxyethylthio) - 1,3,4 - thiadiazol - 5 - yl]thiomethyl - 3 - cephem - 4 - carboxylic acid (6.56 g) (yield: 96.6%).

IR(KBr): 3325, 1780, 1715, 1645, 1530 cm^{-1}

NMR (d_6 -DMSO): δ 1.30—2.40 (6H, m), 3.20—3.80 (6H, m), 4.27 (2H, ABq, $J=12$ Hz), 4.65 (1H, t, $J=9$ Hz), 4.96 (1H, d, $J=5$ Hz), 5.55 (1H, dd, $J=5$ & 8 Hz), 7.87 (4H, s), 8.70 (1H, d, $J=8$ Hz).

(4) In a mixture of water (50 ml) and tetrahydrofuran 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.88 g), 2 - carbamoylmethylthio - 5 - mercapto - 1,3,4 - thiadiazole (2.28 g) and sodium bicarbonate (2.20 g) were dissolved in a mixture of water (50 ml) and tetrahydrofuran (30 ml). The resulting solution was adjusted to pH 5.8 and then reacted at 60°C. for 70 minutes. After cooling, water (30 ml) was added and the aqueous solution was adjusted to pH 5.0, washed twice with ethyl acetate and brought down to pH 2.0 with 4N-HCl. It was then extracted three times with a solvent mixture of ethyl acetate and tetrahydrofuran (2:1), and the organic layer was washed with a saturated aqueous solution of sodium chloride and dried over magnesium sulphate. The solvent was distilled off under reduced pressure and the residue was treated with ethyl acetate. The resulting powder was recovered by filtration, washed with ethyl acetate and dried. The procedure provided 7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (2 - carbamoylmethylthio - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (5.83 g) (yield: 84.2%).

IR(KBr): 3430, 3340, 1776, 1717, 1680, 1535 cm^{-1}

NMR (d_6 -DMSO): δ 1.30—2.40 (6H, m), 3.57 (2H, br), 4.40 (2H, s), 4.32 (2H, ABq, $J=12$ Hz), 4.70 (1H, t, $J=8$ Hz), 5.0 (1H, d, $J=5$ Hz), 5.55 (1H, dd, $J=5$ & 8 Hz), 7.20 (1H, broad), 7.60 (1H, broad), 7.86 (4H, s), 8.74 (1H, d, $J=8$ Hz).

Example 2

(1) 7 β - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (6.69 g) was dissolved in dichloromethane (50 ml). At room temperature, triethylamine (1.01 g) and diketene (1.68 g) were added, and the reaction was carried out for 3.0 hours. After the reaction had been completed, the reaction mixture was treated in the same manner as described in Example 1. The procedure provided 7 β - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.12 g) (yield: 91.3%).

IR(KBr): 3350, 1780, 1735, 1720, 1640, 1530 cm^{-1}

NMR (δ in d_6 -DMSO): 1.45—2.40 (6H, m), 2.18 (3H, s), 3.50 (2H, ABq, J=19 Hz), 4.34 (1H, m), 4.88 (2H, ABq, J=13 Hz), 5.05 (1H, d, J=5 Hz), 5.65 (1H, dd, J=5 & 9 Hz), 7.27—8.0 (5H, m), 8.46 (1H, d, J=8 Hz), 8.75 (1H, d, J=9 Hz).

(2) 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (561 mg), potassium iodide (400 mg) and pyridine (212 mg) were dissolved in water (20 ml). The resulting solution was adjusted to pH 6.5 and then reacted at 60°C. for 50 minutes. Following the addition of water (10 ml), the aqueous solution was adjusted to pH 7.0, washed twice with dichloromethane (5.0 ml), readjusted to pH 6.0 and concentrated under reduced pressure. The residue was subjected to column chromatography on Amberlite XAD-2 (trade mark), followed by elution with water and, then, with a mixture of water and methanol. The eluate was concentrated and lyophilized. The procedure provided N - {7 β - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - cephem - 3 - ylmethyl} - pyridinium - 4 - carboxylate monosodium salt (352 mg).

IR(KBr): 3360, 3250, 1765, 1645, 1630, 1605, 1575, 1530 cm^{-1}

NMR(D_2O): δ 1.50—2.60 (6H, m), 3.14 (2H, ABq, J=19 Hz), 4.36 (1H, m), 5.05 (1H, d, J=5 Hz), 5.32 (2H, ABq, J=15 Hz), 5.60 (1H, dd, J=5 Hz), 7.0—9.0 (10H, m).

(3) 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (561 mg), thiourea (100 mg) and sodium bicarbonate (253 mg) were dissolved in water (6.0 ml). The resulting solution was adjusted to pH 6.5 and reacted at 60°C. for 50 minutes. After cooling, the reaction mixture was subjected to column chromatography on Amberlite XAD-

2 (trade mark), followed by elution with water and water - methanol. The eluate was concentrated and lyophilized. The procedure provided S - {7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - cephem - 3 - yl - methyl} - thiuronium - 4 - carboxylate monosodium salt (464 mg).

IR(KBr): 3350, 3230, 1762, 1645, 1630, 1600, 1580, 1535 cm^{-1}

NMR(D_2O): δ 1.50—2.50 (6H, m), 3.20—3.80 (3H, m), 4.40 (2H, m), 5.05 (1H, d, J=5 Hz), 5.53 (1H, dd, J=5 Hz), 7.30—7.90 (5H, m).

Example 3

7 - [D - 5 - (p - toluenesulphonamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (7.29 g) was dissolved in dichloromethane (50 ml), and triethylamine (0.51 g) and diketene (1.26 g) were added at room temperature. The resulting reaction was carried out for 4.0 hours, after which time the reaction mixture was treated as in Example 1. The procedure provided 7 - [D - 5 - (p - toluenesulphonamide) - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.64 g) (yield: 92.3%).

IR(KBr): 3275, 1780, 1740, 1730, 1715, 1640, 1535 cm^{-1}

NMR (δ in d_6 -DMSO): 1.30—1.80 (4H, m), 2.0—2.4 (2H, m), 2.16 (3H, s), 2.33 (3H, s), 3.45 (2H, ABq, J=19 Hz), 4.91 (2H, ABq, J=12 Hz), 5.0 (1H, d, J=5 Hz), 5.57 (1H, dd, J=5 & 8 Hz), 7.40 (4H, m), 7.81 (1H, d, J=9 Hz), 8.64 (1H, d, J=8 Hz).

Example 4

7 - [D - 5 - (p - tert - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (7.35 g) was dissolved in dichloromethane (50 ml), and triethylamine (1.01 g) and diketene (1.68 g) were added at room temperature. The resulting reaction was carried out for 2 hours, after which time the reaction mixture was treated as in Example 1. The procedure provided 7 - [D - 5 - (p - tert - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (5.78 g) (yield: 93.8%).

IR(KBr): 3280, 1780, 1740, 1725, 1710, 1640, 1530 cm^{-1}

NMR (δ in d_6 -DMSO): 1.32 (9H, s), 1.50—2.40 (6H, m); 2.18 (3H, s), 3.48 (2H, br), 3.57 (2H, s), 4.35 (1H, m), 4.94 (2H, ABq, J=13 Hz), 5.06 (1H, d, J=5 Hz), 5.62 (1H, dd, J=5 & 8 Hz), 7.46 (2H, d, J=8 Hz), 7.85 (2H, d,

J=8 Hz), 8.35 (1H, d, J=8 Hz), 8.78 (1H, d, J=8 Hz).

Example 5

7 - [D - 5 - caprylamino - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid diethylamine salt (6.01 g) was dissolved in dichloromethane (50 ml) followed by the addition of triethylamine (1.01 g) and diketene (1.68 g). The reaction was carried out at room temperature for 3 hours, after which time the reaction mixture was treated as in Example 1. The procedure provided 7 - [D - 5 - caprylamido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyl-oxymethyl - 3 - cephem - 4 - carboxylic acid (4.36 g) (yield: 90.3%).

IR(KBr): 3320, 1780, 1745, 1725, 1715, 1645, 1535 cm^{-1}

NMR (δ in d_6 -DMSO): 0.70—2.40 (2 1H, m), 2.22 (3H, s), 3.53 (2H, broad), 3.60 (2H, s), 4.18 (1H, m), 4.91 (2H, ABq, J=12 Hz), 5.04 (1H, d, J=5 Hz), 5.65 (1H, dd, J=5 & 8 Hz), 7.87 (1H, d, J=8 Hz), 8.70 (1H, d, J=8 Hz).

Example 6

7 - phenylacetamido - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (4.49 g) was dissolved in dichloromethane (50 ml) and, at room temperature, triethylamine (0.51 g) and diketene (1.26 g) were added. The resulting reaction was conducted for 2 hours, after which time the solvent was distilled off under reduced pressure. The residue was diluted with water and adjusted to pH 6.0. The aqueous solution formed was extracted twice with ethyl acetate, brought down to pH 2.0 with 4N-HCl and extracted three times with ethyl acetate. The ethyl acetate solution was washed with a saturated aqueous solution of sodium chloride, treated with magnesium sulphate and filtered. The solvent was distilled off under reduced pressure and the residue was recrystallized from ethyl acetate-diethylether. The above procedure provided 7 - phenylacetamido - 3 - (3 - oxobutyl-oxymethyl - 3 - cephem - 4 - carboxylic acid (4.16 g) (yield: 96.3%).

IR(KBr): 3270, 1785, 1745, 1715, 1655, 1540 cm^{-1}

NMR (δ in d_6 -DMSO): 2.14 (3H, s), 3.52 (6H, broad), 4.86 (2H, ABq, J=13 Hz), 5.00 (1H, d, J=5 Hz), 5.63 (1H, dd, J=5 & 9 Hz), 7.22 (5H, s), 8.93 (1H, d, J=9 Hz).

Example 7

A mixture of dichloromethane (300 ml), triethylamine (27 ml) and dimethylaniline (100 ml) was previously cooled to 10°C, and 7 - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - car-

boxylic acid (50 g) was dissolved therein. To this solution was added dichlorodimethylsilane (36 ml) whereupon the internal temperature increased to 27°C. The resulting mixture was stirred at that temperature for 30 minutes, after which time the internal temperature was brought down to -35°C. Then, phosphorus pentachloride (32.4 g) was added. The mixture was stirred at -25°C. for 40 minutes and, after cooling to -35°C., thioacetamide (20 g) was added. The mixture was further stirred at -20 to -25°C. for 40 minutes and, after cooling to -30°C. methanol (200 ml) was gently added in drops. Then, at the same temperature, sulphur monochloride (17 ml) was gently added dropwise. The mixture was stirred for 20 minutes, after which time it was diluted with water (200 ml) and brought to pH 3.2 with a 40% aqueous potassium carbonate solution. After stirring for 60 minutes, the resulting crystals were collected by filtration and rinsed with water and acetone. The crude crystals thus obtained were suspended in 10% hydrochloric acid (230 ml) and stirred at 30°C. for 1 hour. The insolubles were filtered off and the filtrate was cooled to 5—10°C. and brought to pH 3.3 with potassium carbonate. The filtrate was stirred for one hour, then the precipitated crystals were collected by filtration, rinsed with water and acetone, and dried over phosphorus pentoxide. The procedure just described provided 7 - amino - 3 - (1 - methyltetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (17.0 g).

IR(KBr): 1795 cm^{-1}

NMR (δ in D_2O - NaHCO_3): 3.61 & 3.98 (ABq, J=18 Hz, 2- CH_2), 4.21 (s, tetrazole- CH_3), 5.21 (d, J=4.5 Hz, 6-H), 5.60 (d, J=4.5 Hz, 7-H).

Chlorine gas (2.8 g) was bubbled through a solution of diketene (3.3 g) in methylene chloride (160 ml) under stirring and cooling to maintain the internal temperature at -25° to -35°C. for a period of 100 minutes. Then, the mixture was further stirred at the same temperature for 30 minutes. Separately, 7 - amino - 3 - (1 - methyltetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (10.0 g) and dibutylamine (7.9 g) were dissolved in methylene chloride (60 ml) and the resulting solution was cooled to -10°C. The above reaction mixture was added dropwise to this solution, with stirring and cooling to maintain an internal temperature of -10° to -20°C. over a period of 30 minutes. The reaction was further stirred at that temperature for 40 minutes. Thin-layer chromatography of this reaction mixture revealed the presence of 7 - (4 - chloro - 3 - oxobutyl-oxymethyl - 3 - (1 - methyltetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid. Thiourea (4.64 g) was dissolved in this reac-

tion mixture and the internal temperature was increased to 17–19°C. The mixture was stirred at this temperature, whereupon crystals separated out. The crystals were recovered by suction-filtration, washed with methylene chloride (30 ml) and dried. The procedure provided 7β - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - (1 - methyltetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (12.8 g), melting point: 176–180°C.(decomp.).

IR(KBr): 1762, 1662 cm⁻¹

NMR (δ in d₆-DMSO): 3.39 (s, CH₂CO), 3.55 & 3.77 (ABq, J=18 Hz, 2-CH₂), 3.90 (s, tetrazole 1-CH₃), 4.21 & 4.36 (ABq, J=14 Hz, 3-CH₂), 5.03 (d, J=5 Hz, 6-H), 5.66 (dd, J=9 & 5 Hz, 7-H), 6.23 (s, thiazolin 5-H), 6.2–7.1 (m,



8.85 (d, J=9 Hz, —CONH—).

Example 8

7β - (2 - thienylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (4.55 g) was dissolved in dichloromethane (30 ml), followed by the addition of succinic anhydride (1.50 g). The resulting mixture was stirred at room temperature for 2 hours. The dichloromethane was distilled off and, following the addition of a 3% aqueous phosphoric acid solution (100 ml), the residue was extracted with ethyl acetate (150 ml). The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (100 ml×2), dried (over magnesium sulphate) and concentrated under reduced pressure. The residue was treated with diethyl ether and the resulting powder was recovered by filtration, washed with ether and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7β - (2 - thienylacetamido) - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid (4.00 g).

IR(KBr): 1782 (β-lactam), 1733 (—CO₂H) cm⁻¹

NMR (δ in d₆-DMSO): 2.50 (4H, —CO(CH₂)₂—), 3.40 & 3.63 (2H, ABq, J=18 Hz, 2-CH₂), 3.75 (2H, s, —CH₂CONH—), 4.71 & 5.07 (2H, ABq, J=13 Hz, 3-CH₂), 5.07 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 & 8 Hz, 7-H), 6.90 & 7.30 (3H,



9.10 (1H, d, J=8 Hz, —CONH—)

Example 9

7β - (2 - thienylacetamido) - 3 - hydroxy-

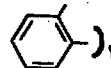
methyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (3.60 g) was dissolved in dichloromethane (25 ml), followed by the addition of phthalic anhydride (1.80 g). The resulting mixture was stirred at room temperature for 2 hours, after which time it was treated in a manner similar to that described in Example 8. The procedure provided 7β - (2 - thienylacetamido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (3.44 g).

IR(KBr): 1777, 1724, 1650 (shoulder) cm⁻¹

NMR (δ in d₆-DMSO): 3.50 & 3.70 (2H, ABq, J=18 Hz, 2-CH₂), 3.77 (2H, s, —CH₂CO—), 4.91 & 5.29 (2H, ABq, J=13 Hz, 3-CH₂), 5.10 (1H, d, J=5 Hz, 6-H), 5.70 (1H, dd, J=5 & 8 Hz, 7-H), 6.92 & 7.32 (3H,



7.64 (4H,



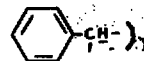
9.12 (1H, d, J=8 Hz, —CONH—).

Example 10

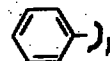
7β - mandelamido - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (4.65 g) was dissolved in dichloromethane (40 ml), followed by addition of succinic anhydride (1.50 g). The resulting mixture was stirred at room temperature for 1 hour and a half. After the reaction had been completed, the mixture was treated as in Example 8 to yield 7β - mandelamido - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid (4.45 g).

IR(KBr): 1776, 1737, 1684 (shoulder) cm⁻¹

NMR (δ in d₆-DMSO): 2.48 (4H, —CO(CH₂)₂—), 3.3–3.7 (2H, 2-CH₂), 4.69 & 5.03 (2H, ABq, J=13 Hz, 3-CH₂), 4.99 (1H,



5.06 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 & 8 Hz, 7-H), 7.35 (5H,



9.31 (1H, d, J=8 Hz, —CONH—).

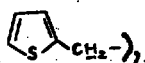
Example 11

7β - (2 - thienylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (0.68 g) was dissolved in di-

chloromethane (7 ml), followed by the addition of glutaric anhydride (0.34 g). The resulting mixture was stirred at room temperature for 2 and a half hours. The dichloromethane was distilled off and, following the addition of 3% aqueous phosphoric acid (15 ml), the residue was extracted with ethyl acetate (25 ml). The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (15 ml $\times$ 2) and dried (over magnesium sulphate). Dropwise addition of a 2N-solution of sodium 2-ethylhexanoate in isopropyl alcohol (1.50 ml), yielded a powder which was recovered by filtration, washed with ethyl acetate - diethylether and dried over phosphorus pentoxide. The procedure provided 7 β - (2-thienylacetamido) - 3 - (4-carboxybutyryloxy)methyl - 3-cephem - 4-carboxylic acid disodium salt (0.42 g).

