

PATENT SPECIFICATION

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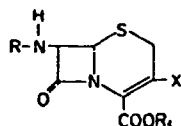
(72) Inventor ROBERT RAYMOND CHAUVETTE

(54) 3-HALO CEPHALOSPORINS

(71) We, ELI LILLY AND COMPANY, a corporation of the State of Indiana, United States of America, having a principal place of business at 307 East McCarty Street, City of Indianapolis, State of Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to 7 - acylamido- and 7 - amino - 3 - halo - 3 - cephem-4 - carboxylic acids, esters and pharmaceutically-acceptable salts and esters thereof, new cephalosporin compounds which are provided by the reaction of a 7 - acylamido - 3 - hydroxy - 3 - cepham - 4 - carboxylic acid ester or a 7 - amino - 3 - hydroxy - 3 - cephem - 4 - carboxylic acid ester with a fluorinating, chlorinating, or brominating reagent. For example, 7 - amino - 3 - chloro-3 - cephem - 4 - carboxylic acid and 7 - phenoxyacetamido - 3 - chloro - 3 - cephem - 4 - carboxylic acid are provided. The 7 - acylamido - 3 - halo cephalosporin acids and pharmaceutically acceptable salts and esters provided are valuable antibiotic compounds having desirable therapeutic properties.

In particular this invention relates to 3-halo cephalosporins represented by the following generalized formula



wherein R is hydrogen or an acyl group derived from a carboxylic acid, R₁ is hydrogen, a carboxylic acid protecting ester group or a

pharmaceutically acceptable ester group, and X represents fluoro, chloro, or bromo or, when R₁ is hydrogen, a pharmaceutically acceptable salt thereof.

The compounds of the invention possess the unique structural characteristic of a halogen atom directly bonded to the carbon atom in the 3-position of the dihydrothiazine ring. According to the cepham nomenclature system the above depicted compounds are named as 7 - amino- or 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acids, salts and esters.

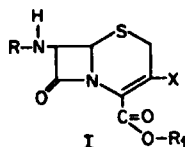
Prior to this invention 3 - bromomethyl-3 - cephem - 4 - carboxylic acid esters and 3 - bromoethyl - 2 - cephem - 4 - carboxylic acids were described. These known 3 - bromomethyl compounds are described as useful intermediates for preparing cephalosporin antibiotics. In contrast with these intermediates, the 7 - acylamido 3 - halo - 3 - cephem-4 - carboxylic acids described herein are especially valuable antibiotics.

According to one aspect of the present invention, 7 - amino-, and 7 - acylamido - 3 - hydroxy - 3 - cephem - 4 - carboxylic acid esters can be halogenated under moderate conditions to provide a 7 - amino-, or 7 - acylamido - 3 - halo - cephem - 4 - carboxylic acid ester. By employing readily removable ester groups well known in the cephalosporin art, the 3 - halo esters provided can then be converted to the free acids by known procedures for removing such ester groups. The 7 - amino - 3 - halo - 3 - cephem-4 - carboxylic acids provided herein can be acylated to provide the 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acid antibiotics. The 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acids provided either by the direct halogenation of a 7 - acylamido-3 - hydroxy - 3 - cephem - 4 - carboxylic

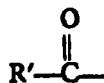
acid ester followed by ester removal of by the acylation of a 7-amino-3-halo-3-cephem-4-carboxylic acid or ester are valuable antibiotic compounds which can be used to inhibit the growth of microorganisms pathogenic to animal and plant life.

It is an object of this invention to provide a novel class of cephalosporin antibiotics. In particular it is an object of this invention to provide 7 - acylamido - 3 - halo - 3-cephem - 4 - carboxylic acid antibiotic compounds. It is a further object of this invention to provide the 3-halo substituted cephalosporin nucleus, the 7 - amino - 3 - halo - 3-cephem - 4 - carboxylic acids as well as the esters and salts thereof. A still further object of the present invention is to provide a process for the preparation of the 7-amino- and 7-acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acids described herein.

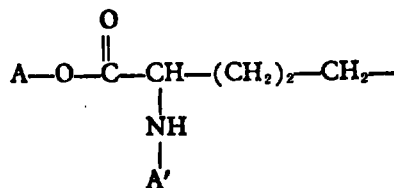
The preferred 3-halo compounds provided by this invention are represented by the following general formula.



wherein R is hydrogen or an acyl group derived from a carboxylic acid and represented by the formula

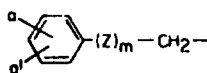


wherein R' is C₁-C₆ alkyl, C₁-C₃ haloalkyl, C₁-C₃ cyanoalkyl, phenyl, methylphenyl, hydroxyphenyl, halophenyl nitrophenyl, aminophenyl, methoxyphenyl, 5-amino - 5 - carboxybutyl, or a 5-substituted-amino - 5 - carboxybutyl ester group of the formula



wherein A is diphenylmethyl, p - nitrobenzyl, benzyl, 2,2,2 - trichloroethyl, t - butyl, or p - methoxybenzyl and A' is C₂-C₄ alkanoyl, C₂-C₄ haloalkanoyl, benzoyl, halobenzoyl, 2,4-dinitrophenyl, or phthaloyl; or

R' is a group of the formula



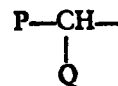
wherein

a and a' independently are hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy, halogen, hydroxy, nitro, amino, or carboxy;

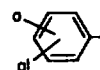
Z is O or S; and

m is 0 or 1; or

R' is a group of the formula



wherein P is 2-thienyl, 3-thienyl, phenyl or a substituted phenyl group of the formula

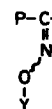


wherein

a and a' are as defined above,

Q is hydroxyl, formyloxy, acetoxy, carboxy, or sulfo; or

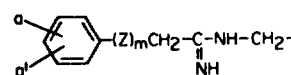
R' is a group of the formula



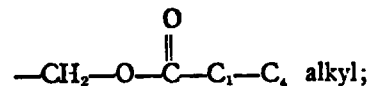
wherein P has the same meanings as defined above and Y is hydrogen, methyl or acetyl; or R' is a group of the formula



wherein R'' is 2-thienyl, 3-thienyl, 2-furyl, 2-oxazolyl, 2-thiazolyl, or 1-tetrazolyl, or R' is a group of the formula



wherein a, a', Z and m have the same meanings as defined above; R₁ is hydrogen, benzyl, 4-methoxybenzyl, 4 - nitrobenzyl, diphenylmethyl, 2,2,2 - trichloroethyl, t-butyl or a pharmaceutically acceptable ester of the formula



and X is fluoro, chloro, or bromo, and when R₁ is hydrogen the pharmaceutically acceptable salts thereof.

In the foregoing definition of the compounds provided by this invention the term "C₁-C₆ alkyl" refers to the straight and branched chain alkyl hydrocarbon groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl,

are hydrogen, 45
oxy, hydrogen,
oxy;

la 50

l, phenyl or a
formula

ive,
etoxy, carboxy,

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ngs as defined
l or acetyl; or

yl, 2-furyl, 2-
olyl, or R' is a

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CH₂-

ame meanings
n, benzyl, 4-
l, diphenyl-
t-butyl or a
r of the for-

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alkyl;

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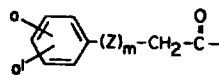
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tically accept-

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ten the term
straight and
groups such
opyl, n-butyl,

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sec-butyl, n-amyl, isoamyl, or n-hexyl;
"C₁-C₄ cyanoalkyl" refers to the groups
cyanomethyl, 2-cyanoethyl, 3-cyanopropyl, or
2-cyanopropyl; "C₂-C₄ alkanoyl" refers to
acetyl, propionyl, or butyryl; "C₂-C₄ halo-
alkanoyl" refers to such groups as chloro-
acetyl, bromoacetyl, 2-chloropropionyl, or
3-bromobutyryl; "C₁-C₄ alkyl" refers to the
straight and branched chain lower alkyl hydro-
carbon groups such as methyl, ethyl, n-
propyl, iso-propyl, n-butyl, or t-butyl;
"C₁-C₄ alkoxy" refers to groups such as
methoxy, ethoxy, isopropoxy, or n-butoxy. As
used herein the term "halogen" refers to
fluoro, chloro, or bromo. The term "halo-
benzoyl" refers to the chloro and bromo sub-
stituted benzoyl groups such as 4-chloro-
benzoyl, 4-bromobenzoyl, or 2,4-dichloro-
benzoyl.

20 Illustrative of the groups in the above
definition represented by the following for-
mula where m is 0 are



phenylacetyl, 4-methylphenylacetyl, 3-
ethylphenylacetyl, 4-isopropylphenylacetyl,
2-methylphenylacetyl, 4-chlorophenyl-
acetyl, 4-nitrophenylacetyl, 4-bromo-
phenylacetyl, 2,4-dichlorophenylacetyl, 3-
bromophenylacetyl, 4-iodophenylacetyl, 2-
fluorophenylacetyl, 3,4-dihydroxyphenyl-
acetyl, 4-hydroxyphenylacetyl, 3-hydroxy-
phenylacetyl, 2,6-dimethoxyphenylacetyl, 3-
carboxyphenylacetyl, 4-aminophenylacetyl,
3-ethoxyphenylacetyl, 4-methoxyphenyl-
acetyl, 3,4-dimethoxyphenylacetyl, 4-t-
butoxyphenylacetyl, 2-carboxyphenylacetyl,
3-chloro-4-methylphenylacetyl, or 3-
nitrophenylacetyl. When in the above formula
m=1 and Z represents —O—, illustrative
groups are the following: Phenoxyacetyl, 4-
hydroxyphenoxyacetyl, 3-hydroxyphenoxy-
acetyl, 4-chlorophenoxyacetyl, 3-bromo-
phenoxyacetyl, 3-ethylphenoxyacetyl, 4-
methylphenoxyacetyl, 3-hydroxy-3-methyl-
phenoxyacetyl, 4-aminophenoxyacetyl, 3-
nitrophenoxyacetyl, 2-carboxyphenoxyacetyl,
2-chlorophenoxyacetyl, 4-t-butylphenoxy-
acetyl, 4-methoxyphenoxyacetyl, 3,4-
dimethoxyphenoxyacetyl, 2-aminophenoxy-
acetyl, 4-isopropoxyphenoxyacetyl, or 4-
nitrophenoxyacetyl. When in the foregoing
formula m=1 and Z represents —S—, illus-
trative groups are the following: Phenylmer-
captoacetyl, 4-chlorophenylmercaptoacetyl,
3-hydroxyphenylmercaptoacetyl, 3,4-di-
methylphenylmercaptoacetyl, 4-aminophenyl-
mercaptoacetyl, 3,4-dichlorophenylmer-
captoacetyl, 3-bromophenylmercaptoacetyl,
4-fluorophenylmercaptoacetyl, 2,6-difluoro-
phenylmercaptoacetyl, 4-nitrophenylmer-

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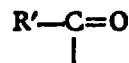
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captoacetyl, or 3-fluorophenylmercapto-
acetyl.

When in the formula I R' is a 5-substituted-
amino-5-carboxybutyl group,



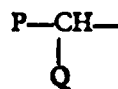
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is representative of esterified amino-protected
adipoyl groups wherein the ester group is
diphenylmethyl, p-nitrobenzyl, benzyl, p-
methoxybenzyl, 2,2,2-trichloroethyl, or t-
butyl, and representative substituted amino
groups are acetamido, propionamido, chloro-
acetamido, benzamido, 2,4-dichlorobenz-
amido, 4-bromobenzamido, phthalimido, or
2,4-dinitroanilino.

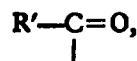
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When in formula I R' represents a group
of the formula

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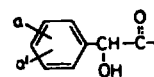


illustrative acyl groups,

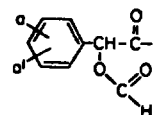


are the mandeloyl group of the formula

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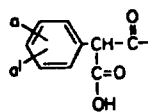


the O-formyl derivative thereof represented
by the following formula



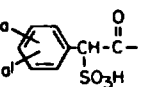
the α-carboxyphenylacetyl group represented
by the following formula

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the α-sulfofenylacetyl group represented by
the formula

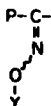
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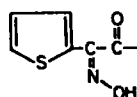
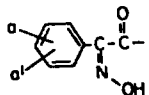
as well as those 2-thienyl and 3-thienyl acyl
groups wherein the above formula the phenyl
group is replaced with a 2-thienyl or 3-thienyl
ring.