IR(KBr): 1760, 1736 (shoulder), 1661, 1609 cm^{-1}

NMR (δ in D_2O): 1.90 (2H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), 2.28 (4H, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), 3.21 & 3.61 (2H, ABq, $J=18$ Hz, 2- CH_2), 3.78 (2H, s,



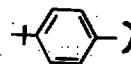
4.4-4.9 (2H, 3- CH_2), 4.98 (1H, d, $J=5$ Hz, 6-H), 5.60 (1H, d, $J=5$ Hz, 7-H), 6.95 & 7.28 (3H,



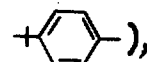
Example 12

7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (7.35 g) was dissolved in dimethylformamide (50 ml), followed by the addition of succinic anhydride (1.50 g). The resulting mixture was stirred at room temperature for 30 minutes. After the reaction had been completed, 3% aqueous phosphoric acid (250 ml) was added, followed by extraction with ethyl acetate (500 ml). The ethyl acetate layer was washed with water (250 ml $\times$ 2), dried (over magnesium sulphate) and concentrated under reduced pressure. Then, upon the addition of diethylether, a powder was obtained which was recovered by filtration, washed with diethylether and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - carboxyvaleramido] - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.20 g).

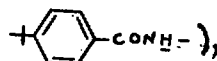
IR(KBr): 1779, 1732, 1640 cm^{-1}
NMR (δ in d_6 -DMSO): 1.28 (9H, s,



1.73 & 2.24 (6H, $-(\text{CH}_2)_3-$), 2.50 (4H, $-\text{COCH}_2\text{CH}_2\text{CO}-$), 3.38 & 3.63 (2H, ABq, $J=18$ Hz, 2- CH_2), 4.37 (1H, $-\text{CH}-\text{NH}-$), 4.71 & 5.06 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.04 (1H, d, $J=5$ Hz, 6-H), 5.66 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.44 & 7.81 (4H,



8.43 (1H, d, $J=8$ Hz,

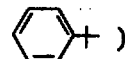


8.80 (1H, d, $J=8$ Hz, $-\text{CONH}-$).

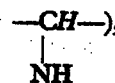
Example 13

7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (7.35 g) was dissolved in dimethylformamide (50 ml), followed by the addition of phthalic anhydride (1.63 g). The resulting mixture was stirred at room temperature for 30 minutes, after which time it was treated as in Example 12. The procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.70 g).

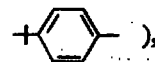
IR(KBr): 1784, 1726, 1642 cm^{-1}
NMR (δ in d_6 -DMSO): 1.27 (9H, s,



1.72 & 2.22 (6H, $-(\text{CH}_2)_3-$), 3.46 & 3.71 (2H, ABq, $J=18$ Hz, 2- CH_2), 4.37 (1H,



4.89 & 5.27 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.08 (1H, d, $J=5$ Hz, 6-H), 5.67 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.43 & 7.81 (4H,



7.62 (4H,



), 8.42 (1H, d, $J=8$ Hz, $-\text{CHNHCO}-$), 8.80 (1H, d, $J=8$ Hz, $-\text{CONH}-$).

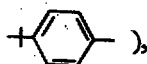
Example 14

7 β - [D - 5 - (p - t - butylbenzamido) - 100

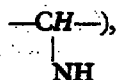
5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid diethylethylamine salt (7.35 g) was dissolved in dimethylformamide (50 ml), followed by the addition of maleic anhydride (1.49 g). The resulting mixture was stirred at room temperature for 30 minutes, after which time it was treated as in Example 12. The procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (3 - carboxyacryloyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.05 g).

IR(KBr): 1780, 1727, 1640 cm⁻¹

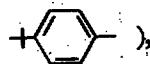
NMR (δ in d₆-DMSO): 1.32 (9H, s,



1.77 & 2.26 (6H, -(CH₂)₃-), 3.41 & 3.65 (2H, ABq, J=18 Hz, 2-CH₂), 4.37 (1H,



4.80 & 5.16 (2H, ABq, J=13 Hz, 3-CH₂), 5.02 (1H, d, J=5 Hz, 6-H), 5.66 (1H, dd, J=5 & 8 Hz, 7-H), 6.34 (2H, -CH=CH-), 7.44 & 7.82 (4H,



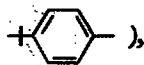
8.40 (1H, d, J=8 Hz, -CHNHCO-), 8.80 (1H, d, J=8 Hz, -CONH-).

Example 15

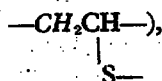
7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid diethylethylamine salt (1.47 g), was dissolved in dichloromethane (20 ml), followed by the addition of p - chlorophenylthiosuccinic anhydride (0.51 g). The resulting mixture was stirred at room temperature for 1 hour, after which time it was treated as in Example 8. The procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - [3 - carboxy - 3 (or 2) - (p - chlorophenylthio)propionyl - oxy]methyl - 3 - cephem - 4 - carboxylic acid (1.50 g).

IR(KBr): 1778, 1728, 1636 cm⁻¹

NMR (δ in d₆-DMSO): 1.28 (9H, s,



1.74 & 2.24 (6H, -(CH₂)₃-), 2.73 (2H,



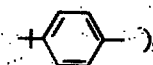
3.3-3.8 (2H, 2-CH₂), 4.03 (1H,



4.40 (1H, -CH-NH-), 4.73 & 5.07 (2H, ABq, J=13 Hz, 3-CH₂), 5.02 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 & 8 Hz, 7-H), 7.42 (4H,



7.44 & 7.82 (4H,



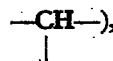
8.44 (1H, d, J=8 Hz, -CHNHCO-), 8.82 (1H, d, J=8 Hz, -CONH-).

Example 16

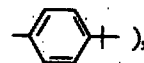
7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid diethylethylamine salt (7.05 g) was dissolved in dichloromethane (50 ml), followed by the addition of succinic anhydride (1.50 g). The resulting mixture was stirred at room temperature for 1 hour and a half, after which time it was treated as in Example 8. The procedure provided 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (3 - carboxypropionyl - oxy)methyl - 3 - cephem - 4 - carboxylic acid (5.43 g).

IR(KBr): 1777, 1710, 1644 cm⁻¹

NMR (δ in d₆-DMSO): 1.52 & 2.17 (6H, -(CH₂)₃-), 2.50 (4H, -(CH₂)₂-), 3.3-3.8 (2H, 2-CH₂), 4.70 & 5.03 (2H, ABq, J=13 Hz, 3-CH₂), 4.72 (1H, t, J=7 Hz,



5.01 (1H, d, J=5 Hz, 6-H), 5.62 (1H, dd, J=5 & 8 Hz, 7-H), 7.86 (4H, s,



8.78 (1H, d, J=8 Hz, -CONH-).

Example 17

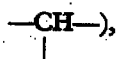
7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid diethylethylamine salt (7.05 g) was dissolved in dichloromethane (50 ml), followed by the addition of phthalic anhydride (2.22 g). The resulting mixture was stirred at room temperature for 1 hour and a half, after which time it was treated as in Example 8. The procedure provided 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.39 g).

13

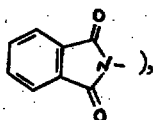
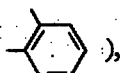
1,544,103

13

IR(KBr): 1772, 1714, 1643 cm^{-1}
NMR (δ in d_6 -DMSO): 1.52 & 2.18 (6H, $-(\text{CH}_2)_3-$), 3.42 & 3.69 (2H, ABq, $J=18$ Hz, 2- CH_2), 4.74 (1H, t, $J=8$ Hz,



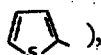
4.89 & 5.28 (2H, ABq, 13 Hz, 3- CH_2), 5.06 (1H, d, $J=5$ Hz, 6-H), 5.66 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.64 (4H,



Example 18

15 7β - (2 - Thienylacetamido) - 3 - (3 - carboxyacryloyloxy)methyl - 3 - cephem - 4 - carboxylic acid was synthesized as in Example 8.

20 IR(KBr): 1780, 1725, 1638 cm^{-1}
NMR (d_6 -DMSO): δ 3.43 & 3.76 (2H, ABq, $J=18$ Hz, 2- CH_2), 3.75 (2H, s, $-\text{CH}_2\text{CO}-$), 4.79 & 5.14 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.07 (1H, d, $J=5$ Hz, 6-H), 5.68 (1H, dd, $J=5$ & 8 Hz, 7-H), 6.35 (2H, s, $-\text{CH}=\text{CH}-$), 6.90 & 7.29 (3H,

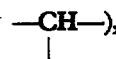


Example 19

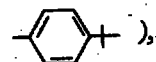
30 Dichloromethane (15 ml) and triethylamine (0.42 ml) were added to a mixture of 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (2.20 g) and 3 - nitrophthalic anhydride (1.16 g). The resulting solution was stirred at room temperature for 1 hour and a half. After the reaction had been completed, the dichloromethane was distilled off under reduced pressure and 3% aqueous phosphoric acid (120 ml) and ethyl acetate (160 ml) were added to the residue. The ethyl acetate layer was washed with water (80 ml $\times$ 2), dried (over magnesium sulphate) and concentrated under reduced pressure. The addition of diethyl-
45 ether, yielded a powder which was recovered by filtration, washed with ether and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7β - (D - 5 -

(p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.10 g). 50

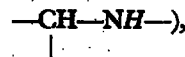
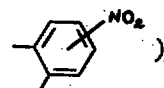
IR(KBr): 1783, 1735, 1640 cm^{-1}
NMR (δ in d_6 -DMSO): 1.28 (9H, s, $-\text{C}(\text{CH}_3)_3$), 1.53 & 2.23 (6H, $-(\text{CH}_2)_3-$), 3.43 & 3.70 (2H, ABq, $J=18$ Hz, 2- CH_2), 4.37 (1H,



4.96 & 5.41 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.07 (1H, d, $J=5$ Hz, 6-H), 5.68 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.42 & 7.80 (4H,



65

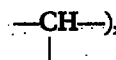


8.84 (1H, d, $J=8$ Hz, $-\text{CONH}-$).

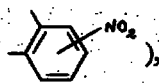
Example 20

70 Dichloromethane (15 ml) and triethylamine (0.42 ml) were added to a mixture of 7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (2.12 g) and 3 - nitrophthalic anhydride (1.16 g). The solution was stirred at room temperature for 1 hour and a half, after which time it was treated as in Example 19. The procedure provided 7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.08 g). 75

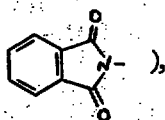
IR(KBr): 1775 (shoulder), 1718, 1642 cm^{-1}
NMR (δ in d_6 -DMSO): 1.54 & 2.19 (6H, $-(\text{CH}_2)_3-$), 3.40 & 3.68 (2H, ABq, $J=18$ Hz, 2- CH_2), 4.73 (1H, t, $J=7$ Hz,



4.98 & 5.42 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.06 (1H, d, $J=5$ Hz, 6-H), 5.66 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.7-8.4 (3H, 90



7.86 (4H, s,



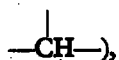
8.81 (1H, d, J=8 Hz, —CONH—).

Example 21

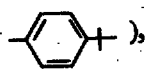
Dichloromethane (15 ml) and triethylamine (0.84 ml) were added to 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (2.20 g) and trimellitic anhydride (1.15 g), and the resulting solution was stirred at room temperature for 1 hour. After the reaction had been completed, the mixture was treated in the same manner as in Example 19. The procedure provided 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2,4 (or 5) - dicarboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.14 g).

IR(KBr): 1777, 1724, 1636 cm⁻¹

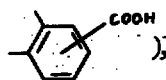
NMR (δ in d₆-DMSO): 1.29 (9H, s, —C(CH₃)₃), 1.54 & 2.25 (6H, —(CH₂)₃—), 3.99 & 3.72 (2H, ABq, J=18 Hz, 2-CH₂), 4.38 (1H,



4.95 & 5.30 (2H, ABq, J=13 Hz, 3-CH₂), 5.11 (1H, d, J=5 Hz, 6-H), 5.76 (1H, dd, J=5 & 8 Hz, 7-H), 7.43 & 7.82 (4H,



7.7—8.3 (3H,



8.43 (1H, d, J=8 Hz,



8.84 (1H, d, J=8 Hz, —CONH—).

Example 22

Desacetylcephalosporin C (2.16 g) was suspended in dimethylformamide (10 ml) and, under cooling with ice, concentrated hydrochloric acid (0.83 ml) was added. Dimethylformamide (10 ml) together with triethylamine (4.20 ml) and 3 - nitrophthalic anhydride

(3.86 g) were added to the resulting solution, and the resulting mixture was stirred at room temperature for 2 hours. Following this reaction, the mixture was diluted with 3% aqueous phosphoric acid (150 ml) and extracted with ethyl acetate (250 ml×2). The ethyl acetate layer was washed with water (200 ml), and a saturated aqueous solution of sodium chloride (200 ml), dried (over magnesium sulphate) and concentrated under reduced pressure. Then, upon the addition of diethylether, there was obtained a powder. This powder was recovered by filtration, washed with ether and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7β - (D - 5 - (2 - carboxy - 6 (or 3) - nitrobenzamido) - 5 - carboxyvaleramido) - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (3.77 g).

IR(KBr): 1780 (shoulder), 1729, 1638, 1534, 1348 cm⁻¹

NMR (δ in d₆-DMSO): 1.67 & 2.23 (6H, —(CH₂)₃—), 3.60 (2H, 2-CH₂), 4.39 (1H,



4.97 & 5.40 (2H, ABq, J=13 Hz, 3-CH₂), 5.08 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 & 8 Hz, 7-H), 7.5—8.5 (7H,



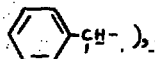
& —CH—NH—), 8.78 (1H, d, J=8 Hz, —CONH—).

Example 23

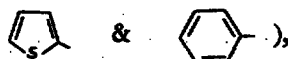
7β - (2 - thienylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid triethylamine salt (4.55 g) was dissolved in dichloromethane (30 ml), followed by the addition of O - carboxymandelic anhydride (2.38 g). The mixture was stirred at room temperature for 1 hour, after which time the dichloromethane was distilled off. 3% aqueous phosphoric acid (100 ml) was added to the residue, followed by extraction with ethyl acetate (150 ml). The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (100 ml×2), dried (over magnesium sulphate) and concentrated under reduced pressure. Then, upon the addition of diethylether, there was obtained a powder. This powder was recovered by filtration, washed with ether and dried over phosphorus pentoxide under reduced pressure. The procedure provided 7β - (2 - thienylacetamido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (4.00 g).

IR(KBr): 1778, 1742, 1666 cm⁻¹

NMR (δ in d_6 -DMSO): 3.24 & 3.45 (2H, ABq, $J=18$ Hz, 2-CH₂), 3.74 (2H, s, —CH₂CONH—), 4.76 & 5.06 (2H, ABq, $J=13$ Hz, 3-CH₂), 5.03 (1H, d, $J=5$ Hz, 6-H), 5.16 (1H, s,



5.68 (1H, dd, $J=5$ & 8 Hz, 7-H), 6.90 & 7.34 (8H,



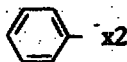
9.09 (1H, d, $J=8$ Hz, —CONH—).

Example 24

Sodium 7 β - mandelamido - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylate (3.86 g), was dissolved in dimethylformamide (40 ml), followed by the addition of O - carboxymandelic anhydride (2.67 g). The mixture was stirred at room temperature for 30 minutes, after which 2% H₃PO₄ (150 ml) was added. The mixture was extracted with ethyl acetate (250 ml) and the ethyl acetate layer was rinsed with water (150 ml $\times$ 2), dried (over magnesium sulphate) and concentrated under reduced pressure. Then, upon the addition of diethylether, there was obtained a powder. This powder was recovered by filtration, washed with ether and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7 β - mandelamido - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (3.68 g).

IR(KBr): 1777, 1745, 1669 cm⁻¹

NMR (δ in d_6 -DMSO): 3.2—3.7 (2H, 2-CH₂), 4.7—5.2 (5H, 3-CH₂, 6-H & —CH—x2), 5.69 (1H, 7-H), 7.2—7.6 (10H,



8.67 (1H, —CONH—).

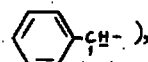
Example 25

7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (7.35 g) was dissolved in dimethylformamide (50 ml), followed by the addition of O - carboxymandelic anhydride (2.67 g). The mixture was stirred at room temperature for 30 minutes, after which time 3% aqueous phosphoric acid (250 ml) was added, followed by extraction with ethyl acetate (500 ml). The ethyl acetate layer was rinsed with water (250 ml $\times$ 2), dried (over magnesium sulphate) and concentrated under reduced pressure. Then, upon the addition of diethylether, there was obtained a powder. This powder was recovered by filtration, washed with ether and

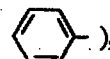
dried under reduced pressure over phosphorus pentoxide. The procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (6.30 g).

IR(KBr): 1778, 1736, 1642 cm⁻¹

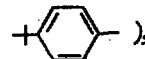
NMR (δ in d_6 -DMSO): 1.29 (9H, s, —C(CH₃)₃—), 1.73 & 2.23 (6H, —(CH₂)₃—), 3.2—3.6 (2H, 2-CH₂), 4.38 (1H, —CH—NH—), 4.75 & 5.04 (2H, ABq, $J=13$ Hz, 3-CH₂), 5.02 (1H, d, $J=5$ Hz, 6-H), 5.16 (1H, s,



5.66 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.2—7.5 (5H,



7.43 & 7.81 (4H,



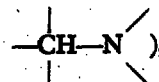
8.43 (1H, d, $J=8$ Hz, —CH—NH—), 8.80 (1H, d, $J=8$ Hz, —CONH—).

Example 26

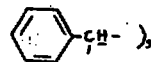
7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (7.05 g) was dissolved in dichloromethane (50 ml), followed by the addition of O - carboxymandelic anhydride (2.38 g). The mixture was stirred at room temperature for 1 hour, after which time it was treated as in Example 23. The procedure provided 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (6.25 g).

IR(KBr): 1773, 1715, 1647 (shoulder) cm⁻¹

NMR (δ in d_6 -DMSO): 1.54 & 2.22 (6H, —(CH₂)₃—), 3.27 & 3.49 (2H, ABq, $J=18$ Hz, 2-CH₂), 4.73 (1H, t, $J=7$ Hz,



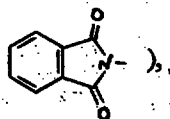
4.74 & 5.03 (2H, ABq, $J=13$ Hz, 3-CH₂), 4.98 (1H, d, $J=5$ Hz, 6-H), 5.16 (1H, s,



5.61 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.2—7.5 (5H,



7.86 (4H, s,



8.77 (1H, d, J=8 Hz, —CONH—).

Example 27

7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.51 g) was suspended in dichloromethane (75 ml). Then, at a temperature not exceeding 10°C, triethylamine (4.20 ml) was added. N,N - dimethylaniline (10.0 ml) together with dimethyldichlorosilane (4.40 ml) were added to the solution thus obtained. The mixture was stirred at 20–25°C. for 30 minutes. The mixture was then cooled to –30°C., followed by the addition of phosphorus pentachloride (4.20 g). It was then reacted at –25 ± 2°C. for 30 minutes, after which time methanol (25 ml) was added dropwise at a temperature not exceeding –20°C. The mixture was reacted at –15 to –10°C. for 20 minutes and, after the addition of water (50 ml), was stirred vigorously for 5 minutes. The reaction mixture was separated and the aqueous layer was collected, washed with ethyl acetate and adjusted to pH 3.2 with a 40% aqueous potassium carbonate solution. The resulting crystals were collected by filtration, washed with water, 50% aqueous methanol and acetone in the order mentioned and dried. The procedure provided 7 β - amino - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (3.43 g).

IR(KBr): 3170, 1798, 1730, 1700, 1615 cm⁻¹NMR (δ in D₂O+NaOD): 3.55 (2H, ABq, J=18 Hz), 4.6–5.6 (4H, m), 7.30–7.90 (4H, m).

Example 28

7 β - [D - 5 - phthalimido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy) - methyl - 3 - cephem - 4 - carboxylic acid (5.88 g) was suspended in dichloromethane (70 ml). Then, at a temperature not exceeding 10°C., triethylamine (2.80 ml) was added, followed by the addition of N,N - dimethylaniline (10.0 ml) and dimethyldichlorosilane (3.13 ml). The mixture was stirred at 20–25°C. for 30 minutes, after which phosphorus pentachloride (4.20 g) was added at –30°C. The mixture was reacted at –25 ± 2°C. for 30 minutes, after which time methanol (25 ml) was added dropwise at a temperature not exceeding –20°C. The reaction was further

allowed to proceed at from –15 to –10°C. for 20 minutes and water (50 ml) was then added at –15 to –10°C., followed by vigorous stirring for 5 minutes. The water layer was taken, washed with dichloromethane and adjusted to pH 3.5 with a 40% aqueous solution of potassium carbonate. The resulting crystals were recovered by filtration, washed with water, 50% aqueous methanol and acetone, and finally dried. The procedure provided 7 β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.84 g).

IR(KBr): 3200, 1800, 1745, 1720, 1622 cm⁻¹NMR (δ in D₂O+NaOD): 2.27 (3H, s), 3.48 (2H, ABq, J=18 Hz), 4.6–5.6 (4H, m).

Example 29

Desacetylcephalosporin C (13.7 g) was suspended in dimethylformamide (70 ml), followed by the addition of concentrated sulphuric acid (2.4 ml). Triethylamine (29 ml) was added to the resulting solution, together with N - carboethoxyphthalimide (8.5 g). The mixture was stirred at 30°C. for 50 minutes, followed by the addition of succinic anhydride (3.0 g). The mixture was stirred for 30 minutes, at the end of which time a further amount (0.6 g) of succinic anhydride was added. The mixture was further stirred for 1 hour and, then, poured into a cold saturated aqueous solution of sodium chloride (200 ml). The solution was made acidic with phosphoric acid and extracted three times with ethyl acetate. The extract was extracted back into a solution of sodium bicarbonate (8 g) in water (150 ml). The water layer was adjusted to pH 1.7 with phosphoric acid and extracted with a mixture of tetrahydrofuran and dichloromethane (1:4). The extract was dried over magnesium sulphate, filtered and, with the addition of triethylamine (18 ml), concentrated to dryness. Dichloromethane (200 ml) was added to the residue, together with triethylamine (6 ml) and dimethylaniline (30 ml). Then, following the addition of dimethyldichlorosilane (21 ml), the mixture was stirred for 30 minutes, after which it was cooled to –30°C. and phosphorus pentachloride (20 g) was added. The mixture was stirred at –30°C. for 30 minutes and, following the addition of methanol (63 ml), it was further stirred for 30 minutes. It was then diluted with water (120 ml), brought to pH 3.0 and allowed to cool. The resulting crystals were recovered by filtration. The above procedure provided 7 - amino - 3 - (3 - carboxypropionyl-oxy)methyl - 3 - cephem - 4 - carboxylic acid (5.4 g).

IR(KBr): 1802, 1735, 1720 (shoulder) cm⁻¹

Example 30

Dichloromethane (60 ml) was added to 7 β -

[D - 5 - (*p* - *t* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (6.67 g), and, under cooling with ice, dimethylaniline (3.78 ml) and triethylamine (4.20 ml) were also added. Dimethyldichlorosilane (3.87 g) was added to the resulting solution, followed by stirring at 8—15°C. for 1 hour. Then, at -30°C., dimethylaniline (1.26 ml) and phosphorus pentachloride (4.17 g) were added. The mixture was stirred at from -30° to -20°C. for 2 hours. Then, at -45°C., methanol (30 ml) was added dropwise over a period of 10 minutes. After the dropwise addition had been completed, the mixture was stirred at from -10°C. to -5°C. for 40 minutes and water (20 ml) was then added dropwise over 5 minutes. The mixture was adjusted to pH 3.3 with concentrated aqueous ammonia, whereupon a white slurry separated. After an hour of standing under ice-cooling, the precipitate was recovered by filtration, washed with water, methanol and ether in the order mentioned and dried under reduced pressure over phosphorus pentoxide.

The procedure provided 7 β - amino - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (2.31 g).

IR(KBr): 1800, 1740, 1621 cm⁻¹

Example 31

Dichloromethane (60 ml) was added to 7 β - [D - 5 - (*p* - *t* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid (7.26 g), and, under cooling with ice, dimethylaniline (3.78 ml) and triethylamine (4.20 ml) were added. Dimethyldichlorosilane (3.87 g) was added to the resulting solution, followed by stirring at 7—15°C. for 1 hour. The mixture was cooled to -30°C. and dimethylaniline (1.26 ml) and phosphorus pentachloride (4.17 g) were added. The mixture was stirred at -30 to -20°C. for 2 hours, after which time it was cooled to -45°C. and methanol (30 ml) was added dropwise over a period of 15 minutes. After the dropwise addition had been completed, the mixture was stirred at -10 to -8°C. for 40 minutes and water (20 ml) was then added dropwise over 10 minutes. The mixture was adjusted to pH 3.4 with concentrated aqueous ammonia, whereupon a substantially white slurry separated. After 45 minutes' standing under ice-cooling, the precipitate was recovered by filtration and washed with water, methanol and ether in the order mentioned. It was then dried under reduced pressure over phosphorus pentoxide. The procedure provided 7 β - amino - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid (2.93 g).

IR(KBr): 1787, 1734, 1614, 1535, 1350 cm⁻¹

Example 32

7 β - amino - 3 - (2 - carboxybenzoyloxy)-methyl - 3 - cephem - 4 - carboxylic acid (7.33 g) was suspended in water (48 ml), and at 0—3°C., 2N-sodium hydroxide (19.5 ml) was added in small instalments, care being used not to allow the pH to exceed 8.5. Then, following the addition of sodium bicarbonate (3.65 g), a solution of D - α - sulphophenylacetyl chloride (5.0 g) in ethyl acetate (8.8 ml) was added dropwise at 0—5°C. over a period of 1 hour. After the dropwise addition had been completed, the reaction was carried out at 0—5°C. for 20 minutes. The reaction mixture was adjusted to pH 5.5 and separated. The water layer was collected, degassed and, after confirmation that the pH was within the range of 5.5 to 6.5, ethanol (800 ml) was added over a period of 1 hour. Thereafter, the mixture was stirred for 30 minutes, followed by cooling to a temperature not exceeding 5°C. The resulting crystals were recovered by filtration, washed with ethanol-water (10:1) and ethanol in the order mentioned and dried. The procedure provided 7 β - (D - α - sulphophenylacetamido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid trisodium salt (11.6 g).

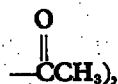
IR(KBr): 3350, 1768, 1735, 1670, 1610 cm⁻¹

NMR (δ in D₂O): 3.44 (2H, ABq, J=18 Hz), 5.00 (2H, ABq, J=13 Hz), 5.06 (1H, d, J=5 Hz), 5.08 (1H, s), 5.67 (1H, d, J=5 Hz), 7.3—7.9 (m, 9H).

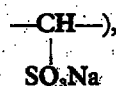
Example 33

7 β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.09 g) was suspended in water (48 ml), and, at 0—3°C., 2N-sodium hydroxide (10.4 ml) was added in small instalments, care being taken not to allow the pH to exceed 8.5. Then, following the addition of sodium bicarbonate (3.65 g), a solution of D - α - sulphophenylacetyl chloride (5.0 g) in ethyl acetate (8.8 ml) was added dropwise at 0—5°C. over a period of 1 hour. After the dropwise addition had been completed, the reaction was carried out at 0—5°C. for 20 minutes. Then, the reaction mixture was adjusted to pH 5.5 and separated. The water layer was collected, degassed and, after confirmation that the pH was within the range of from 5.5 to 6.5, ethanol (800 ml) was added to this aqueous solution (about 80 ml). Thereafter, the mixture was stirred for 30 minutes, at the end of which time it was cooled to a temperature not exceeding 5°C. The resulting crystals were recovered by filtration, washed with ethanol-water (10:1) and ethanol, and dried. The procedure provided 7 β - (D - α - sulphophenylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid disodium salt (8.8 g).

IR(KBr): 3300, 1763, 1740, 1680, 1610, 1215, 1047 cm^{-1}
NMR (δ in D_2O): 2.27 (3H, s,



3.29 (2H, ABq, $J=18$ Hz, 2- CH_2), 4.84 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.00 (1H, d, $J=5$ Hz, 6-H), 5.07 (1H, s,

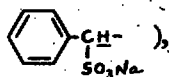


5.70 (1H, d, $J=5$ Hz, 7-H), 7.25—7.80 (5H, m).

Example 34

7 β - (D - α - sulphophenylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid disodium salt (940 mg) together with glutaric anhydride (580 mg) and triethylamine (404 mg) was dissolved in dimethylformamide (10 ml). The mixture was stirred at room temperature for 2 hours, after which time the dimethylformamide was distilled off. A small amount of water was added to the residue and the resulting aqueous solution was desalted with Amberlite IR-120 (H) (trade mark), adjusted to pH 6.0 with 1N-sodium hydroxide solution and lyophilized. The lyophilizate was re-dissolved in water and purified by column chromatography on Amberlite XAD-2 (trade mark). This procedure provided 7 β - (D - α - sulphophenylacetamido) - 3 - (4 - carboxybutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid trisodium salt.

IR(KBr): 1760, 1675, 1620 cm^{-1}
NMR (δ in D_2O): 1.65—2.60 (6H, m), 3.41 (2H, q, 2- CH_2), 4.83 (2H, d, 3- CH_2), 5.0 (1H, s,



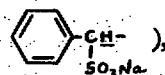
5.08 (1H, d, 6-H), 5.75 (1H, d, 7-H), 7.47 (5H, m).

Example 35

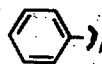
7 β - (D - α - sulphophenylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid disodium salt (470 mg) was dissolved in dimethylformamide (3 ml) together with succinic anhydride (250 mg) and triethylamine (200 mg). The mixture was stirred at room temperature for 2 hours. It was then diluted with a small amount of water and the dimethylformamide was distilled off under reduced pressure. The residue was dissolved by the addition of water and purified by column-

chromatography on Amberlite XAD-2 (trade mark). The procedure provided 7 β - (D - α - sulphophenylacetamido) - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid trisodium salt.

IR(KBr): 1765, 1685, 1600 cm^{-1}
NMR (δ in D_2O): 2.62 (4H, s, $-\text{CO}(\text{CH}_2)_2\text{CO}-$), 3.38 (2H, q, 2- CH_2), 4.95 (2H, 3- CH_2), 5.10 (1H, s,



5.21 (1H, d, 6-H), 5.91 (1H, d, 7-H), 7.78 (5H, m).



Example 36

7 β - (D - α - sulphophenylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid ditriethylamine salt (480 mg) was dissolved in chloroform (5 ml), followed by the addition of diketene (300 mg). The mixture was stirred at room temperature for 2 hours, after which time the solvent was distilled off. The residue was diluted with water and desalted with Amberlite IR-120 (H) (trade mark). The desalted solution was adjusted to pH 5.7 with 1N-sodium hydroxide solution and lyophilized. Yield: 400 mg. The lyophilizate was purified by column chromatography on Amberlite XAD-2 (trade mark). The procedure provided 7 β - (D - α - sulphophenylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid disodium salt.

In IR and NMR spectra, this product was found in good agreement with the product obtained in Example 33.

Example 37

7 β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (15.7 g) was suspended in dichloromethane (80 ml). At -10°C , triethylamine (10.1 g) was added. A solution of 4 - chloro - 3 - oxobutyryl chloride (1.41 mMol/g) (44.9 g) was added dropwise to the resulting solution at -20 to 15°C . over a period of 20 minutes. After the dropwise addition had been completed, the reaction was carried out at a temperature not exceeding -5°C . for 1 hour. After the reaction, the dichloromethane was distilled off and the residue was dissolved by the addition of tetrahydrofuran (50 ml), ethyl acetate (100 ml) and 10% aqueous phosphoric acid. The resulting solution was separated and the organic layer was collected. The water layer was extracted with a solvent mixture of ethyl acetate-tetrahydrofuran (5:1). The extracts were pooled, washed with a saturated aqueous solution of sodium chloride, dried over magnesium

19
 5 sulphate and filtered. The solvent was distilled off and the residue was treated with ethyl acetate - ether. The resulting powder was recovered by filtration, washed with ether and dried. The procedure provided 7β - (4 - chloro - 3 - oxobutylamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (20.4 g).

10 IR(KBr): 3325, 1782, 1740, 1732, 1715, 1685, 1650 cm⁻¹
 NMR (δ in d₆-DMSO): 2.14 (3H, s), 3.55 (4H, s), 3.54 (2H, br), 4.44 (2H, s), 4.90 (2H, ABq, J=13 Hz), 5.05 (1H, d, J=5 Hz), 5.54 (1H, q, J=5 & 8 Hz), 8.96 (1H, d, J=8 Hz).

15 The dichloromethane solution of 4 - chloro - 3 - oxobutyl chloride used in this reaction was prepared by dissolving diketene (84.0 g) in dichloromethane (420 ml) and introducing chlorine gas (78.1 g) at -30 to -35°C. for one hour.

Example 38

25 While a solution of diketene (0.91 g) in dichloromethane (2 ml) was stirred at -40 to -30°C., bromine (1.82 g) was added dropwise. Separately, 7β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.6 g) and triethylamine (1.7 g) were dissolved in dichloromethane (20 ml) and cooled to -40°C. This solution was added to the above reaction mixture. After stirring for 20 minutes, the mixture was further stirred under cooling with ice. Following the addition of water (7 ml), phosphoric acid and ethyl acetate, the mixture was stirred vigorously and the organic layer was washed with an aqueous solution of sodium chloride, dried and decolorized with activated carbon. Then, the solvent was distilled off and ether was added. The procedure provided 7β - (4 - bromo - 3 - oxobutylamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.4 g).

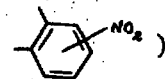
30 IR(KBr): 1790, 1730, 1645, 1545 cm⁻¹
 35 NMR (δ in d₆-DMSO): 2.17 (3H, s, COCH₃), 3.60 (2H, s, -COCH₂CO-), 3.3-3.8 (2H, broad, 2-CH₂), 4.36 (2H, s, BrCH₂CO-), 4.76 & 5.06 (2H, ABq, J=12 Hz, 3-CH₂), 5.07 (1H, d, J=4.5 Hz, 6-H), 5.68 (1H, dd, J=4.5 & 8 Hz, 7-H), 9.04 (1H, d, J=8 Hz, -CONH-).