When R' is a group of the formula in the
syn or anti form

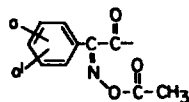
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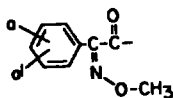
illustrative acyl groups include



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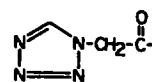
and



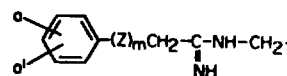
- Illustrative of the foregoing acyl groups are
- 4 - methylmandeloyl, 4 - hydroxymandeloyl,
 - 10 3 - hydroxymandeloyl 4 - aminomandeloyl, 3-bromomandeloyl, 4 - chloromandeloyl, 3-methyl - 4 - fluoromandeloyl, 2 - fluoromandeloyl, 4 - fluoromandeloyl, 4 - methoxymandeloyl, 3,4-dimethyl-O-formylmandeloyl, 4-
 - 15 chloro - O - formylmandeloyl, 3 - amino - O-formylmandeloyl, 3 - bromo - O - formylmandeloyl, 3,4 - dimethoxy - O - formylmandeloyl, O - acetyl mandeloyl, O - acetyl 4 - hydroxymandeloyl, α - carboxy - 4 - methylphenylacetyl, α - carboxy - 3,4 - dichlorophenylacetyl, α - carboxy - 4 - hydroxyphenylacetyl, α - carboxy - 2 - methoxyphenylacetyl, α - carboxy - 4 - isopropoxyphenylacetyl, α - carboxy - 3 - hydroxyphenylacetyl, α - carboxy - 4 - aminophenylacetyl, α - sulfo - 4 - methylphenylacetyl, α -
 - 20 sulfo - 3,4 - dichlorophenylacetyl, α - sulfo - 4 - chlorophenylacetyl, α - sulfo - 4 - hydroxyphenylacetyl, α - sulfo - 3 - methoxyphenylacetyl, α - oximino - 4 - hydroxyphenylacetyl, α - oximino - 3 - chlorophenylacetyl, α - oximino - 4 - carboxyphenylacetyl, α - methoximino - 4 - methylphenylacetyl, α -
 - 25 methoximino - 3,5 - dichlorophenylacetyl, α - methoximino - 4 - hydroxyphenylacetyl, α - methoximino - 2 - aminophenylacetyl, α -acetyloximinophenylacetyl, α - acetyloximino - 2 - thienylacetyl, α - acetyloximino - 4 - hydroxyphenylacetyl, α - oximino - 2 - thienylacetyl, α - carboxy - 2 - thienylacetyl, α -
 - 30 carboxy - 3 - thienylacetyl, α - methoximino - 2 - thienylacetyl, α - hydroxy - 2 - thienylacetyl, α - hydroxy - 3 - thienylacetyl, α -sulfo - 2 - thienylacetyl, α - formyloxy - 2-

thienylacetyl, α - acetoxy - 2 - thienylacetyl or α - methoximino - 2 - thienylacetyl.

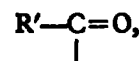
When in the foregoing formula R' represents a group of the formula R''-CH₂-, illustrative of the acyl groups of the formula I are the following. 2 - thienylacetyl, 3-thienylacetyl, 2 - furylacetyl, oxazyl - 2-acetyl, thiazyl - 2 - acetyl, and the tetrazyl-1 - acetyl group represented by the following formula



When in Formula I, R' represents the group

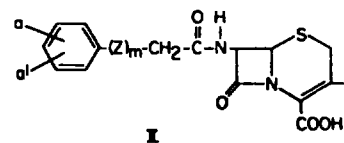


illustrative of the acyl groups,



represented thereby are N-(phenylacetimidoyl)aminoacetyl, N - phenoxyacetamidoyl, aminoacetyl, N - (phenylmercaptoacetimidoyl)aminoacetyl, N-(4-chlorophenylmercaptoacetimidoyl)aminoacetyl, N - (4 - methoxyphenylacetimidoyl)aminoacetyl, N - (2,6-dimethoxyphenylacetimidoyl)aminoacetyl, N-(4 - hydroxyphenoxyacetimidoyl)aminoacetyl, N - (4 - chlorophenoxyacetimidoyl)aminoacetyl, N - (4 - nitrophenylacetimidoyl)aminoacetyl, N - (3,4 - dimethylphenylacetimidoyl)aminoacetyl, N - (4 - fluorephenoxyacetimidoyl)aminoacetyl, and like mono and disubstituted groups.

A preferred group of 3-halo cephalosporin antibiotics of this invention are represented by the following formula II,



wherein a, a', Z and m have the same meanings as defined above and X is chloro. Illustrative of these preferred compounds are the following:

- 7 - phenylacetamido - 3 - chloro - 3-cephem - 4 - carboxylic acid,
- 7 - phenoxyacetamido - 3 - chloro - 3-cephem - 4 - carboxylic acid,
- 7 - (4 - hydroxyphenylacetamido) - 3-chloro - 3 - cephem - 4 - carboxylic acid,
- 7 - (4 - chlorophenoxyacetamido) - 3-chloro - 3 - cephem - 4 - carboxylic acid,
- 7 - (4 - methoxyphenoxyacetamido) - 3-chloro - 3 - cephem - 4 - carboxylic acid, and

thienylacetyl 45
 thienyl
 mula $\sim$ repre-
 la $R''-CH_2-$
 of the formula
 thienylacetyl, 3- 50
 oxaz - 2-
 id the tetrazyl-
 y the following

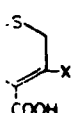
sents the group

$-CH_2-$

s

phenylacetimid- 60
 oxyacetamidoyl,
 rcaptoacetimid-
 phenylmercapto-
 (4 - methoxy- 65
 N - (2,6-
 ninoacetyl, N-
 yl)aminoacetyl,
 imido)amino-
 vlacetimidoyl)-
 thylphenylacet- 70
 - fluorphenoxy-
 like mono and

cephalosporin 75
 represented by



he same mean- 80
 s chloro. Illus-
 pounds are the

- chloro - 3-

- chloro - 3-

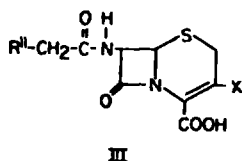
amido) - 3-
 xylic acid,

amido) - 3-
 xylic acid,

etamido) - 3- 90
 xylic acid, and

the pharmaceutically acceptable esters and salts thereof.

Another preferred group of compounds represented by the following formula III.



III

wherein R'' represents 2-thienyl, 3-thienyl, 2-furyl, and 1-tetraoyl and X represents chloro. Illustrative of the foregoing preferred compounds represented by the formula III are the following:

7 - (2 - thienylacetamido) - 3 - chloro-
 3 - cephem - 4 - carboxylic acid,

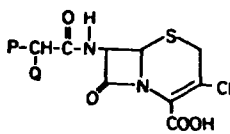
7 - (2 - furylacetamido) - 3 - chloro - 3-
 cephem - 4 - carboxylic acid,

7 - (3 - thienylacetamido) - 3 - chloro-
 3 - cephem - 4 - carboxylic acid,

7 - (1 - tetrazolacetamido) - 3 - chloro-
 3 - cephem - 4 - carboxylic acid, and the

pharmaceutically acceptable esters and salts thereof.

A further preferred group of compounds represented by the formula I are those represented by the following formula IV.