Example 39

40 A solution of diketene (0.20 ml) in dichloromethane (1 ml) was stirred at -30°C.; a 1.5M solution of chlorine in carbon tetrachloride (2.0 g) was then added dropwise over a period of 10 minutes. The mixture was further stirred at -25 to -35°C for 30 minutes. Separately, 7β - amino - 3 - [2 - carboxy - 6 - (or 3) - nitrobenzoyloxy]methyl - 3 - cephem -

4 - carboxylic acid (0.90 g) and triethylamine (0.84 ml) were dissolved in dichloromethane (5 ml) and cooled to -5 to -10°C. To this solution was added the above reaction mixture dropwise at -20 to -30°C. over a period of 15 minutes. The mixture was then stirred at the same temperature for 45 minutes. Then, the reaction mixture was distilled under reduced pressure and the residue was stirred vigorously with ethyl acetate (25 ml), tetrahydrofuran (5 ml) and 10% aqueous phosphoric acid (20 ml). The organic layer was washed with a saturated aqueous solution of sodium chloride (15 ml), dried over sodium sulphate and concentrated under reduced pressure. A powder was obtained upon addition of ether. This powder was recovered by filtration, washed with ether (10 ml) and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7β - (4 - chloro - 3 - oxobutylamido) - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid (0.70 g).

65 NMR (δ in d₆-DMSO): 3.4-3.8 (2H, 2-CH₂), 3.54 (2H, s, -COCH₂CO-), 4.52 (2H, s, ClCH₂-), 4.94 & 5.22 (2H, ABq, J=13 Hz, 3-CH₂), 5.04 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 & 8 Hz, 7-H), 7.7-8.5 (3H, m,

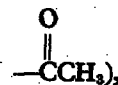


9.04 (1H, d, J=8 Hz, -CONH-).

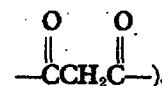
Example 40

7β - (4 - chloro - 3 - oxobutylamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (4.33 g) was dissolved in acetone (10 ml), and, under cooling with ice, water (10 ml) and thiourea (0.84 g) were added. Sodium bicarbonate (0.84 g) and water (10 ml) were then added. The mixture was reacted at room temperature for 5 hours, after which time it was cooled with ice. The resulting crystals were collected by filtration, washed with water, acetone and ether and dried. This procedure provided 7β - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (4.22 g).

100 IR(KBr): 1775, 1740, 1710, 1661 cm⁻¹
 105 NMR (δ in d₆-DMSO): 2.17 (3H, s,



3.38 (2H, s, -CH₂CONH-), 3.51 (2H, 2-CH₂), 3.59 (2H, s,



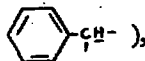
4.77 & 5.06 (2H, ABq, J=13 Hz, 3-CH₂), 5.05 (1H, d, J=5 Hz, 6-H), 5.69 (1H, dd, J=5 & 9 Hz, 7-H), 6.23 (1H, s, thiazoline-H), 8.82 (1H, d, J=9 Hz, —CONH—).

Example 41

7β - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid sodium salt (1.37 g) was dissolved in dimethylformamide (3.5 ml), followed by the addition of O - carboxymandelic anhydride (0.90 g). The mixture was stirred at room temperature for one hour, after which time most of the dimethylformamide was distilled off under reduced pressure. Ethyl acetate (50 ml) was added to the residue, followed by vigorous stirring. The resulting powder was recovered by filtration, and thereafter washed with ethyl acetate (20 ml), dichloromethane (20 ml) and ether (20 ml) in that order. This procedure provided 7β - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (1.60 g).

IR(KBr): 1780, 1743, 1665, 1643, 1537 cm⁻¹

NMR (δ in d₆-DMSO + D₂O): 3.33 & 3.65 (2H, ABq, J=18 Hz, 2-CH), 3.37 (2H, s, —CH₂CO—), 4.8—5.3 (2H, 3-CH₂), 4.97 (1H, d, J=5 Hz, 6-H), 5.21 (1H, s,



5.64 (1H, d, J=5 Hz, 7-H), 6.25 (1H, s, thiazolin-H), 7.2—7.6 (5H,



Example 42

7β - (2 - thienylacetamido) - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid sodium salt (3.86 g) was dissolved in N,N - dimethylformamide (20 ml) and, at -5°C, triethylamine (1.40 ml) and diketene (1.50 ml) were added. The reaction was carried out at from -5 to 0°C. for 1 hour, after which time the reaction mixture was poured into ice-water (200 ml). The resulting mixture was adjusted to pH 2.0 with 4N-hydrochloric acid and extracted with ethyl acetate. The ethyl acetate layer was rinsed with water, diluted with water, brought to pH 7.0 with 5% aqueous sodium bicarbonate solution and separated. The water layer was collected, concentrated and subjected to column-chromatography on Amberlite XAD-2 (trade mark), elution being carried out with a mixture of water and methanol. The eluate was lyophilized. The above procedure provided 7β - (2 - thienylacetamido) - 3 - (3 - oxo-

butyryloxy)methyl - 3 - cephem - 4 - carboxylic acid sodium salt (4.48 g).

IR(KBr): 3300, 1765, 1745, 1670, 1613 cm⁻¹

NMR (δ in D₂O): 2.31 (3H, s), 3.47 (2H, ABq, J=18 Hz), 3.85 (2H, s), 4.88 (2H, ABq, J=13 Hz), 5.08 (1H, d, J=5 Hz), 5.60 (1H, d, J=5 Hz), 6.9—7.5 (3H, m).

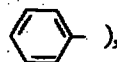
Example 43

7β - phenylacetamido - 3 - hydroxymethyl - 3 - cephem - 4 carboxylic acid triethylamine salt (4.5 g) was dissolved in dichloromethane (50 ml), followed by the addition of succinic anhydride (1.5 g). The mixture was stirred at room temperature for 5 hours, after which time the solvent was distilled off under reduced pressure. Following the addition of water and ethyl acetate, the residue was adjusted to pH 2.0 with phosphoric acid. The ethyl acetate layer was dried and concentrated. The resulting crystals were collected by filtration (2.9 g). The mother fluid was further concentrated and, after the addition of ether, the concentrate was allowed to stand, whereupon 1.6 g of crystals were obtained. These crystals were recrystallized from ethyl acetate.

The procedure provided 7β - phenylacetamido - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid, melting point: 86—89°C.

IR(KBr): 1800, 1735, 1692, 1660 cm⁻¹

NMR (δ in d₆-DMSO): 2.48 (4H, —(CH₂)₂—), 3.51 (4H, 2-CH₂, —CH₂CO—), 4.69 & 5.02 (2H, ABq, J=13 Hz), 5.00 (1H, d, J=5 Hz, 6-H), 5.63 (1H, dd, J=5 & 9 Hz, 7-H), 7.23 (5H,



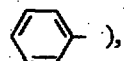
9.02 (1H, d, J=9 Hz, —CONH—).

Example 44

The reaction procedure of Example 43 was repeated except that phthalic anhydride (2.2 g) was used instead of succinic anhydride. This procedure provided 7β - phenylacetamido - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid. Melting point: 128—129°C. (ethyl acetate - ether).

IR(KBr): 1788, 1731, 1695, 1662 cm⁻¹

NMR (δ in d₆-DMSO): 3.53 (2H, s, —CH₂CO—), 3.61 (2H, 2-CH₂), 4.90 & 5.27 (2H, ABq, J=13 Hz, 3-CH₂), 5.08 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 & 8 Hz, 7-H), 7.25 (5H,



7.62 (4H,



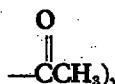
9.07 (1H, d, J=8 Hz, —CONH—).

Example 45

Dichloromethane (20 ml) was added to a mixture of 7β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (3.14 g) and dimethylacetamide (1.8 g). Then, under ice-cooling and stirring, phenoxyacetyl chloride (1.8 g) was added. The mixture was stirred for 1 hour, after which the insolubles were filtered off and the filtrate washed with an aqueous solution of sodium chloride. The organic layer was extracted with aqueous sodium bicarbonate solution. The water layer was made acidic with phosphoric acid and extracted with ethyl acetate. The extract was washed with aqueous sodium chloride solution, dried and distilled to remove the solvent. Ether was added to the residue and the resulting powder was recovered by filtration and washed with ether.

The procedure provided 7β - phenoxyacetamido - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 1788, 1722 cm⁻¹
NMR (δ in d₆-DMSO): 2.18 (3H, s,



3.60 (2H, 2-CH₂), 3.66 (2H, s,



4.67 (2H, s, —OCH₂—), 4.99 (2H, 3-CH₂), 5.18 (1H, d, J=5 Hz, 6-H), 5.78 (1H, dd, J=5 & 8 Hz, 7-H), 6.8—7.7 (5H, m), 9.07 (1H, d, J=8 Hz).

Example 46

7β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (314 mg) was suspended in dichloromethane (5 ml), and at -10°C., triethylamine (0.28 ml) was added. To the diketene (0.2 ml) was added to the resulting solution, and the reaction was carried out at a temperature not exceeding 0°C. for 2 hours. After this reaction, the dichloromethane was distilled off and the residue was dissolved in water - ethyl acetate, adjusted to pH 2.0 with 4N - hydrochloric acid and separated. The ethyl acetate layer was collected, diluted with water, adjusted to pH 7.0 with 5% aqueous sodium bicarbonate, and separated. The water layer was collected, concentrated and subjected to column

chromatography on Amberlite XAD-2 (trade mark), elution being carried out with water - methanol. The eluate was lyophilized. The above procedure provided 7β - (3 - oxobutyrylamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid sodium salt (375 mg).

IR(KBr): 3320, 1770, 1745, 1660, 1610 cm⁻¹

NMR (δ in D₂O): 2.27 (6H, s), 3.55 (2H, ABq, J=18 Hz), 4.93 (2H, ABq, J=13 Hz), 5.15 (1H, d, J=5 Hz), 5.70 (1H, d, J=5 Hz).

Example 47

7β - (4 - bromo - 3 - oxobutylamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (2.4 g) was dissolved in acetonitrile (5 ml), followed by the addition of thiocarbamic acid O - methyl ester (0.6 g). The mixture was stirred at room temperature overnight and the resulting crystals were recovered by filtration. The procedure provided 7β - [2 - (2 - oxo - 4 - thiazolin - 4 - yl)acetamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 1780, 1722, 1675, 1629 cm⁻¹

NMR (δ in d₆-DMSO): 2.17 (3H, s, —COCH₃), 3.32 (2H, s, —CH₂CONH—), 3.41 & 3.64 (2H, ABq, J=18 Hz, 2-H), 3.58 (2H, s, —COCH₂CO—), 4.75 & 5.06 (2H, ABq, J=13 Hz), 5.06 (1H, d, J=4.5 Hz, 6-H), 5.68 (1H, dd, J=4.5 & 8 Hz, 7-H), 5.99 (1H, s, thiazolin-H), 8.94 (1H, d, J=8 Hz, —CONH—), 11.06 (1H, thiazolin-NH).

Example 48

7β - amino - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid (660 mg) was suspended in dichloromethane (7 ml), followed by the addition of N,N - dimethylacetamide (0.7 ml). Then, under ice-cooling and stirring, a solution of (1H, tetrazol - 1 - yl)acetyl chloride (294 mg) in dichloromethane (2 ml) was added. The mixture was stirred at room temperature for 1 hour, after which time it was poured into an aqueous solution of sodium bicarbonate and separated. The water layer was collected, washed with dichloromethane, made acidic with phosphoric acid and extracted with ethyl acetate. The extract was re-extracted with aqueous sodium bicarbonate solution to bring the desired compound into the aqueous phase. This aqueous solution was purified by column-chromatography on Sephadex LH-20 (trade mark). The fractions containing the dominant product were pooled and lyophilized. The procedure provided 7β - [2 - (1H - tetrazol - 1 - yl)acetamido] - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid disodium salt.

IR(KBr): 1765, 1620 cm^{-1}

NMR (δ in D_2O): 2.60 (4H, m, $-(\text{CH}_2)_2-$), 3.44 & 3.74 (2H, ABq, $\text{J}=17$ Hz, $2-\text{CH}_2$), 5.20 (1H, d, $\text{J}=5$ Hz, 6-H), 5.59 (2H, s, $\text{NCH}_2\text{CO}-$), 5.76 (1H, d, $\text{J}=5$ Hz, 7-H), 9.33 (1H, s, tetrazol-H).

Example 49

7 β -amino-3-(3-oxobutyryloxy)methyl-3-cephem-4-carboxylic acid (942 mg) was suspended in dichloromethane (10 ml), followed by the addition of N,N-dimethylacetamide (1 ml). Then, under ice-cooling and stirring, a solution of (1H-tetrazol-1-yl)acetyl chloride (441 mg) in dichloromethane (3 ml) was added. The mixture was further stirred at room temperature for 30 minutes, after which it was poured into an aqueous solution of sodium bicarbonate. The water layer, i.e., aqueous extract, was purified by column-chromatography on Sephadex LH-20 (trade mark). The fractions rich in the desired product were pooled, concentrated, made acidic with phosphoric acid and extracted with ethyl acetate. The extract was dried, concentrated and treated with ether. The procedure provided 7 β -[2-(1H-tetrazol-1-yl)acetamido]-3-(3-oxobutyryloxy)methyl-3-cephem-4-carboxylic acid.

IR(KBr): 1782, 1707 cm^{-1}

NMR (δ in d_6 -DMSO): 2.17 (3H, s, $-\text{CH}_3$), 3.55 (2H, broad, $2-\text{CH}_2$), 3.59 (2H, s,



4.78 & 5.08 (2H, ABq, $\text{J}=13$ Hz, $3-\text{CH}_2$), 5.09 (1H, d, $\text{J}=5$ Hz, 6-H), 5.34 (2H, s, $\text{NCH}_2\text{CO}-$), 5.71 (1H, dd, $\text{J}=5$ & 8 Hz, 7-H), 9.28 (1H, s, tetrazol-H), 9.46 (1H, d, $\text{J}=8$ Hz, $-\text{CONH}-$).

Example 50

D- α -t-butoxycarbonylamino- α -(p-hydroxyphenyl)acetic acid (2.68 g) was dissolved in dry tetrahydrofuran (30 ml), followed by the addition of 2,6-lutidine (1.08 g). While cooling at -10°C . and stirring, ethyl chloroformate (1.08 g) was gently added and the mixture was stirred at -10°C . for 20 minutes. An ice-cooled mixed solution of 7 β -amino-3-(3-oxobutyryloxy)methyl-3-cephem-4-carboxylic acid (3.14 g) and sodium bicarbonate (1.0 g) in water (30 ml) was added in a single dose to the above mixture. The entire mixture was stirred at -3°C . for 10 minutes and, then, under cooling with ice-water, for 2 hours. Following the addition of water (60 ml), the mixture was washed with ethyl acetate (50 ml) and, under stirring in the presence of ethyl acetate (100 ml), 50%.

phosphoric acid was gently added so as to bring the pH to 3.0. The ethyl acetate layer was collected, rinsed with water (100 ml), dried over anhydrous sodium sulfate and treated with a 2N-solution of sodium 2-ethylhexanoate in isopropyl alcohol (6 ml). The resulting precipitate was collected by decantation, loosened with ethyl acetate, recovered by filtration and dried under reduced pressure over phosphorus pentoxide. The brown powder thus obtained was purified by column-chromatography on Amberlite XAD-2 (trade mark). The procedure provided 7 β -(D- α -t-butoxycarbonylamino- α -(p-hydroxyphenyl)acetamido)-3-(3-oxobutyryloxy)methyl-3-cephem-4-carboxylic acid sodium salt.

IR(KBr): 3400, 1770, 1680 1610 cm^{-1}

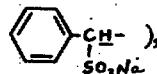
NMR (δ in D_2O): 1.45 (9H, s), 2.30 (3H, s), 3.43 (2H, broad), 5.65 (1H, d, $\text{J}=5$ Hz, 7-H), 6.76-7.40 (4H, m).

Example 51

7 β -(D- α -sulphophenylacetamido)-3-(3-oxobutyryloxy)methyl-3-cephem-4-carboxylic acid disodium salt (278 mg) was dissolved in water (0.3 ml), together with pyridine (60 mg) and KSCN (1.2 g). The mixture was heated at 60°C for 1 hour, after which it was subjected to column-chromatography on Amberlite XAD-2 (trade mark), elution being carried out with water. The fractions rich in the desired product were pooled, lyophilized and recrystallized from methanol. The procedure provided 7-(D- α -sulphophenylacetamido)-3-cephem-3-pyridiniummethyl-4-carboxylate sodium salt.

IR(KBr): 1760, 1665, 1610 cm^{-1}

NMR (δ in D_2O): 2.97, 3.35 (2H, ABq, $\text{J}=18$ Hz, $2-\text{CH}_2$), 5.27, 5.40 (2H, $3-\text{CH}_2$), 5.07 (1H, d, $\text{J}=5.2$ Hz, 6-H), 5.71 (1H, d, $\text{J}=5.2$ Hz, 7-H), 5.10 (1H, s,



7.47 (5H, m), 8.04, 8.55, 8.90 (5H,

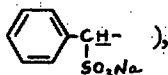


Example 52

The reaction procedure of Example 51 was repeated using isonicotinamide (90 mg) instead of pyridine and the reaction product was treated in the same manner as Example 51. The procedure provided 7-(D- α -sulphophenylacetamido)-3-cephem-3-(4-carbamoylpyridinium)methyl-4-carboxylate sodium salt. It was purified by recrystallization from ethanol-water. Melting point: 175°C . (decomp.).

IR(KBr): 1765, 1692, 1645, 1615, 1029 cm^{-1}

NMR (δ in D_2O): 2.99, 3.56 (2H, ABq, $J=18$ Hz, 2- CH_2), 5.40, 5.51 (2H, 3- CH_2), 5.13 (1H, d, $J=4.8$ Hz, 6-H), 5.73 (1H, d, $J=4.8$ Hz, 7-H), 5.10 (1H, s,



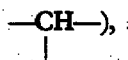
7.40 (5H, m), 8.31, 9.07 (4H).

Example 53

7 β - (2 - thienylacetamido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (0.49 g) was dissolved in 50% aqueous acetone (8 ml) together with acetylacetone (0.50 g) and sodium bicarbonate (0.17 g), and the resulting solution was stirred at 60°C. for 1 hour. The reaction mixture was brought to room temperature and most of the acetone was distilled off under reduced pressure. 5% aqueous phosphoric acid solution (10 ml) was added to the residue together with ethyl acetate (20 ml). The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (20 ml), dried over a magnesium sulphate and concentrated under reduced pressure. Then, upon the addition of ether, a powder was obtained. This powder was suspended in water (3 ml) and dissolved by the addition of sodium bicarbonate. The solution was subjected to column-chromatography on Sephadex LH-20 (trade mark), elution being carried out with water. The desired fractions were pooled and lyophilized. The procedure provided 7 β - (2 - thienylacetamido) - 3 - (2 - acetyl - 3 - oxo)butyl - 3 - cephem - 4 - carboxylic acid sodium salt (0.28 g). This product was dissolved in water (10 ml), followed by the addition of 10% aqueous phosphoric acid (2 ml) and ethyl acetate (20 ml). The ethyl acetate layer was rinsed with water, dried over magnesium sulphate and concentrated under reduced pressure. Then, upon the addition of ether, there was obtained a powder. This powder was recovered by filtration, washed with ether and dried under reduced pressure over phosphorus pentoxide. The procedure provided 7 β - (2 - thienylacetamido) - 3 - (2 - acetyl - 3 - oxo)butyl - 3 - cephem - 4 - carboxylic acid (0.23 g).

IR(KBr): 1765, 1718 cm^{-1}

NMR (δ in d_6 -DMSO): 2.13 & 2.18 (6H, s, $(\text{COCH}_3)_2$), 2.6—3.1 (2H, m, 3- CH_2), 3.31 & 3.56 (2H, ABq, 2- CH_2), 3.75 (2H, s, $-\text{CH}_2\text{CO}-$), 4.14 (1H,



5.01 (1H, d, $J=5$ Hz, 6-H), 5.58 (1H,

dd, $J=5$ & 8 Hz, 7-H), 6.92 & 7.30 (3H,



9.04 (1H, d, $J=8$ Hz, $-\text{CONH}-$). 60

Example 54

7 β - (2 - thienylacetamido) - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid (2.65 g) was dissolved in 50% aqueous acetone (40 ml) together with acetylacetone (2.42 g) and sodium bicarbonate (1.22 g). The mixture was stirred at 60°C. for 1 hour, after which time it was treated by a procedure similar to that described in Example 53. The procedure provided 7 β - (2 - thienylacetamido) - 3 - (2 - acetyl - 3 - oxo)butyl - 3 - cephem - 4 - carboxylic acid (1.26 g). In IR and NMR spectra, this product was in good agreement with the product according to Example 53. 65 70 75

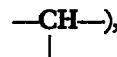
Example 55

7 β - [D - 5 - (*p* - *t* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid (0.73 g) was dissolved in 50% aqueous acetone (8 ml) together with acetylacetone (0.50 g) and sodium bicarbonate (0.34 g). The mixture was stirred at 60°C for 1 hour, after which it was treated by a procedure similar to that described in Example 53. 80 85

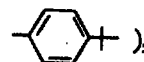
This procedure yielded 7 β - [D - 5 - (*p* - *t* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - acetyl - 3 - oxo)butyl - 3 - cephem - 4 - carboxylic acid (0.29 g). 90

IR(KBr): 1767, 1721, 1655, 1635 cm^{-1}

NMR (δ in d_6 -DMSO): 1.30 (9H, s, $-\text{C}(\text{CH}_3)_3$), 1.76 & 2.25 (6H, $-(\text{CH}_2)_4$), 2.6—3.1 (2H, m, 3- CH_2), 3.2—3.6 (2H, 2- CH_2), 4.13 (1H, $-\text{CH}(\text{COCH}_3)_2$), 4.37 (1H,



4.99 (1H, d, $J=5$ Hz, 6-H), 5.57 (1H, dd, $J=5$ & 8 Hz, 7-H), 7.43 & 7.83 (4H,



8.40 (1H, d, $J=8$ Hz, $-\text{CH}-\text{NH}-$), 8.77 (1H, d, $J=8$ Hz, $-\text{CONH}-$). 100

Example 56

7 β - [D - 5 - (*p* - *t* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (0.67 g) was dissolved in 50% aqueous acetone (8 ml) together with acetylacetone (0.50 g) and sodium bicarbonate (0.25 g). The mixture was stirred

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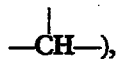
at 60°C. for 1.5 hours, after which it was treated by a procedure similar to that described in Example 53. The procedure provided 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - acetyl - 3 - oxo) - butyl - 3 - cephem - 4 - carboxylic acid (0.33 g). In IR and NMR spectra, this product was in agreement with the product obtained in Example 55.

Example 57

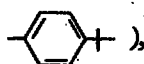
7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - [2 - carboxy - 6 (or 3) - nitrobenzoyloxy]methyl - 3 - cephem - 4 - carboxylic acid (0.73 g), pyrrole (0.20 g) and sodium bicarbonate (0.25 g) were dissolved in 50% aqueous acetone (14 ml). The mixture was stirred at 60°C. for 1 hour, after which it was brought down to room temperature and most of the acetone was distilled off under reduced pressure. Then, 5% aqueous phosphoric acid solution (15 ml) and ethyl acetate (30 ml) were added. The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (20 ml), dried (over magnesium sulphate) and concentrated under reduced pressure. A powder was obtained upon the addition of ether. This powder was suspended in water (3 ml) and dissolved by the addition of sodium bicarbonate (0.17 g). The solution was subjected to column-chromatography on Sephadex LH-20 (trade mark) (250 ml), elution being carried out with water. The fractions containing the desired product were pooled and lyophilized. The above procedure provided 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - pyrrolyl)methyl - 3 - cephem - 4 - carboxylic acid disodium salt (0.29 g).

IR(KBr): 1760, 1600 cm⁻¹

NMR (δ in D₂O): 1.23 (9H, s, -C(CH₃)₃), 1.86 & 2.42 (6H, -(CH₂)₃-), 2.86 & 3.25 (2H, ABq, J=18 Hz, 2-CH₂), 3.42 & 3.73 (2H, ABq, J=15 Hz, 3-CH₂), 4.51 (1H,



4.96 (1H, d, J=5 Hz, 6-H), 5.61 (1H, d, J=5 Hz, 7-H), 5.93 (1H, pyrrole - 3 - H), 6.07 (1H, pyrrole - 4 - H), 6.77 (1H, pyrrole - 5 - H), 7.38 & 7.76 (4H,



7.80 (1H, pyrrole - 1 - H).

Example 58

7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (0.73 g) was dissolved in 50% aqueous acetone (14 ml), together with N - methylpyrrole (0.24 g) and sodium hydro-

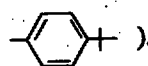
gen carbonate (0.25 g). The mixture was stirred at 60°C. for 1 hour. After the reaction had been completed, the mixture was treated in the same manner as in Example 57. The procedure provided 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (N - methylpyrrol - 2 - yl) - methyl - 3 - cephem - 4 - carboxylic acid disodium salt (0.21 g).

IR(KBr): 1757, 1597 cm⁻¹

NMR (δ in D₂O): 1.21 (9H, s, -C(CH₃)₃), 1.87 & 2.45 (6H, -(CH₂)₃-), 2.79 & 3.07 (2H, ABq, J=18 Hz, 2-CH₂), 3.46 (3H, s, N-CH₃), 3.51 & 3.87 (2H, ABq, J=15 Hz, 3-CH₂), 4.51 (1H,



4.91 (1H, d, J=5 Hz, 6-H), 5.58 (1H, d, J=5 Hz, 7-H), 5.86 (1H, pyrrole - 3 - H), 5.99 (1H, pyrrole - 4 - H), 6.61 (1H, pyrrole - 5 - H), 7.39 & 7.79 (4H,



Example 59

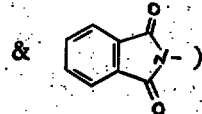
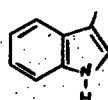
7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (0.64 g) was dissolved in 50% aqueous acetone (12 ml) together with indole (0.35 g) and sodium hydrogen carbonate (0.17 g). The mixture was stirred at 60°C. for 1 hour. Following the completion of the reaction, the mixture was treated by a procedure similar to that described in Example 57. The procedure provided 7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (3 - indolyl)methyl - 3 - cephem - 4 - carboxylic acid disodium salt (0.24 g).

IR(KBr): 1758, 1702, 1600 cm⁻¹

NMR (δ in D₂O): 1.68 & 2.26 (6H, -(CH₂)₃-), 2.45 & 2.82 (2H, ABq, J=18 Hz, 2-CH₂), 3.61 & 3.86 (2H, ABq, J=15 Hz, 3-CH₂), 4.6-4.9 (2H,



& 6-H), 5.46 (1H, d, J=5 Hz, 7-H), 7.0-7.8 (10H,



Example 60

7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (0.70 g) was dissolved in 50%

aqueous acetone (14 ml) together with indole (0.35 g) and sodium hydrogen carbonate (0.25 g). The mixture was stirred at 60°C. for 45 minutes and, after the reaction had been completed, the mixture was treated by a procedure similar to that described in Example 57. The procedure provided 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (3 - indolyl)methyl - 3 - cephem - 4 - carboxylic acid disodium salt (0.22 g). In the IR spectrum, this product was found to be the same as the product obtained in Example 59.

Example 61

7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - [2 - carboxy (6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (0.73 g) was dissolved in water (7 ml) together with sodium azide (0.26 g) and sodium hydrogen carbonate (0.25 g). The mixture was stirred at 60°C. for 40 minutes. The reaction mixture was then brought to room temperature and, following the addition of 10% aqueous phosphoric acid (10 ml), extracted with ethyl acetate (30 ml). The ethyl acetate layer was washed with a saturated solution of sodium chloride (20 ml), dried over magnesium sulphate and concentrated under reduced pressure. Upon addition of ether a powder was obtained. This powder was suspended in water (3 ml) and dissolved by the addition of sodium hydrogen carbonate (0.17 g). This solution was subjected to column-chromatography on Sephadex LH-20 (250 ml), elution being carried out with water. The fractions containing the desired product were pooled and then lyophilized.

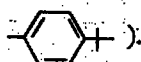
The above procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - azidomethyl - 3 - cephem - 4 - carboxylic acid disodium salt (0.36 g).

IR(KBr): 2100, 1766, 1606 cm⁻¹

NMR (δ in D₂O): 1.32 (9H, s, —C(CH₃)₃), 1.92 & 2.48 (6H, —(CH₂)₃—), 3.05 & 3.53 (2H, ABq, J=18 Hz, 2-CH₂), 4.03 & 4.20 (2H, ABq, J=13 Hz, 3-CH₂), 4.52 (1H,

—CH—),

5.08 (1H, d, J=5 Hz, 6-H), 5.68 (1H, d, J=5 Hz, 7-H), 7.56 & 7.86 (4H,



Example 62

7 - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - (3 - oxobutyl-oxymethyl) - 3 - cephem - 4 - carboxylic acid (908 mg) was dissolved in phosphate buffer (40 ml) of pH 6.4 together with a nitrogen-containing heterocyclic thiol (2.2 mMol) and sodium hydrogen

carbonate (336 mg). The solution was stirred at 60°C. for 1 hour, after which time it was concentrated under reduced pressure to about 20 ml. The concentrate thus obtained was subjected to column-chromatography on Amberlite XAD-2, elution being carried out with water, 5% ethanol, and 10% ethanol, respectively. The fractions containing the desired product were pooled and lyophilized to obtain compounds corresponding to one of the following. The reaction yield figure was the value determined by liquid chromatography immediately following the reaction:

(1) 7 - 2 - (2 - Imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - (2 - carboxymethyl - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid disodium salt; yield 85%.

IR(KBr): 1761 cm⁻¹

NMR (δ in D₂O): 3.56 & 3.92 (2H, ABq, J=18 Hz, 2-CH₂), 3.76 (2H, s, —CH₂CO), 4.16 (2H, s, —CH₂CO), 4.20 & 4.62 (2H, ABq, J=13 Hz, 3-CH₂), 5.24 (1H, d, J=5 Hz, 6-H), 5.79 (1H, d, J=5 Hz, 7-H), 6.65 (1H, s, thiazolin - 5 - H).

(2) 7 - [2 - (2 - Imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - (3 - hydroxymethyl - 4 - methyl - 1,2,4 - triazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid sodium salt; yield 82%.

IR(KBr): 1760 cm⁻¹

NMR (δ in D₂O): 3.40 & 3.82 (2H, ABq, J=18 Hz, 2-CH₂), 3.62 (2H, s, —CH₂CO), 3.74 (3H, s, —CH₃), 3.72 & 4.34 (2H, ABq, J=13 Hz, 3-CH₂), 4.82 (2H, s, CH₂OH), 5.08 (1H, d, J=5 Hz, 6-H), 5.64 (1H, d, J=5 Hz, 7-H), 6.52 (1H, s, thiazolin - 5 - H).

Example 63

5.61 g of 7 - [D - 5 - (benzamido)adipin-amido] - 3 - (3 - oxobutyl-oxymethyl) - 3 - cephem - 4 - carboxylic acid was dissolved in 50 ml of water together with 1.50 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 2.20 g of sodium bicarbonate. After the pH was adjusted to 5.2, the reaction was carried out at 60°C. for 50 minutes. After cooling, 100 ml of a saturated aqueous solution of sodium chloride was added and the pH was adjusted to pH 1.5 with 4N-HCl. The solid precipitate was recovered by filtration, rinsed with 20 ml of a saturated aqueous solution of sodium chloride and dissolved in 100 ml of ethyl acetate - tetrahydrofuran (2:1) and 20 ml of water. The organic layer was dried and the solvent was distilled off under reduced pressure. To the residue was added ether - ethyl acetate and the resultant powder was recovered by filtration, rinsed with ether and then dried. The procedure provided 5.45 g

(yield 94.8%) of 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazo - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3340, 1783, 1730, 1645, 1535 cm^{-1}

NMR (δ in d_6 -DMSO): 1.50—2.0 (4H, m), 2.05—2.45 (2H, m), 3.70 (2H, broad), 3.93 (3H, s, NCH_3), 4.15—4.55 (3H, m), 5.10 (1H, d, $J=5$ Hz, 6-H), 5.66 (1H, dd, $J=5$ & 9 Hz, 7-H), 7.32—7.97 (5H, m),



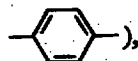
8.43 (1H, d, $J=8$ Hz, $-\text{CONH}-$), 8.73 (1H, d, $J=9$ Hz, $-\text{CONH}-$).

Example 64

6.11 g of 7 - [D - 5 - (*p* - *t* - toluenesulfonamido)adipinamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 50 ml of water together with 1.50 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 2.20 g of sodium bicarbonate. After the solution was adjusted to pH 5.0, the reaction was conducted at 60°C for 50 minutes. Following reaction, the reaction mixture was treated in the same manner as in Example 63. The procedure provided 5.96 g (yield 95.1%) of 7 - [D - 5 - (*p* - toluenesulfonamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3275, 1780, 1727, 1635, 1535 cm^{-1}

NMR (δ in d_6 -DMSO): 1.45—1.78 (4H, m), 2.0—2.3 (2H, m), 2.41 (3H, s, $-\text{CH}_3$), 3.71 (2H, broad, 2- CH_2), 3.95 (3H, s, $-\text{NCH}_3$), 4.28 (2H, broad), 5.06 (1H, d, $J=5$ Hz, 6-H), 5.62 (1H, dd, $J=5.0$ & 9.0 Hz, 7-H), 7.47 (4H, m),



7.91 (1H, d, $J=9.0$ Hz), 8.68 (1H, d, $J=9.0$ Hz).

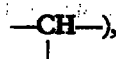
Example 65

6.17 g of 7 [D - 5 - (*p* - *tert* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 50 ml of water together with 1.74 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 2.50 g of sodium bicarbonate. After the solution was adjusted to pH 5.0, the reaction was conducted at 60°C for 50 minutes. Following reaction, the mixture was treated in the same manner as in Example 63 to obtain 6.02 g (yield 95.3%) of 7 - [D - 5 - (*p* - *tert* - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H -

tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3350, 1780, 1725, 1643, 1532, 1504 cm^{-1}

NMR (δ in d_6 -DMSO): 1.30 (9H, s, $-\text{C}(\text{CH}_3)_3$), 1.50—2.45 (6H, m, $-(\text{CH}_2)_3-$), 3.64 (2H, broad, 2- CH_2), 3.93 (3H, s, $-\text{NCH}_3$), 4.27 (2H, broad, 3- CH_2), 4.36 (1H, m,



5.0 (1H, d, $J=5$ Hz, 6-H), 5.62 (1H, dd, $J=5.0$ & 8.0 Hz, 7-H), 7.35 (2H, d, $J=8.0$ Hz), 7.70 (2H, d, $J=8$ Hz), 8.36 (1H, d, $J=8.0$ Hz, $-\text{CONH}-$), 8.76 (1H, d, $J=8.0$ Hz, $-\text{CONH}-$).