IV

wherein P represents phenyl or a substituted phenyl group as defined in formula I and Q is hydroxy or carboxy. Illustrative of the preferred compounds represented by formula IV are the following:

7 - D - mandelamido - 3 - chloro - 3-
 cephem - 4 - carboxylic acid,

7 - D - (4 - chloromandelamido) - 3-
 chloro - 3 - cephem - 4 - carboxylic acid,

7 - D - (4 - hydroxymandelamido) 3 -
 chloro - 3 - cephem - 4 - carboxylic acid,

7 - D - (4 - methoxymandelamido) - 3-
 chloro - 3 - cephem - 4 - carboxylic acid,

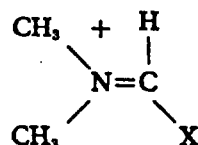
7 - (α - carboxyphenylacetamido) - 3-
 chloro - 3 - cephem - 4 - carboxylic acid,

and the pharmaceutically acceptable esters and salts thereof.

The 3-halo cephalosporins described herein and represented by the formula I are prepared either by the direct halogenation of a 7 - acylamido - 3 - hydroxy - 3 - cephem-4 - carboxylic acid ester or by the acylation of a 7 - amino - 3 - halo - 3 - cephem - 4 - carboxylic acid or ester thereof (formula I, $R=H$). The 7 - amino - 3 - halo - 3 - cephem-4 - carboxylic acid or ester can be prepared either by the direct halogenation of the corresponding 7 - amino - 3 - hydroxy ester or

by the cleavage of the 7 - acylamido side chain of a 7 - acylamido - 3 - halo cephem ester.

The compounds represented by the formula I wherein X is chloro or bromo can be prepared by reacting a 7-acylamido-3-hydroxy-3 - cephem ester or a 3-hydroxy - 3-cephem nucleus ester in dimethylformamide (DMF) with a reactive chloro or bromo compound which forms with DMF the chloro or bromo dimethyliminium chloride or bromide as represented by the formula



wherein X and X^- represent chloro or bromo and chloride or bromide respectively. Solvents other than DMF which are capable of forming the iminium halide can be used in conjunction with or instead of DMF. The reactive iminium halide of the above formula is formed in situ and is a highly reactive chlorinating or brominating intermediate. Chloro and bromo compounds which form the above iminium halide include the commonly used chlorinating agents such as phosgene (carbonyl chloride), oxalyl chloride, thionyl chloride, and the phosphorus chlorides, for example, phosphorus trichloride and phosphorus oxychloride (phosphoryl chloride). Brominating reagents which can be employed in the present invention include carbonyl dibromide, oxalyl bromide, thionyl bromide (sulfurous oxybromide), and the phosphorus bromides, phosphorus oxybromide, and phosphorus tribromide. Phosphorus pentachloride can be employed in the preparation of the 3-chloro - 3 - cephem compounds of the invention, however this reagent concurrently reacts with the 7 - acylamido side chain of the starting material to form the imine chloride, the reactive intermediate in the well known cephalosporin side chain cleavage reaction. Accordingly, it is preferable to use one of the other named chlorinating agents.

The chlorination and bromination of a 3-hydroxy sephem ester is conveniently carried out by employing dry DMF as the solvent. The DMF is preferably dried over a molecular sieve before use. A cosolvent can be employed along with excess DMF although such is not essential. For example, a cosolvent such as tetrahydrofuran dioxane, methylene chloride, dimethylacetamide or dimethyl sulfoxide can be used along with DMF. The brominating or chlorinating agent such as one of those enumerated above is desirably used in an amount corresponding to two equivalents of the amount of 3 - hydroxy cephem ester starting material used. The reaction can

be carried out by adding the halogenating reagent to a solution of the 3 - hydroxycephem ester in dry DMF maintained at a temperature by 5° to 15°C. and allowing the reaction mixture to stand at room temperature for between 4 and 8 hours or longer. The reaction is initially exothermic and accordingly the reaction vessel is maintained in an ice-water bath to maintain the temperature below about 25°C. during the initial phase of the reaction. Thereafter the reaction mixture is allowed to stand at or about room temperature for the duration of the reaction. The extent to which the reaction has proceeded can be determined by thin layer chromatography.

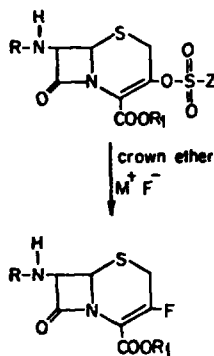
Alternatively, the chlorination and bromination can be carried out by first preparing a mixture of the halogenating reagent in DMF to preform the haloiminium halide, and then adding the mixture to a solution of the 3-hydroxy - 3 - cephem ester in DMF, a mixture of DMF and a co-solvent or in a solvent such as dimethylacetamide or tetrahydrofuran.

The 3 - chloro- or 3 - bromo - 3 - cephem esters are recovered from the reaction product mixture by pouring the mixture into a water-ethyl acetate mixture and separating the organic phase containing the product. The organic phase is washed, dried and is evaporated to afford the 3 - halo - 3 - cephem ester as an amorphous residue. The product is obtained crystalline in many instances by trituration of the residue with ether or with n-hexane.

The preferred chlorinating and brominating reagents are phosphorus trichloride and phosphorus tribromide.

The 7 - amino - 3 - halo - 3 - cephem-4 - carboxylic acids are preferably obtained by the cleavage of the 7-acyl group of a 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acid ester followed by removal of the carboxylic acid protecting ester group.

The compounds represented by the formula I wherein X is fluoro are prepared from the alkyl sulfonate or arylsulfonate esters of the 3 - hydroxy - 3 - cephem esters by the following generalized reaction scheme.



Crown ethers are described in *Tetrahedron Letters*, 18 1793—1796 (1972).

The reaction is carried out in an inert solvent, specifically a nitrile or a nitroalkane, preferably acetonitrile, propionitrile, nitromethane, or nitroethane, under substantially anhydrous conditions at a temperature of from -20°C. to room temperature and preferably at about room temperature. The 3 - sulfonate ester cephalosporin present in the selected solvent is mixed with a maximum of one equivalent of the fluoride source based on the sulfonate ester and at least an equivalent amount of crown ethers based upon the fluoride which is present. The reaction is completed generally within a period of from 30 minutes to 3 hours, with time generally being dependent upon the temperature of reaction. Typical crown ethers include 18-crown-6-ether as well as corresponding substituted 18 - crown - 6 - ether, such as dicyclohexyl-18 - crown - 6 - ether. Preferably, the fluoride source is an inorganic fluoride salt, M⁺F⁻ hereinabove being, for example, sodium fluoride, or silver fluoride.

The product which is obtained from the reaction will correspond structurally in every respect to the sulfonate ester starting material with the exception that the group

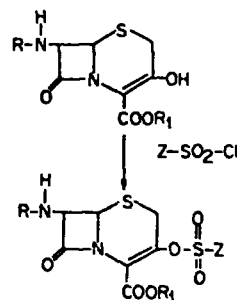


will have been displaced by a fluorine atom.

It has been discovered that the initial 3-sulfonate ester cephalosporin reactant can be either a Δ^3 cephalosporin, a Δ^2 cephalosporin, or a mixture of the two. The active reactant is the Δ^3 -cephalosporin. However, it has been discovered that, under the conditions of reaction, any Δ^2 -cephalosporin which may be present is isomerized to the Δ^3 -cephalosporin, and thus the active reactant is formed *in situ* if not present initially in the reaction mixture.

Isolation of the resulting product can be accomplished employing generally recognized techniques. Preferably, the product is isolated by means of preparative thin layer chromatography employing customary procedures.

The preparation of the alkylsulfonate and arylsulfonate esters of the 3 - hydroxy - 3 - cephem esters is illustrated in the following generalized reaction scheme.



In the above formula, the terms R and R₁

in an inert solvent, such as a n-alkane, nitrobenzene, or nitro-
 order substantially
 perature of from
 and preferably
 the 3 - sulfonate
 in the selected
 aximum of one
 rce based on the
 an equivalent
 upon the fluor-
 reaction is com-
 period of from
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 nperature of re-
 clude 18-crown-
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 as dicyclohexyl-
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 t the initial 3-
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dsulfonate and
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ms R and R₁

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have the same meanings as previously defined and Z is C₁-C₆ lower alkyl, such as methyl, ethyl, propyl, n-butyl, or n-hexyl, or Z is a phenyl group or a substituted phenyl group such as methylphenyl, nitrophenyl, or halophenyl. Representative of the sulfonyl halides which can be employed in the preparation of these sulfonate esters are methanesulfonyl chloride, toluenesulfonyl chloride, p - fluoro-benzene sulfonyl chloride, ethanesulfonyl chloride, or butanesulfonyl chloride.

The reaction is carried out in an inert solvent at a temperature between -5 and 35°C., and preferably between 15 and 25°C. Inert solvents which can be employed in the sulfonation reaction are generally aprotic solvents, and those solvents which are unreactive with the sulfonyl halide. Solvents which can be employed include the ether solvents such as tetrahydrofuran and dioxane. A preferred solvent for the sulfonation reaction is dimethylacetamide. The reaction is carried out in the presence of a hydrogen halide acceptor, and the commonly employed tertiary amines such as pyridine and triethylamine can be used, however, the preferred hydrogen halide acceptor in this process is propylene oxide. Other alkylene oxides can be employed, however, propylene oxide is much preferred. The tertiary amine hydrogen halide acceptors are less desirable than the alkylene oxides in that the double bond in the delta 3 position of the cephem ring is susceptible to isomerization to the delta 2 position in the presence of these amines. However, should isomerization to the delta 2 isomer occur during sulfonate ester formation, the double bond can be isomerized back to the delta 3 position in the following manner. The delta 2 isomer of the sulfonate ester is first oxidized with a peracid, such as m-chloroperbenzoic acid or peracetic acid to the sulfoxide. During sulfoxide formation the double bond shifts from the delta 2 to the delta 3 position and thereafter the sulfoxide is reduced, for example, with phosphorus trichloride, according to the well known method to provide the 3-cephem sulfonate ester.

A preferred sulfonate ester for the conversion of the 3 - hydroxy - 3 - cephem - 4 - carboxylic acid esters to the corresponding 3 - fluoro - 3 - cephem compounds is the methylsulfonate ester mesylate). Another preferred ester is that formed with p-toluenesulfonyl chloride (tosylate ester). A preferred carboxylic acid protecting ester group in the above described process is the p-nitrobenzyl ester.

Alternatively, the compounds represented by the formula I wherein X is fluoro are prepared by reacting a 7 - acylamido - 3 - hydroxy cephem ester in an inert solvent with the fluorinated tertiary amine, N - (2 - chloro-1,1,2 - trifluoroethyl) - diethylamine. The

fluorinated tertiary amine is prepared by reacting diethylamine with chlorotrifluoroethylene as described in *J. Org. Chem.* 29, 2187 (1964); *Tetrahedron Lett.* 23, 1065 (1962) and *Tetrahedron Lett.* 26, 1249 (1962). The fluorination is carried out by reacting the 3 - hydroxy cephem ester with an equivalent amount of the fluorinated tertiary amine in an inert solvent, for example, methylene chloride, chloroform, tetrahydrofuran, or any unreactive compound in which the starting material and fluorinating reagent are substantially soluble. The reaction mixture containing the starting 3 - hydroxy cephem ester and the fluorinating reagent is heated at a temperature between 30 and 60°C. for up to about 1 hour.

As previously mentioned the 3-chloro or 3-bromo cephalosporin compounds of the invention can be prepared either by halogenating a 7-acylamido - 3 - hydroxy - 3 - cephem 4 - carboxylic acid ester or by acylating a 3 - halo - 7 amino - 3 - cephem - 4 - carboxylic acid or ester. When the former method for preparing the compounds of the invention is chosen, the 7 - acylamido group of the starting material is desirably one which does not react with the halogenating agent under the conditions described above. For example, when the acyl moiety in the 7-position of the starting material contains reactive functional groups such as the carboxyl group, the amino group, or the sulfonic acid (sulfo) group, such groups are blocked by the formation of an unreactive derivative thereof prior to the halogenation reaction described above. Illustrative of the 7 - acylamido - 3 - hydroxy - 3 - cephem - 4 - carboxylic acid esters which can be halogenated without concurrent halogenation in the side chain are the compounds represented by the formula I wherein R is C₂-C₇ alkanoyl, C₂-C₃ haloalkanoyl, C₂-C₃ cyanoalkanoyl, phenoxyacetyl, 2 - thienylacetyl, 3 - thienylacetyl, and 2 - furylacetyl.

The 7 - amino - 3 - halo - 3 - cephem compounds of the Formula I, R=H, are best prepared by the cleavage of the 7 - acylamido side chain from a 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acid ester by the well known phosphorous pentachloride cleavage reaction. For example, 7 - [2 - (2 - thienyl) - acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid p - nitrobenzyl ester is reacted with phosphorus pentachloride in methylene chloride in the presence of pyridine to provide the imino chloride derivative thereof. The imino chloride intermediate is reacted with an alcohol, for example methanol or isobutanol to provide the corresponding imino ether derivative. Hydrolysis of the imino ether provides the p - nitrobenzyl 7-amido - 3 - exomethylenecephem - 4 - carboxylate hydrochloride.