Example 66

4.83 g of 7 - [D - 5 - (caprylamido) - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy) - methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 50 ml of water together with 1.74 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 2.50 g of sodium bicarbonate. After the solution was adjusted to pH 5.0 and 35.0 g of sodium bromide was added, the reaction was conducted at 60°C for 45 minutes.

Following this reaction period, the reaction mixture was treated in the same manner as in Example 63. The procedure provided 4.78 g (yield 96.3%) of 7 - [D - 5 - (caprylamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3300, 1775, 1725, 1655, 1640, 1545, 1533 cm^{-1}

NMR (δ in d_6 -DMSO): 0.60—2.40 (21H, m), 3.70 (2H, broad, 2- CH_2), 3.95 (3H, s, NCH_3), 4.15 (1H, m), 4.27 (2H, broad, 3- CH_2), 5.03 (1H, d, $J=5$ Hz, 6-H), 5.65 (1H, dd, $J=5.0$ & 8.0 Hz, 7-H), 7.97 (1H, d, $J=8.0$ Hz, $-\text{CONH}-$), 8.76 (1H, d, $J=8.0$ Hz, $-\text{CONH}-$).

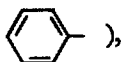
Example 67

4.32 g of 7 - phenylacetamido - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 50 ml of water together with 1.50 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 1.68 g of sodium bicarbonate. The reaction was conducted at 60°C for 50 minutes. After cooling, the reaction mixture was adjusted to pH 5.0 and washed with ethyl acetate. It was then brought to pH 2.0 and extracted three times with ethyl acetate. The ethyl acetate solutions were washed with a saturated aqueous solution of sodium chloride, treated with magnesium sulphate, filtered and distilled under reduced pressure to remove the solvent. The residue

was recrystallized from ethyl acetate - ether. The above procedure provided 4.29 g (yield 96.2%) of 7 - phenylacetamido - 3 - (1 - methyl - 1H - tetrazol - 5 - yl) thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3270, 1785, 1733, 1662, 1628, 1542 cm^{-1}

NMR (δ in d_6 -DMSO): 3.55 (2H, s, $-\text{CH}_2\text{CO}-$), 3.60 (2H, broad, $2-\text{CH}_2$), 3.92 (3H, s, NCH_3), 4.26 (2H, broad, $3-\text{CH}_2$), 5.00 (1H, d, $J=5.0$ Hz, 6-H), 5.60 (1H, dd, $J=5.0$ & 8.0 Hz, 7-H), 7.23 (5H, s,



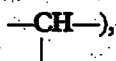
8.98 (1H, d, $J=8$ Hz, $-\text{CONH}-$).

Example 68

561 mg of 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 10 ml of water together with 252 mg of sodium bicarbonate and 212 mg of 2 - methylthio - 5 - mercapto - 1,3,4 - thiadiazole. The reaction was conducted at 60°C . for 50 minutes and, after cooling, the reaction mixture was washed with ethyl acetate and freeze-dried. The resultant solid was dissolved in a small quantity of methanol and treated with acetone. The crystals formed were collected by filtration and rinsed with ether. The procedure provided 614 mg of 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (2 - methylthio - 1,3,4 - thiadiazol - 5 - yl) thiomethyl - 3 - cephem - 4 - carboxylic acid disodium salt.

IR(KBr): 3400, 1766, 1640, 1600, 1530 cm^{-1}

NMR (δ in D_2O): 1.40—2.55 (6H, m, $-(\text{CH}_2)_3-$), 2.68 (3H, s, SCH_3), 3.33 (2H, ABq, $J=18$ Hz, $2-\text{CH}_2$), 4.14 (2H, ABq, $J=14$ Hz, $3-\text{CH}_2$), 4.30 (1H, m,



5.01 (1H, d, $J=4.5$ Hz, 6-H), 5.58 (1H, d, $J=4.5$ Hz, 7-H), 7.25—7.95 (5H, m,



Example 69

561 mg of 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 5 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 10 ml of water together with 252 mg of sodium carbonate and 224 mg of 5 - mercapto - 2 - ethoxycarbonylmethyl - 1H - 1,3,4 - triazole. The reaction was conducted at 60°C . for 50

minutes and, after cooling, the reaction mixture was treated in the same manner as in Example 68. The procedure provided 642 mg of 7 - [D - 5 - benzamido - 5 - carboxyvaleramido] - 3 - (2 - ethoxycarbonylmethyl - 1H - 1,3,4 - triazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid disodium salt.

IR(KBr): 3400, 3280, 1765, 1745, 1640, 1603, 1535 cm^{-1}

NMR (δ in D_2O): 1.20 (3H, t, $J=8.0$ Hz, $-\text{CH}_2\text{CH}_3$), 1.50—2.50 (6H, m, $-(\text{CH}_2)_3-$), 3.32 (2H, ABq, $J=19$ Hz, $2-\text{CH}_2$), 3.80—4.50 (7H, m), 4.95 (1H, d, $J=4.5$ Hz, 6-H), 5.52 (1H, d, $J=4.5$ Hz, 7-H), 7.20—7.90 (5H, m),



Example 70

1.27 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid was dissolved in 12 ml of water together with 0.25 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 0.68 g of sodium hydrogen carbonate, followed by stirring for one and a half hours at 60°C . The reaction solution was allowed to cool to room temperature and 30 ml of a 4% aqueous solution of phosphoric acid was then added, followed by extraction with 60 ml of ethyl acetate. The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (40 ml $\times$ 2) and dried over magnesium sulphate, followed by condensation under reduced pressure. The condensate had ether added thereto and the resultant powder was recovered by filtration, washed with ether and dried over phosphorus pentoxide under reduced pressure to give 1.02 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid.

In both IR and NMR spectra, this product was found to be in good agreement with the product obtained in Example 65.

Example 71

1.26 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (3 - carboxyacryloxy)methyl - 3 - cephem - 4 - carboxylic acid, 0.25 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 0.68 g of sodium hydrogen carbonate were dissolved in 12 ml of water and the solution was then stirred for 2 hours at 60°C ., followed by treatment in the same manner as in Example 70. The procedure provided 0.88 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid, of which both the IR and NMR spectra were

found to be in good agreement with those of the product obtained in Example 65.

Example 72

The reaction of Example 71 was repeated but employing 1.36 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid as starting compound. The procedure provided 0.97 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid, of which IR and NMR were found in good agreement with that of the product obtained in Example 65.

Example 73

1.55 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (3 - carboxy - 3 (or 2) - (p - chlorophenylthio)propionyloxy)methyl - 3 - cephem - 4 - carboxylic acid, 0.25 g of 5 - mercapto - 1 - methyl - 1H - tetrazole and 0.68 g of sodium hydrogen carbonate were dissolved in 12 ml of water, and the solution was stirred for one and a half hours at 60°C., followed by treatment in the same manner as in Example 70. The procedure provided 0.99 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid, of which both the IR and NMR spectra were found to be in good agreement with that of the product obtained in Example 65.

Example 74

The reaction of Example 73 was repeated but employing 1.21 g of 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid as starting compound. The procedure provided 1.00 g of 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid, of which both the IR and NMR spectra were found to be in good agreement with that of the product obtained in Example 1 (2).

Example 75

The reaction of Example 73 was repeated but employing 1.30 g of 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid as starting compound. The procedure provided 0.94 g of 7 β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (1 - methyl - 1 - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid, of which both the IR and NMR spectra were found to be in good agreement with that of the product obtained in Example 1 (2).

Example 76

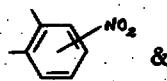
7 β - [D - 5 - (2 - carboxy - 6 (or 3) - nitrobenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (0.78 g) was dissolved in water (6 ml) together with 5 - mercapto - 1 - methyl - 1H - tetrazole (0.12 g) and sodium hydrogen carbonate (0.42 g). The solution was stirred at 60°C. for 30 minutes, after which time it was treated as in Example 70. The procedure provided 7 β - [D - 5 - (2 - carboxy - 6 (or 3) - nitrobenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (0.50 g).

IR(KBr): 1782, 1731, 1645, 1537, 1351 cm⁻¹

NMR (d₆-DMSO): δ 1.73 & 2.26 (6H, —(CH₂)₃), 3.69 (2H, 2-CH₂), 3.94 (3H, s, >N—CH₃), 4.32 (2H, 3-CH₂), 4.52 (1H,



5.06 (1H, d, J=5 Hz, 6-H), 5.67 (1H, dd, J=5 & 8 Hz, 7-H), 7.6—8.4 (4H,



8.79 (1H, d, J=8 Hz, —CONH—).

Example 77

7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (0.73 g) was dissolved in water (6 ml) together with 5 - mercapto - 1 - methyl 1H tetrazole (0.12 g) and sodium hydrogen carbonate (0.34 g). The mixture was stirred at 60°C. for 30 minutes, after which time it was treated as in Example 70. The procedure provided 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (0.53 g).

In both IR(KBr) and NMR (d₆-DMSO) spectra, the compound obtained was in good agreement with the product obtained in Example 65.

Example 78

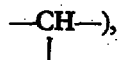
The reaction of Example 76 was repeated but employing 0.73 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2,4 (or 5) - dicarboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid as starting compound to give 0.52 g of 7 β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] -

- 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thio-
methyl - 3 - cephem - 4 - carboxylic acid. In
both IR(KBr) and NMR (d_6 -DMSO) spectra,
this compound was in good agreement with
the product obtained in Example 65.

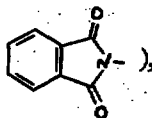
Example 79

- 7 β - (D - 5 - phthalimido - 5 - carboxy-
valeramido) - 3 - (2 - carboxy - 6 (or 3) -
nitrobenzoyloxy)methyl - 3 - cephem - 4 -
carboxylic acid (0.70 g) was dissolved in water
(6 ml) together with 5 - mercapto - 2 -
methyl - 1,3,4 - thiadiazole (0.13 g) and
sodium hydrogen carbonate (0.34 g). The solu-
tion was stirred at 60°C. for 30 minutes, after
which time it was treated as in Example 65.
The procedure provided 7 β - (D - 5 - phthal-
imido - 5 - carboxyvaleramido) - 3 - (2 -
methyl - 1,3,4 - thiadiazol - 5 - yl)thiomethyl -
3 - cephem - 4 - carboxylic acid (0.51 g).

- IR(KBr): 1733 (sh), 1715, 1648 (sh) cm^{-1}
NMR (d_6 -DMSO): δ 1.53 & 2.15 (6H,
—(CH₂)₃—), 2.67 (3H, s, —CH₃), 3.45
& 3.72 (2H, ABq, J=18 Hz, 2-CH₂),
4.19 & 4.50 (2H, ABq, J=13 Hz,
3-CH₂), 4.72 (1H, t, J=7 Hz,



5.02 (1H, d, J=5 Hz, 6-H), 5.61 (1H,
dd, J=5 & 8 Hz, 7-H), 7.87 (4H, s,

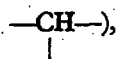


- 8.74 (1H, d, J=8 Hz, —CONH—).

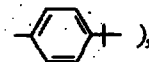
Example 80

- The reaction of Example 79 was repeated
but employing 0.73 g of 7 β - [D - 5 - (p - t -
butylbenzamido) - 5 - carboxyvaleramido] -
3 - (2 - carboxy - 6 (or 3) - nitrobenzoyloxy)-
methyl - 3 - cephem - 4 - carboxylic acid as
starting compound to give 0.55 g of 7 β - [D -
5 - (p - t - butylbenzamido) - 5 - carboxy-
valeramido] - 3 - (2 - methyl - 1,3,4 - thia-
diazol - 5 - yl)thiomethyl - 3 - cephem - 4 -
carboxylic acid.

- IR(KBr): 1780, 1728, 1644 cm^{-1}
NMR (d_6 -DMSO): δ 1.28 (9H, s,
—C(CH₃)₃), 1.74 & 2.23 (6H,
—(CH₂)₃—), 2.66 (3H, s, —CH₃), 3.50
& 3.75 (2H, ABq, J=18 Hz, 2-CH₂),
4.20 & 4.50 (2H, ABq, J=13 Hz,
3-CH₂), 4.39 (1H,



5.05 (1H, d, J=5 Hz, 6-H), 5.65 (1H,
dd, J=5 & 8 Hz, 7-H), 7.44 & 7.80 (4H,



8.42 (1H, d, J=8 Hz,

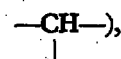


8.80 (1H, d, J=8 Hz, —CONH—).

Example 81

- 7 β - (D - mandelamido) - 3 - (3 - carboxy-
propionyloxy)methyl - 3 - cephem - 4 - car-
boxylic acid (0.46 g) was dissolved in water (5
ml) together with 5 - mercapto - 1 - methyl -
1H - tetrazole (0.12 g) and sodium hydrogen
carbonate (0.25 g). The solution was stirred at
60°C. for 1 and a half hours. After cooling
in air, the reaction mixture was subjected to
column chromatography on Amberlite XAD-2,
elution being carried out with water and,
with a solvent mixture of water and methanol.
The fractions containing the desired com-
pound were pooled, concentrated and
lyophilized. The procedure provided 7 β - (D -
mandelamido) - 3 - (1 - methyl - 1H -
tetrazol - 5 - yl)thiomethyl - 3 - cephem -
4 - carboxylic acid sodium salt (0.31 g).

IR(KBr): 1761, 1675, 1604 cm^{-1}
NMR (D₂O): δ 3.25 & 3.68 (2H, ABq,
J=18 Hz, 2-CH₂), 3.95 (3H, s,
>N—CH₃), 4.02 & 4.29 (2H, ABq, J=
13 Hz, 3-CH₂), 4.97 (1H, d, J=5 Hz,
6-H), 5.18 (1H, s,



5.51 (1H, d, J=5 Hz, 7-H), 7.37 (5H, s,



Example 82

- 7 β - (2 - thienylacetamido) - 3 - (2 -
carboxybenzoyloxy)methyl - 3 - cephem - 4 -
carboxylic acid (0.50 g) was dissolved in water
(5 ml) together with sodium hydrogen car-
bonate (0.17 g), potassium iodide (0.40 g)
and pyridine (0.21 g). The solution was
adjusted to pH 6.5 and then stirred at 60°C.
for one and a half hours. After cooling in air,
the reaction mixture was subjected to column
chromatography on Amberlite XAD-2, elution
being carried out with water and, then, with
a solvent mixture of water and methanol. The
fractions containing the desired product were
pooled, concentrated and lyophilized.
The procedure provided 7 β - (2 - thienyl

acetamido) - 3 - (1 - pyridylmethyl) - 3 -
cephem - 4 - carboxylic acid betaine (0.23 g),

IR(KBr): 1763, 1698, 1617 cm^{-1}

NMR (D_2O): δ 3.17 & 3.67 (2H, ABq, $J=17$ Hz, 2-CH_2), 3.38 (2H, s, $-\text{CH}_2\text{CO}-$), 5.19 (1H, d, $J=5$ Hz, 6-H), 5.41 & 5.67 (2H, ABq, $J=14$ Hz, 3- CH_2), 5.75 (1H, d, $J=5$ Hz, 7-H), 7.01 & 7.28 (3H,



8.15 & 8.62 & 9.04 (5H,



Example 83

The following compounds were synthesized by procedures similar to that described in Example 79.

(1) 7β - (D - 5 - Phthalimido - 5 - carboxyvaleramido) - 3 - [2 - (2 - hydroxyethylthio) - 1,3,4 - thiadiazol - 5 - yl]thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3325, 1780, 1715, 1645, 1530 cm^{-1}

NMR ($\text{d}_6\text{-DMSO}$): δ 1.30—2.40 (m, 6H), 3.20—3.80 (m, 6H), 4.27 (AB-q, 2H, $J=12$ Hz); 4.65 (t, 1H, $J=9$ Hz), 4.96 (d, 1H, $J=5$ Hz), 5.55 (q, 1H, $J=5$ & 8 Hz), 7.87 (s, 4H), 8.70 (d, 1H, $J=8$ Hz).

(2) 7β - (D - 5 - Phthalimido - 5 - carboxyvaleramido) - 3 - (2 - carbamoylmethylthio - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 3430, 3340, 1776, 1717, 1680, 1535 cm^{-1}

NMR ($\text{d}_6\text{-DMSO}$): δ 1.30—2.40 (m, 6H), 3.57 (broad; 2H), 4.40 (s, 2H), 4.32 (AB-q, 2H, $J=12$ Hz), 4.70 (t, 1H, $J=8.0$ Hz), 5.0 (1H, d, $J=5$ Hz), 5.55 (q, 1H, $J=5$ & 8 Hz), 7.20 (br. 1H), 7.60 (br. 1H), 7.86 (s, 4H), 8.74 (d, 1H, $J=5$ Hz).

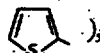
Example 84

7β - (2 - thienylacetamido) - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid was prepared by a procedure similar to that described in Example 81. The yield was 0.37 g, starting from 0.50 g of 7β - (2 - thienylacetamido) - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid.