The starting materials employed in the preparation of the compounds of the present

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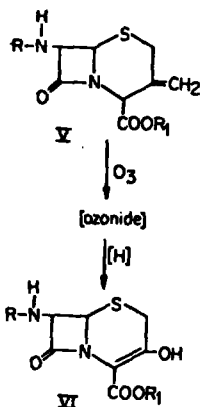
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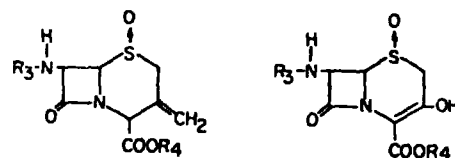
invention are prepared by reacting a 7-acylamido - 3 - exomethylenecepham - 4 - carboxylic acid ester or a 7 - amino - 3 - exomethylenecepham - 4 - carboxylic acid ester with ozone in an inert solvent at a temperature between -80 and 0°C . to form the ozonide derivative of the 3 - exomethylene double bond. The ozonide intermediate, which is not isolated, is decomposed by reacting the ozonide in situ with a mild reducing agent such as sodium bisulfite, or preferably sulfur dioxide, to provide the corresponding 3-hydroxy - 3 - cephem - 4 - carboxylic acid ester.

The ozonolysis of a 7 - amino - 3 - exomethylenecepham - 4 - carboxylic acid ester or a 7 - acylamido - 3 - exomethylenecepham - 4 - carboxylic acid ester of the following Formula V is carried out by passing ozone through a solution of the 3 - exomethylenecepham ester in an inert solvent at a temperature between -80 and 0°C . The exomethylene double bond reacts with ozone to form in situ an intermediate ozonide which is decomposed, as hereinafter described, to form the 3-hydroxy - 3 - cepham ester of the Formula VI.



In the above formulae, R is hydrogen or an acyl group derived from a carboxylic acid and which acyl group is non-oxidizable under the described ozonolysis conditions, R_1 is an ester forming group and preferably one which is easily removed under hydrogenolysis, or acid or base hydrolysis conditions.

Although the 3-exomethylene cephalosporins can also undergo oxidation with ozone to form the sulfoxide, under the described ozonization conditions the exo double bond reacts preferentially with ozone to form the ozonide. The formation of the sulfoxide occurs as a result of over oxidation. Whereas the exo double bond reacts rapidly with ozone, the reaction at the sulfur atom of the dihydrothiazine ring to form the sulfoxide occurs at a much slower rate. However, the following over oxidation products can be formed in the ozonolysis reaction.



Ozone gas is prepared by means of an ozone generator of the type commonly used in synthetic and analytical chemical work to produce ozone by the action of an electric discharge on oxygen. One such ozone generator is that manufactured by the Welsback Corporation. The ozone is generated in a stream of oxygen which is then passed directly into the reaction vessel. The percentage of ozone contained in the oxygen stream can be varied as desired, for example, by varying the rate of flow of oxygen through the ozonizer as well as by varying the intensity of the electric discharge. The percentage of ozone in the oxygen stream can be determined iodometrically by titrating the sodium thiosulfate the amount of iodine liberated from a standard solution of potassium iodide by ozone from the generator. The percentage of ozone in the oxygen stream is not critical, however for convenience in carrying out the ozonolysis method of this invention an estimate of the amount of ozone flowing into the reaction mixture enables one to determine the time at which the desired reaction should be complete and thus minimizes the formation of over oxidation products.

Alternatively, the ozonolysis reaction can be followed chromatographically. For instance, a small aliquot of the reaction mixture is withdrawn, the ozonide decomposed, and the amount of unreacted starting material and 3-hydroxy - 3 - cephem product present in the sample is assessed by a comparison of the thin layer chromatogram with that of a known amount of starting material and 3 - hydroxy - 3 - cephem compound.

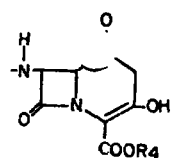
Inert solvents which can be used in the ozonolysis are those solvents in which the 3-exomethylene cepham esters are at least partially soluble and which are unreactive with ozone under the described conditions. Commonly used organic solvents such as methanol, ethanol, ethyl acetate, methyl acetate, and methylene chloride are satisfactory.

The concentration of the starting material in the inert solvent is not critical and it is preferred to use a solvent volume sufficient to form a complete solution.

The preferred temperature in the ozonolysis reaction is between -80 and -50°C .

When ozonide formation is complete as determined by either method described above, any excess ozone present in the reaction mixture is purged from the mixture by bubbling nitrogen or oxygen through the mixture.

Following the removal of any excess ozone, the ozonide is decomposed by adding to the reaction mixture a mild reducing reagent



means of an ozone 50
only used in syn-
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selected from the group consisting of sodium bisulfite, sulfur dioxide, and trimethyl phosphite to provide the 3 - hydroxy - 3 - cephem - 4 - carboxylic acid ester. The decomposition is carried out by adding an excess of the reducing reagent and then stirring the reaction mixture at a temperature of -80 to 0°C. until the reaction mixture is negative in the potassium iodide-starch test.

A preferred reagent for decomposing the intermediate ozonide is gaseous sulfur dioxide. This reagent is preferred since it is completely volatilized from the reaction mixture during the subsequent work-up and thus does not complicate the recovery of the reaction product.

The 7 - acylamido - 3 - hydroxy - 3 - cephem - 4 - carboxylic acid esters are recovered from the reaction mixture by first evaporating the mixture to dryness and thereafter extracting the product from the residue. Alternatively, N-acylated 3 - hydroxy - 3 - cephem esters can be recovered from the organic liquid phase of the decomposition mixture by separating the liquid phase from insolubles, and after washing and drying, the organic layer is evaporated to yield the 3 - hydroxy ester.

The 3-hydroxy nucleus ester, a 7 - amino - 3 - hydroxy - 3 - cephem - 4 - carboxylic acid ester, is best isolated in the form of a salt as for example, the hydrochloride or hydrobromide salt.

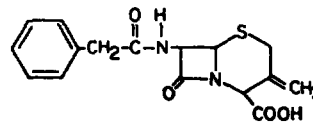
When an ester of 7 - amino - 3 - exomethylenecepham - 4 - carboxylic acid (Formula V, R=H) is ozonized it is preferable to use a salt of this nucleus, for example, the hydrochloride or p-toluenesulfonate salt.

In a specific example of the preparation of a 3 - hydroxy - 3 - cephem ester, p - methoxybenzyl 7 - phenoxyacetamido - 3 - exomethylenecepham-4-carboxylate is dissolved in ethyl acetate and is reacted with ozone at a temperature of about -78°C. The excess ozone is expelled by bubbling oxygen through the cold solution. The ozonide is decomposed by adding excess sodium bisulfite to the reaction mixture at 0°C. with stirring. The organic layer is decanted from the insolubles and is washed, dried and evaporated to yield p - methoxybenzyl 7 - phenoxyacetamido - 3 - hydroxy - 3 - cephem - 4 - carboxylate.