IR(KBr): 1776, 1734, 1672 cm^{-1}

NMR ($\text{d}_6\text{-DMSO}$): δ 3.56 & 3.78 (2H,

ABq, $J=18$ Hz, 2-CH_2), 3.73 (2H, s, $-\text{CH}_2\text{CO}-$), 3.92 (3H, s, $>\text{N}-\text{CH}_3$), 4.21 & 4.37 (2H, ABq, $J=13$ Hz, 3- CH_2), 5.05 (1H, d, $J=5$ Hz, 6-H), 5.66 (1H, dd, $J=5$ & 8 Hz, 7-H), 6.90 & 7.29 (3H,



9.10 (1, d, $J=8$ Hz, $-\text{CONH}-$).

Example 85

7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (1.33 g) was dissolved in water (12 ml) together with 5 - mercapto - 1 - methyl - 1H - tetrazole (0.25 g) and sodium hydrogen carbonate (0.51 g). The mixture was stirred at 60°C . for 30 minutes, after which time it was brought to room temperature. Following the addition of a 4% aqueous phosphoric acid solution (30 ml), the reaction mixture was extracted with ethyl acetate (60 ml). The ethyl acetate layer was washed with a saturated aqueous solution of sodium chloride (40 ml $\times$ 2), dried (over magnesium sulphate) and concentrated under reduced pressure. Upon addition of ether, a powder was obtained. This powder was recovered by filtration, washed with ether and then dried under reduced pressure over phosphorus pentoxide. The procedure provided 7β - [D - 5 - (p - t - butylbenzamido) - 5 - carboxyvaleramido] - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (1.14 g).

In both IR(KBr) and NMR ($\text{d}_6\text{-DMSO}$) spectra, this compound was in good agreement with the product of Example 65.

Example 86

7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (1.27 g) was dissolved in water (12 ml) together with 5 - mercapto - 1 - methyl - 1H - tetrazole (0.25 g) and sodium hydrogen carbonate (0.51 g). The solution was stirred at 60°C . for 30 minutes. After the reaction was completed, the reaction mixture was treated in the same manner as in Example 85. The procedure provided 7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (1.12 g).

In both IR(KBr) and NMR ($\text{d}_6\text{-DMSO}$) spectra, this compound was in good agreement with the product obtained in Example 1 (3).

Example 87

7β - (D - mandelamido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (0.50 g) was dissolved in water (5 ml) together with 5 - mercapto - 1 - methyl - 1H -

5 tetrazole (0.12 g) and sodium hydrogen carbonate (0.17 g). The solution was stirred at 60°C. for 30 minutes. After cooling in air, the reaction mixture was subjected to column chromatography on Amberlite XAD-2, elution being carried out with water and a solvent mixture of water and methanol. The fractions containing the desired product were pooled, concentrated and then lyophilized. The procedure provided 7β - (D - mandelamido) - 3 - 1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid sodium salt (0.34 g).

10 In both IR and NMR spectra, this compound was in good agreement with the product obtained in Example 81.

Example 88

20 7β - (2 - thienylacetamido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (488 mg) was dissolved in water (5 ml) together with sodium hydrogen carbonate (84 mg), potassium iodide (400 mg) and pyridine (212 mg). The solution was adjusted to pH 6.5 and reacted at 60°C. for 45 minutes. After cooling, the reaction mixture was subjected to column chromatography on Amberlite XAD-2, elution being carried out with water and, then, a solvent mixture of water and methanol. The fraction containing the desired product were pooled, concentrated and then lyophilized. The procedure provided 7β - (2 - thienylacetamido) - 3 - (1 - pyridylmethyl) - 3 - cephem - 4 - carboxylic acid betaine (250 mg).

35 In both IR and NMR spectra, this compound was in good agreement with the product obtained in Example 82.

Example 89

40 7β - (2 - thienylacetamido) - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid (0.49 g) was dissolved in water (4 ml) together with 5 - mercapto - 1 - methyl - 1H - tetrazole (0.12 g) and sodium hydrogen carbonate (0.17 g). The solution was stirred at 60°C. for 30 minutes. After the reaction had been completed, the reaction mixture was treated by a procedure similar to that described in Example 85. The procedure provided 7β - (2 - thienylacetamido) - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (0.40 g).

50 In both IR and NMR spectra, this compound was in good agreement with the product obtained in Example 84.

Example 90

55 The following compounds were synthesized by procedures similar to those described in Examples 85 and 86.

60 (1) 7β - [D - 5 - (p - t - Butylbenzamido) - 5 - carboxyvaleramido] - 3 - (2 - methyl - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid. Yield 84%.

In both IR and NMR spectra this compound was in good agreement with the product obtained in Example 80.

(2) 7β - (D - 5 - Phthalimido - 5 - carboxyvaleramido) - 3 - (2 - methyl - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid. Yield 86%.

In both IR and NMR spectra, this compound was in good agreement with the product obtained in Example 79.

(3) 7β - (D - 5 - Phthalimido - 5 - carboxyvaleramido) - 3 - [2 - (2 - hydroxyethylthio) - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid. Yield 81%.

In both IR and NMR spectra, this compound was in good agreement with the product obtained in Example 83 (1).

(4) 7β - (D - 5 - Phthalimido - 5 - carboxyvaleramido) - 3 - (2 - carbamoylmethylthio - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid. Yield 88%.

In both IR and NMR spectra, this compound was in good agreement with the product obtained in Example 83 (2).

Example 91

7β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (3.14 g), sodium hydrogen carbonate (1.84 g) and 5 - mercapto - 1 - methyl - 1H - tetrazole (1.4 g) were dissolved in water (30 ml) and the solution was adjusted to pH to 5.5, followed by stirring and heating at 60°C. for one hour. After cooling, the reaction solution was washed with dichloromethane (20 ml) and the aqueous layer was adjusted to pH 3.3, followed by stirring for one hour under ice-cooling. The resultant precipitates were collected by filtration and washed with water, methanol and acetone, respectively, followed by drying to give 7β - amino - 3 - (1 - methyl - 1H - tetrazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (2.7 g).

IR(KBr): 1795 cm⁻¹

NMR (δ in D₂O + NaHCO₃): 3.61 & 3.98 (2H, ABq, J=18 Hz, 2-CH₂), 4.21 (s, -NCH₃), 5.21 (d, J=4.5 Hz, 6-H), 5.60 (d) J=4.5 Hz, 7-H).

Example 92

7β - amino - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (3.14 g), sodium hydrogen carbonate (0.84 g) and 5 - mercapto - 1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazole (2.60 g) were dissolved in water (30 ml) and the resultant solution was stirred for 60 minutes at 55°C. After cooling, acetone (15 ml) was added to the reaction solution and the mixture was passed through a column of active alumina (10 g). The column was washed with water - acetone (1:1) (30 ml) and the washing was combined with the eluate, followed by distilling off acetone under reduced pressure. To the

residual solution was added Amberlite IR-120 (acid form) (6.0 ml) and the mixture was stirred for 30 minutes under ice-cooling. The insolubles were filtered off and the filtrate was condensed. The condensate was added dropwise into ethanol (about 30 times volume of the condensate) and precipitated solid was collected by filtration, followed by washing with ethanol and drying to give 7 β - amino - 3 - 1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazol - 5 - yl + thiomethyl - 3 - cephem - 4 - carboxylic acid (3.28 g).

IR(KBr): 3450, 1780, 1620, 1540 cm⁻¹
NMR (δ in D₂O): 3.07 (6H, s), 3.70 (2H, ABq, J=17 Hz), 3.85 (2H, t, J=6 Hz), 4.25 (2H, ABq, J=12 Hz), 4.8—5.2 (4H, m).

Example 93

7 β - amino - 3 - (3 - oxobutyryloxy)-methyl - 3 - cephem - 4 - carboxylic acid (3.14 g) was dissolved in water (30 ml) together with sodium hydrogen carbonate (1.84 g) and 2 - mercapto - 5 - methyl - 1,3,4 - thiadiazole (1.6 g) and the resultant solution was adjusted its pH to 6.4, followed by stirring for one hour at 60°C. After cooling, the reaction solution was washed with dichloromethane and the pH of the aqueous layer was adjusted to 3.5 under ice-cooling, followed by stirring for one hour. The precipitated materials were collected by filtration and washed with water, methanol and acetone, respectively, followed by drying to give 7 β - amino - 3 - (2 - methyl - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (2.9 g).

IR(KBr): 1795 cm⁻¹
NMR (δ in D₂O + NaHCO₃): 2.87 (3H, s, thiadiazole —CH₃), 3.53 & 3.95 (2H, ABq, J=18 Hz, 2-CH₂), 4.10 & 4.46 (2H, ABq, J=13 Hz, 3-CH₂), 5.17 (1H, d, J=4.5 Hz, 6-H), 5.58 (1H, d, J=4.5 Hz, 7-H).

Example 94

7 β - amino - 3 - (3 - oxobutyryloxy)-methyl - 3 - cephem - 4 - carboxylic acid (282 mg) was dissolved under ice-cooling in water (1 ml) containing sodium salt of 5 - mercapto - 1H - 1,2,3 - triazole (120 mg) and sodium hydroxide (40 mg) and to the resultant solution was added 1N-HCl with stirring to adjust its pH to 5.5, followed by further stirring for one hour at 55°C. Methanol (5 ml) was added to the reaction solution and the mixture was then allowed to cool to room temperature. The cooled mixture was adjusted to pH 3.9 by adding 1N-HCl with stirring and the resultant mixture was further stirred for one hour under ice-cooling. The precipitated insolubles were collected by filtration and washed with water and methanol, respectively. The insolubles were dried naturally and then over

phosphorus pentoxide to give 7 β - amino - 3 - (1H - 1,2,3 - triazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid (190 mg).

IR(KBr): 1800, 1525 cm⁻¹

Example 95

7 β - amino - 3 - (3 - oxobutyryloxy)-methyl - 3 - cephem - 4 - carboxylic acid (314 mg) was dissolved in water (1 ml) containing sodium hydrogen carbonate (84 mg) and to the resultant solution were added isonicotinamide (185 mg) and potassium iodide (1.0 g), followed by stirring for one hour at 55°C. To the reaction mixture was added ethanol (20 ml) with stirring and resulting precipitates were collected by filtration, followed by washing with ethanol. After natural drying, the brown powder was dissolved in water (3 ml) and the solution was chromatographed on a column packed with Amberlite XAD-2. The eluate was freeze-dried to give 7 β - amino - 3 - (4 - carbamoylpyridinium)methyl - 4 - carboxylate (150 mg).

IR(KBr): 3500, 1760, 1600 cm⁻¹

Example 96

7 β - (2 - thienylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid (876 mg) and sodium sulfite (504 mg) were dissolved in a mixture of tetrahydrofuran (5 ml) and water (10 ml) and the resultant solution was stirred for 120 minutes at 60°C. Water (10 ml) was added to the reaction mixture and the mixture was adjusted to pH to 7.2 with 2N-HCl, followed by condensation under reduced pressure. The residue was subjected to column - chromatography on silica-gel and eluted with acetonitrile - water (7:1—5:1). The fractions containing the desired compound were combined, and the mixture was subjected to distillation to remove acetonitrile. The residue was chromatographed on column packed with Amberlite XAD-2 and eluted with water and then water-methanol. The fractions containing the desired compound were combined and the mixture was condensed under reduced pressure, followed by freeze-drying to give the disodium salt of 7 β - (2 - thienylacetamido) - 3 - sulfomethyl - 3 - cephem - 4 - carboxylic acid (310 mg).

IR(KBr): 3450, 1760, 1665, 1605, 1190, 1055 cm⁻¹

NMR (δ in D₂O): 3.67 (2H, ABq, J=17 Hz), 3.92 (2H, s), 4.16 (2H, ABq, J=16 Hz), 5.20 (1H, d, J=5 Hz), 5.64 (1H, d, J=5 Hz), 7.05 & 7.40 (3H, m).

Example 97

7.05 g of 7 - [D - phthalimido - 5 - carboxyvaleramido] - 3 - hydroxymethyl - 3 - cephem - 4 - carboxylic acid diethylethylamine

salt was dissolved in 50 ml of dichloromethane and, at 0°C., triethylamine (1.5 ml) was added to the solution, followed by dropwise addition of dropping diketene (2.0 ml) over 10 minutes at -5 to 0°C. The mixture was further stirred for 50 minutes at -5 to 0°C. and 40 ml of water was then added, followed by adjusting the pH to 6.0 with 2N-HCl. The aqueous layer was washed with dichloromethane (10 ml) and 2.25 g of 5 - mercapto - 1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazole was added thereto, followed by adjusting to pH 5.5. The solution was stirred at 60°C. for 40 minutes, and then the insolubles were filtered off. To the filtrate were added sodium chloride (15.0 g) and a saturated aqueous solution of sodium chloride (50 ml) and the mixture was adjusted to pH 2.0 with 4N-HCl. The solid materials precipitated were collected by filtration and washed with a saturated aqueous solution of sodium chloride and water, respectively, followed by drying to give 6.75 g of 7β - (D - 5 - phthalimido - 5 - carboxyvaleramido) - 3 - [1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazol - 5 - yl]thiomethyl - 3 - cephem - 4 - carboxylic acid hydrochloride.

IR(KBr): 3370, 1775, 1715, 1640 cm⁻¹

NMR (δ in d₆-DMSO): 1.30-2.40 (6H, m), 3.5-4.8 (9H, m), 5.04 (1H, d, J=5 Hz), 5.60 (1H, q, J=5.8 Hz), 7.90 (4H, s), 8.86 (1H, d, J=8 Hz).

Example 98

5 - mercapto - 2 - methyl - 1,3,4 - thiadiazole (79 mg), sodium hydrogen carbonate (50 mg) and 7β - [2 - (1H - tetrazol - 1 - yl)acetamido] - 3 - (3 - carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid disodium salt (243 mg) were dissolved in a phosphate buffer solution having pH of 6.4 (3 ml) and the resultant solution was heated for one hour at 60°C. After cooling, the reaction solution was concentrated under reduced pressure and the residue was subjected to column chromatography on Sephadex LH-20 (250 ml), elution being carried out with water. The fractions containing the desired product were pooled and then lyophilized. The procedure provided sodium 7β - [2 - (1H - tetrazol - 1 - yl)acetamido] - 3 - (2 - methyl - 1,3,4 - thiadiazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylate.

NMR (δ in D₂O): 2.78 (3H, s, -CH₃), 3.60 (2H, ABq, J=18 Hz, 2-CH₂), 4.25 (2H, ABq, J=13 Hz, 3-CH₂), 5.12 (1H, d, J=4.5 Hz, 6-H), 5.58 (2H, s, -CH₂CO-), 5.70 (1H, d, J=4.5 Hz, 7-H), 9.15 (1H, s, tetrazole-H).

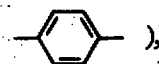
Example 99

(1) Sodium 7β - [D - α - t - butyoxycar-

bonylamino - α - (p - hydroxyphenyl)acetamido] - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylate (280 mg) and sodium salt of 5 - mercapto - 1H - 1,2,3 - triazole (120 mg) were dissolved in a phosphate buffer having a pH of 6.4 (3 ml) and the resulting solution was heated at 60°C. for 60 minutes. After cooling down to room temperature, the reaction solution was chromatographed on a column packed with Amberlite XAD-2 and the combined eluates containing the desired product were freeze-dried to give sodium 7β - [D - α - t - butyoxycarbonylamino - α - (p - hydroxyphenyl)acetamido] - 3 - (1H, 1,2,3 - triazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylate.

IR(KBr): 3400, 1762, 1678 cm⁻¹

NMR (D₂O): δ 1.46 [9H, s, -C(CH₃)₃], 3.00-4.12 (4H, m, 2-CH₂, 3-CH₂), 5.00 (1H, d, J=4.5 Hz, 6-H), 5.60 (1H, d, J=4.5 Hz, 7-H), 6.78-7.42 (4H, m,



7.71 (1H, s, triazole - 4 - H).

(2) The thus obtained sodium 7β - [D - α - t - butyoxycarbonylamino - α - (p - hydroxyphenyl)acetamido] - 3 - (1H, 1,2,3 - triazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylate (175 mg) was dissolved in formic acid (4 ml) and the resultant solution was stirred for 2 hours at room temperature. The reaction solution was subjected to distillation under reduced pressure and then to azeotropy (3 times) with toluene to remove formic acid, followed by drying over phosphorous pentoxide overnight. The thus obtained foamy material was stirred with water - methanol (8:2 (15 ml) and the mixture was subjected to filtration treatment with activated carbon and then filtration, using Celite. The filtrate was freeze-dried to give 7 - [D - α - amino - α - (p - hydroxyphenyl)acetamido] - 3 - (1H - 1,2,3 - triazol - 5 - yl)thiomethyl - 3 - cephem - 4 - carboxylic acid. This product was in good agreement with the standard in thin-layer chromatography and liquid chromatography.

Example 100

7β - [2 - (2 - imino - 4 - thiazolin - 4 - yl) - acetamido] - [1 - [2 - (N,N - dimethyl - 3 - cephem - 4 - carboxylic acid (908 mg), 5 - mercapto - 1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazole (450 mg) and sodium hydrogen carbonate (168 mg) were dissolved in water (10 ml) and the resultant solution was heated at 55°C. for 60 minutes. The reaction solution was found to contain 7β - [2 - (2 - imino - 4 - thiazolin - 4 - yl)acetamido] - 3 - [1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazol - 5 - yl]thiomethyl - 3 - cephem - 4 - carboxylic acid

in 81% yield relative to the starting cephalosporin by liquid chromatography. The reaction solution was adjusted to pH to 5.8 and purified by column - chromatography using Amberlite XAD-2.