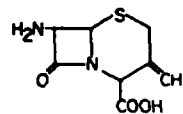
In a further example p-nitrobenzyl-7-amino 3 - methylenecepham - 4 - carboxylate hydrochloride is dissolved in methanol and ozone is bubbled through the solution at a temperature of about -78°C. Excess ozone is purged from the mixture with nitrogen and the ozonide is decomposed by bubbling sulfur dioxide through the mixture. The reaction mixture is evaporated to dryness and the residue, p-nitrobenzyl 7 - amino - 3 - hydroxy - 3 - cephem - 4 - carboxylate is obtained as the hydrochloride salt.

The starting materials for the preparation of

the 3 - exomethylenecepham esters are prepared by reacting a 7 - acylamido cephalosporanic acid with a sulfur containing nucleophile according to known procedures to effect the nucleophilic displacement of the acetoxy group of the cephalosporanic acid and provide a 7 - acylamido - 3 - thiosubstituted - methyl-3 - cephem - 4 - carboxylic acid. The 3-thiosubstituted cephem product is then reduced with hydrogen in the presence of Raney nickel or with zinc/formic acid in the presence of dimethylformamide to produce the 3 - exomethylenecepham acid. For example, 7 - phenylacetamidocephalosporanic acid is reacted with potassium ethyl xanthate to yield 7 - phenylacetamido - 3 - ethoxythionocarbonylthiomethyl - 3 - cephem - 4 - carboxylic acid which on reduction with zinc/formic acid in the presence of DMF yields, 7-phenylacetamido - 3 -exomethylenecepham - 4 - carboxylic acid of the formula 70
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Likewise, the 3-exomethylenecepham nucleus of the formula



is prepared by reacting a 7 - acylamido - 3 - exomethylenecepham - 4 - carboxylic acid ester with phosphorus pentachloride (PCl₅) in methylene chloride in the presence of pyridine to obtain the intermediate imino chloride. The ester group is then removed to yield anol in the cold to afford the imino ether. The imino ether readily undergoes hydrolysis to provide the 7 - amino - 3 - exomethylenecepham - 4 - carboxylic acid ester hydrochloride. The ester group is then removed to yield the 3 - exomethylenecepham nucleus. 95
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As described above the compounds of the invention as represented by the formula I can be prepared by the acylation of a 7 - amino - 3 - halo - 3 - cephem - 4 - carboxylic acid or an ester thereof. 105

The acylation of these nuclei can be carried out by the known methods used for the acylation of 7 - aminocephalosporanic acid or 7-aminodeacetoxycephalosporanic acid. The cephalosporin - 3 - halo nucleus acids or esters (Formula I, R=H) can be acylated under anhydrous acylation methods as well as in the presence of water. Accordingly, the cephalosporin 3-halo nucleus free acid or an ester thereof can be acylated with a carboxylic acid halide in an aqueous solvent system, for example aqueous acetone, in the presence 110
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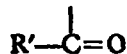
of a hydrogen halide acceptor such as propylene oxide, pyridine or sodium bicarbonate. The acylation can also be effected by reacting an ester of the halo nucleus with a carboxylic acid in the presence of a condensing agent such as N - ethoxycarbonyl - 2 - ethoxy-1,2 - dihydroquinoline (EEDQ) or dicyclohexylcarbodiimide. Also the 3-halo nucleus ester can be acylated in a mixed anhydride reaction. By yet another known acylation method, the halo nucleus can be acylated with an active ester of a carboxylic acid, for example the pentachlorophenyl ester of a carboxylic acid.

For example, p - nitrobenzyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate is reacted with phenylacetyl chloride in cold aqueous acetone containing sodium bicarbonate to provide p - nitrobenzyl 7 - phenylacetamido - 3 - chloro - 3 - cephem - 4 - carboxylate.

Diphenylmethyl 7 - amino - 3 - bromo - 3 - cepham - 4 - carboxylate is reacted with phenoxycetyl chloride in the presence of pyridine to provide diphenylmethyl 7 - phenoxyacetamido - 3 - bromo - 3 - cephem - 4 - carboxylate.

p - Methoxybenzyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate is reacted with mandelic O - carboxy - anhydride in ethyl acetate to provide p - methoxybenzyl 7 - (D- α - mandelamido) - 3 - chloro - 4 - carboxylate.

Illustrative of these derivatives of the acyl groups



which can be employed in the acylation of the halo nucleus esters of free acids provided by this invention are the following: thiophene-2 - acetyl chloride, phenoxycetyl chloride, phenylacetyl chloride, oxazole - 2 - acetyl bromide, thiazole - 2 - acetyl chloride, tetrazole - 1 - acetic acid, mandelic acid O - carboxyanhydride, 4 - hydroxymandelic acid O - carboxy anhydride, 4 - chlorophenoxyacetyl bromide, benzoyl chloride, 2,6 - dimethoxybenzoyl chloride, thiophene - 3 - acetyl chloride, furyl - 2 - acetyl chloride, the pentachlorophenyl ester of phenylmercapto acetic acid, or 4 - chlorophenylmercaptoacetyl chloride.

Illustrative of the antibiotic cephalosporins of the Formula I wherein R' is represented by R''CH₂- are the following:

7 - [2 - (2 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,
7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylic acid,
7 - [2 - (3 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,
7 - [2 - (2 - furyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

7 - [2 - (2 - furyl)acetamido] - 3 - bromo - 3 - cephem - 4 - carboxylic acid,

7 - [2 - (2 - oxazolyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - [2 - (2 - thiazoyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - [2 - (2 - thiazolyl)acetamido] - 3 - bromo - 3 - cephem - 4 - carboxylic acid,

7 - [2 - (1 - tetrazolyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - [2 - (1 - tetrazolyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylic acid, and

the benzyl, diphenylmethyl, m - methoxybenzyl, p - nitrobenzyl, 2,2,2 - trichloroethyl and tert-butyl esters and the pharmaceutically acceptable esters and non-toxic base addition salts thereof.