IR(KBr): 1765 cm^{-1}

NMR (D_2O): δ 3.06 (6H, s, $-\text{N}(\text{CH}_3)_2$), 3.5-4.8 (10H, m), 5.12 (1H, d, $J=5$ Hz, 6-H), 5.65 (1H, d, $J=5$ Hz, 7-H), 6.62 (1H, s, thiazolin-H).

Example 101

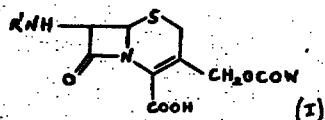
Monosodium salt monohydrate of deacetylcephalosporin C (4.13 g) was suspended in dimethylformamide (16.0 ml) and the resultant suspension was dissolved by adding concentrated hydrochloric acid (1.66 ml) at a temperature lower than 0°C , followed by adding dimethylformamide (16.0 ml), triethylamine (4.90 ml) and phthalic anhydride (2.96 g), respectively. The resultant mixture was stirred for one and a half hours at 20°C and the reaction solution was poured onto a mixture of an aqueous solution of sodium chloride (200 ml) and dichloromethane (40 ml). The mixture was adjusted to pH to 6.5 and the aqueous layer was separated. The layer was washed with dichloromethane and extracted by use of a mixed solution of ethyl acetate - tetrahydrofuran (3:1) (50 ml $\times$ 3) and the combined extract was washed with a saturated aqueous solution of sodium chloride, followed by drying over magnesium sulphate. The dried extract was concentrated under reduced pressure and ether was added to the concentrate. The above procedure provided 7 β - [D - 5 - (2 - carboxybenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.22 g).

IR(KBr): 1780, 1735, 1725, 1715, 1640 cm^{-1}

NMR (δ in d_6 -DMSO): 1.40-2.40 (6H, m), 2.62 (2H, ABq, $J=18$ Hz), 4.35 (1H, m), 5.09 (2H, ABq, $J=13$ Hz), 5.10 (1H, d, $J=5$ Hz), 5.72 (1H, dd, $J=5$ & 8 Hz), 7.30-7.9 (8H, m), 8.53 (1H, d, $J=8$ Hz), 8.82 (1H, d, $J=8$ Hz).

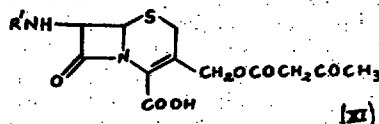
WHAT WE CLAIM IS:—

1. A compound (I) represented by the formula [I]:



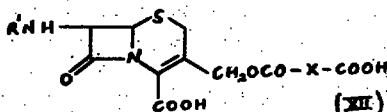
wherein R^1 is hydrogen or an acyl group; and W is an acetyl group or a group represented by $-\text{X}-\text{COOH}$ or $-\text{X}-\text{OH}$ (X is an organic residue), or a salt thereof.

2. A compound represented by the formula (XI):



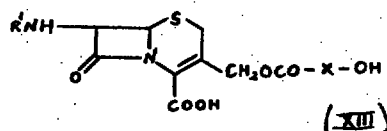
wherein R^1 is hydrogen or an acyl group, or a salt thereof.

3. A compound represented by the formula (XII):



wherein R^1 is hydrogen or an acyl group and X is an organic residue, or a salt thereof.

4. A compound represented by the formula (XIII):

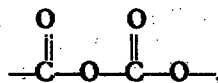


wherein R^1 is hydrogen or an acyl group and X is an organic residue, or a salt thereof.

5. A compound as claimed in any of claims 1, 3 and 4, wherein the organic residue is a carbon chain which is able to form a five- or six-membered ring with



or



which may include a double bond or hetero atom, and to which carbon chain a suitable substituent or substituents may be attached.

6. A compound as claimed in claim 5, in which the hetero atom is oxygen, nitrogen or sulphur.

7. A compound as claimed in claim 5 or 6, wherein the substituent on the carbon chain is carboxyl, halogen or nitro, an alkyl group having up to 3 carbon atoms, an alkenyl group having up to 2 carbon atoms, or an aralkyl, or aryl group or hydroxy or mercapto substituted by one of the aforementioned groups and, when two or more such substituents are present, they may form a cyclic structure with the carbon chain.

8. A compound as claimed in any of claims 1 to 7, wherein the symbol R^1 represents hydrogen.

in 81% yield relative to the starting cephalosporin by liquid chromatography. The reaction solution was adjusted to pH to 5.8 and purified by column - chromatography using Amberlite XAD-2.

IR(KBr): 1765 cm^{-1}

NMR (D_2O): δ 3.06 (6H, s, $-\text{N}(\text{CH}_3)_2$), 3.5—4.8 (10H, m), 5.12 (1H, d, $J=5$ Hz, 6-H), 5.65 (1H, d, $J=5$ Hz, 7-H), 6.62 (1H, s, thiazolin-H).

Example 101

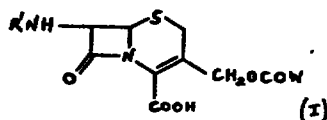
Monosodium salt monohydrate of deacetylcephalosporin C (4.13 g) was suspended in dimethylformamide (16.0 ml) and the resultant suspension was dissolved by adding concentrated hydrochloric acid (1.66 ml) at a temperature lower than 0°C , followed by adding dimethylformamide (16.0 ml), triethylamine (4.90 ml) and phthalic anhydride (2.96 g), respectively. The resultant mixture was stirred for one and a half hours at 20°C . and the reaction solution was poured onto a mixture of an aqueous solution of sodium chloride (200 ml) and dichloromethane (40 ml). The mixture was adjusted to pH to 6.5 and the aqueous layer was separated. The layer was washed with dichloromethane and extracted by use of a mixed solution of ethyl acetate - tetrahydrofuran (3:1) (50 ml $\times$ 3) and the combined extract was washed with a saturated aqueous solution of sodium chloride, followed by drying over magnesium sulphate. The dried extract was concentrated under reduced pressure and ether was added to the concentrate. The above procedure provided 7 β - [D - 5 - (2 - carboxybenzamido) - 5 - carboxyvaleramido] - 3 - (2 - carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid (6.22 g).

IR(KBr): 1780, 1735, 1725, 1715, 1640 cm^{-1}

NMR (δ in d_6 -DMSO): 1.40—2.40 (6H, m), 2.62 (2H, ABq, $J=18$ Hz), 4.35 (1H, m), 5.09 (2H, ABq, $J=13$ Hz), 5.10 (1H, d, $J=5$ Hz), 5.72 (1H, dd, $J=5$ & 8 Hz), 7.30—7.9 (8H, m), 8.53 (1H, d, $J=8$ Hz), 8.82 (1H, d, $J=8$ Hz).

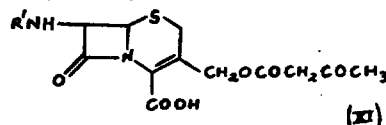
WHAT WE CLAIM IS:—

1. A compound (I) represented by the formula [I]:



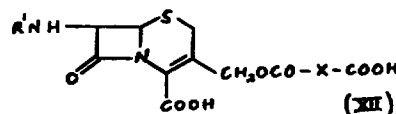
wherein R^1 is hydrogen or an acyl group; and W is an acetyl group or a group represented by $-\text{X}-\text{COOH}$ or $-\text{X}-\text{OH}$ (X is an organic residue), or a salt thereof.

2. A compound represented by the formula (XI):



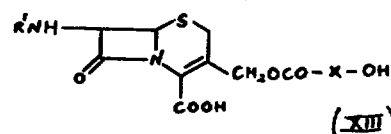
wherein R^1 is hydrogen or an acyl group, or a salt thereof.

3. A compound represented by the formula (XII):



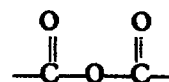
wherein R^1 is hydrogen or an acyl group and X is an organic residue, or a salt thereof.

4. A compound represented by the formula (XIII):

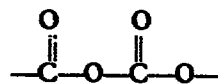


wherein R^1 is hydrogen or an acyl group and X is an organic residue, or a salt thereof.

5. A compound as claimed in any of claims 1, 3 and 4, wherein the organic residue is a carbon chain which is able to form a five- or six-membered ring with



or



which may include a double bond or hetero atom, and to which carbon chain a suitable substituent or substituents may be attached.

6. A compound as claimed in claim 5, in which the hetero atom is oxygen, nitrogen or sulphur.

7. A compound as claimed in claim 5 or 6, wherein the substituent on the carbon chain is carboxyl, halogen or nitro, an alkyl group having up to 3 carbon atoms, an alkenyl group having up to 2 carbon atoms, or an aralkyl, or aryl group or hydroxy or mercapto substituted by one of the aforementioned groups and, when two or more such substituents are present, they may form a cyclic structure with the carbon chain.

8. A compound as claimed in any of claims 1 to 7, wherein the symbol R^1 represents hydrogen.



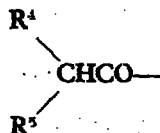
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9. A compound as claimed in any of claims 1 to 7, wherein the symbol R^1 represents an acyl group of the formula:



5 wherein R^4 represents acetyl, halogenoacetyl, phenyl, *p*-hydroxyphenyl, thienyl, 2-imino-4-thiazolin-4-yl, 2-oxo-4-thiazolin-4-yl, tetrazolyl, phenoxy or 3-amino-3-carboxypropyl; and R^5 represents hydrogen, sulpho, amino or hydroxy, and each of amino and carboxyl group in R^4 and R^5 may be protected.

10 10. A compound as claimed in claim 9, wherein the protective group of the amino group is phthaloyl, benzoyl, *o*-carboxybenzoyl, *p*-nitrobenzoyl, toluoyl, naphthoyl, *p*-tert-butylbenzoyl, *p*-tert-butylbenzenesulphonyl, phenylacetyl, benzenesulphonyl, phenoxyacetyl, toluenesulphonyl, chlorobenzoyl, acetyl, valeryl, capryl, *n*-decanoyl, acryloyl, pivaloyl, camphorsulphonyl, methanesulphonyl, chloroacetyl, tert-butoxycarbonyl, ethoxycarbonyl, isobornyloxycarbonyl, phenyloxycarbonyl, trichloroethoxycarbonyl, benzoyloxycarbonyl, β -methylsulphonyl-ethoxycarbonyl, methylcarbamoyl, phenylcarbamoyl, naphthylcarbamoyl or 2-methoxycarbonyl-1-methylvinyl.

15 11. 7β - [5-amino-5-carboxyvaleramido] - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

20 12. 7β - amino - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

25 13. 7β - (α -sulphophenyl acetamido) - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

30 14. 7β - (4-chloro-3-oxobutylamido) - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

35 15. 7β - (4-bromo-3-oxobutylamido) - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

40 16. 7β - [2-(2-imino-4-thiazolin-4-yl)acetamido] - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

45 17. 7β - (2-thienylacetamido) - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

50 18. 7β - [2-(1H-tetrazol-1-yl)acetamido] - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

55 19. 7α - [α -amino- α -(*p*-hydroxyphenyl)acetamido] - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

20. 7β - (2-thienylacetamido) - 3 - (2-carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

21. 7β - (5-amino-5-carboxyvaleramido) - 3 - (3-carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

22. 7β - (5-amino-5-carboxyvaleramido) - 3 - (2-carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

23. 7β - (5-amino-5-carboxyvaleramido) - 3 - (3-carboxyacryloyloxymethyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

24. 7β - (5-amino-5-carboxyvaleramido) - 3 - (2-carboxy-6(or 3)-nitrobenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

25. 7β - (5-amino-5-carboxyvaleramido) - 3 - (2,4(or 5)-dicarboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof, wherein the amino group may be protected.

26. 7β - amino - 3 - (2-carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

27. 7β - amino - 3 - (3-carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

28. 7β - amino - 3 - [2-carboxy-6(or 3)-nitrobenzoyloxymethyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

29. 7β - (α -sulphophenylacetamido) - 3 - (2-carboxybenzoyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

30. 7β - (α -sulphophenylacetamido) - 3 - (3-carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

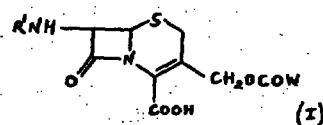
31. 7β - [2-(1H-tetrazol-1-yl)acetamido] - 3 - (3-carboxypropionyloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

32. 7β - amino - 3 - mandelyloxymethyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

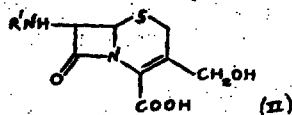
33. 7β - (D- α -sulphophenylacetamido) - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

34. 7β - (D-5-phthalimido-5-carboxyvaleramido) - 3 - (3-oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof.

35. A process for producing a compound of the formula (I):



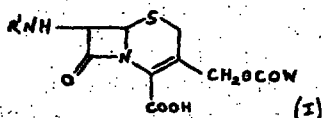
wherein R^1 represents an acyl group, and W represents an acetyl group, or a salt thereof, which comprises reacting with diketene a compound of the formula (II):



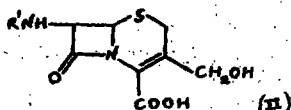
wherein R^2 represents hydrogen or an acyl group, or a salt thereof.

36. A process as claimed in claim 35 wherein R^1 is a D - 5 - phthalimido - 5 - carboxyvaleryl group.

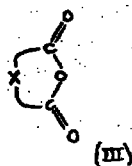
37. A process for producing a compound of the formula (I)



wherein W represents $-X-COOH$ (X is an organic residue) and R^1 represents an acyl group, or a salt thereof, which comprises reacting a compound of the formula (II):

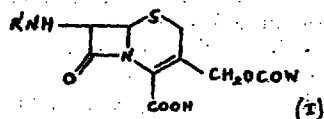


wherein R^2 represents hydrogen or an acyl group, or a salt thereof, with a compound of the formula (III):

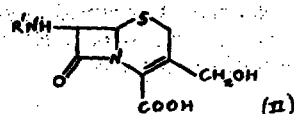


wherein X has the same meaning as defined hereinbefore.

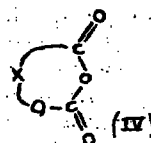
38. A process for producing a compound of the formula (I)



wherein R^1 represents an acyl group and W represents $-X-OH$ (X is an organic residue), or a salt thereof, which comprises reacting a compound of the formula (II):

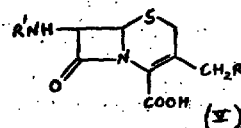


wherein R^2 represents hydrogen or an acyl group, or a salt thereof, with a compound of the formula (IV):

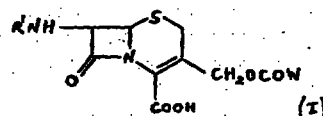


wherein X has the same meaning as defined hereinbefore.

39. A process for producing a compound of the formula (V):



wherein R^1 represents hydrogen or an acyl group; and R^3 represents a residue of a nucleophilic compound, or a salt thereof, which comprises reacting with a nucleophilic compound a compound of the formula (I)



wherein W represents acetyl or a group represented by $-X-COOH$ or $-X-OH$ (X is an organic residue); and R^1 has the same meaning as defined above,

or a salt thereof.

40. A process as claimed in claim 39 wherein R^1 is a D - α - sulphophenylacetyl group, W is an acetyl group, R^3 is a 4 - carbamoylpyridinium group and the nucleophilic compound is isonicotinamide.

41. A process as claimed in claim 39 wherein R^1 is a D - 5 - phthalimido - 5 - carboxyvaleryl group, W is an acetyl group, R^3 is a {1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazol - 5 - yl}thio group and the nucleophilic compound is 5 - mercapto - 1 - [2 - (N,N - dimethylamino)ethyl] - 1H - tetrazole.

42. A process as claimed in claim 39 wherein R^1 is a D - 5 - phthalimido - 5 - carboxyvaleryl group, W is an acetyl group, R^3 is a {1 - methyl - 1H - tetrazol - 5 - yl}thio group, and the nucleophilic compound is 5 - mercapto - 1 - methyl - 1H - tetrazole.



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43. A compound of the formula (I) as defined in claim 1 substantially as herein described with reference to any one of the specific examples 1 to 101.
- 5 44. A process according to claim 35 substantially as herein described with reference to any of the specific examples 1(1), 2(1), 3 to 6, 36 or 42.
- 10 45. A process according to claim 37 substantially as herein described with reference to any of the specific examples 8 to 22, 29, 34, 35, 43, 44 or 101.
- 15 46. A process according to claim 38 substantially as herein described with reference to any of the specific examples 23 to 26 or 41.
- 20 47. A process according to claim 39 substantially as herein described with reference to any of the specific examples 1(2), 1(3), 1(4), 2(2), 2(3), 51 to 99(1) or 100.
- 25 48. Compounds (I) as defined in claim 1 when made by a process as claimed in any of claims 35, to 37, 38 and 44 to 46.
49. Compounds (V) as defined in claim 39 when made by a process as claimed in claim 39 or 47.

50. A pharmaceutical composition comprising a compound (I) as claimed in any of claims 1 to 34 and 48, together with a non-toxic, pharmaceutically acceptable carrier and/or diluent therefor. 30

51. A pharmaceutical composition comprising a compound (V) as claimed in claim 49 together with a non-toxic, pharmaceutically acceptable carrier and/or diluent therefor. 35

52. A process for the production of 7 β - (D - α - sulphophenylacetamido) - 3 - (3 - oxobutyryloxy)methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof which comprises reacting D - α - sulphophenylacetyl chloride with 7 β - amino - 3 - (3 - oxobutyryloxy)-methyl - 3 - cephem - 4 - carboxylic acid or a salt thereof. 40

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