The compounds of the Formula I wherein Q is a carboxylic acid group can be prepared for example, by the acylation of a 3-halo-3-cephem nucleus ester with tert-butyl phenylmalonic acid chloride, or with tert-butyl 2-thienylmalonic acid chloride in the presence of a hydrogen halide acceptor such as sodium bicarbonate. For example, tert-butyl - 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate is reacted with 2 equivalents of tert-butyl phenylmalonyl chloride in acetone at a temperature of about 5°C. and in the presence of excess sodium bicarbonate to provide, tert-butyl 7 - (α - tert - butyloxycarboxylphenylacetamido) - 3 - chloro - 3 - cephem - 4 - carboxylate. Removal of both tert-butyl ester groups with 90% formic acid provides the diacid, 7 - (α - carboxyphenylacetamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

Illustrative compounds represented by the Formula I wherein Q is a carboxy group are 7 - (α - carboxyphenylacetamido)-3-chloro-3-cephem - 4 - carboxylic acid, 7 - [2 - carboxy-2 - (2 - thienyl) - acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid, 7 - (α - carboxy - 4 - hydroxyphenylacetamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid, 7 - (α - carboxyphenylacetamido) - 3 - fluoro - 3 - cephem - 4 - carboxylic acid, 7 - [2 - carboxy - 2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylic acid, 7 - (α - carboxyphenylacetamido) - 3 - bromo - 3 - cephem - 4 - carboxylic acid, 7 - (α - carboxy - 4 - chlorophenylacetamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid, 7 - (α - carboxy - 3,4 - dimethoxyphenylacetamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid, 7 - [2 - carboxy - 2 - (3 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid, 7 - (α - carboxy - 3 - hydroxyphenylacetamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid and the benzyl, diphenylmethyl, p-methoxybenzyl, p-nitrobenzyl, 2,2,2-trichloroethyl and tert-butyl esters and the pharmaceutically acceptable non-toxic base addition salts thereof.

The α - sulfo - acylamido - 3 - halo-

lo] - 3 - bromo-
id,
etamid - 3 - 65
boxylic acid,
acetamido] - 3
boxylic acid,
etamid - 1 - 3-
boxylic acid, 70
etamido] - 3-
boxylic acid,
etamido] - 3 -
oxylic acid, and
m - methoxy- 75
- trichloroethyl
pharmaceutically
c base addition

mula I wherein
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t-butyl phenyl-
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n the presence
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rt-butyl - 7 -
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valents of tert-
in acetone at a
in the presence
o provide, tert-
carboxylphenyl-
- cephem - 4 -
tert-butyl ester
d provides the
etamido) - 3 -
oxylic acid.

esented by the
oxy group are
lo]-3-chloro-3-
- [2 - carboxy-
- 3 - chloro-
acid, 7 - (α-
etamido) - 3 -
oxylic acid, 7-
3 - fluoro - 3 -
7 - [2 - carb-
- 3 - fluoro-
acid, 7 - (α- 110
- bromo - 3 -
7 - (α - carb-
- 3 - chloro-
acid, 7 - (α-
ylacetamido)- 115
arboxylic acid,
ylacetamido]-
arboxylic acid,
oxyphenylacet-
m - 4 - carb- 120
phenylmethyl,
4, 2,2,2-tri-
ers and the
on-toxic base
- 3 - halo- 125

cephem compounds of the Formula I where-
in Q is a sulfo group ($-\text{SO}_3\text{H}$) are prepared
by following the acylation procedures em-
ployed for the preparation of α-sulfobenzyl-
penicillins described in the *J. Med. Chem.*, 15
(11), 11-5 (1972); *ibid.*, p. 1108. For ex-
ample, 7 - amino - 3 - chloro - 3 - cephem -
4 - carboxylic acid is reacted with α - sulfo-
phenylacetyl chloride in a mixture of acetone
and water containing an excess of sodium
bicarbonate to yield 7 - (α - sulfophenylacet-
amido) - 3 - chloro - 3 - cephem - 4 -
carboxylic acid. Examples of α - sulfoacyl-
amido - 3 - halo - cepheems of the present
invention are,

7-(α - sulfo - 3 - chlorophenylacetamido)-3-
chloro - 3 - cephem - 4 - carboxylic acid,
7 - (α - sulfo - 4 - hydroxyphenylacet-
amido) - 3 - chloro - 3 - cephem - 4 - carb-
oxylic acid,

7 - (α - sulfo - 4 - methoxyphenylacet-
amido) - 3 - bromo - 3 - cephem - 4 - carb-
oxylic acid,

7 - [2 - sulfo - 2 - (2 - thienyl)acetamido]-
3 - chloro - 3 - cephem - 4 - carboxylic acid,

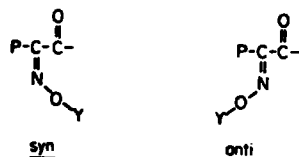
7 - [2 - sulfo - 2 - (3 - thienyl)acetamido]-
3 - chloro - 3 - cephem - 4 - carboxylic acid.

7 - [2 - sulfo - (2 - thienyl)acetamido]-
3 - fluoro - 3 - cephem - 4 - carboxylic acid,

7 - (α - sulfo - 3,4 - dimethylphenylacet-
amido) - 3 - chloro - 3 - cephem - 4 - carb-
oxylic acid and the benzyl, diphenylmethyl,

p - methoxybenzyl, p - nitrobenzyl, 2,2 - tri-
chloroethyl and tert-butyl esters and the phar-
maceutically acceptable non-toxic base addi-
tion salts thereof.

The compounds of the Formula I wherein
R is an acyl group of the formula



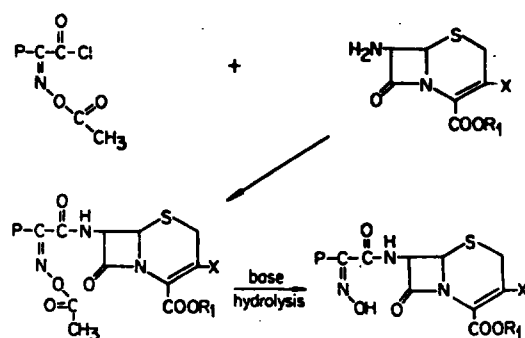
are prepared by the acylation of a 3 - halo-
3 - cephem - nucleus ester with an α-meth-
oximino or α-acetyloximino glyoxalmoyl chlor-
ide wherein Y is methyl or acetyl. The α-
oximino compounds, Y=H, are prepared by
the base hydrolysis of the α - acetyloximino
compounds. For example, p - nitrobenzyl
7 - (α - methoximinophenylglyoxamido) - 3 -
chloro - 3 - cephem - 4 - carboxylate is pre-
pared by the acylation of p - nitrobenzyl
7 - amino - 3 - chloro - 3 - cephem - 4 -
carboxylate with α - methoxyiminophenyl-
glyoxalmoyl chloride in an acetone-water mix-
ture containing an excess of sodium bicarbon-
ate.

p - Nitrobenzyl 7 - amino - 3 - chloro-
3 - cephem - 4 - carboxylate is acylated with
α - acetyloximinophenylglyoxalmoyl chloride in
acetone in the presence of pyridine to yield

p - nitrobenzyl 7 - (α - acetyloximinophenyl-
glyoxamido) - 3 - chloro - 3 - cephem - 4 -
carboxylate. 60

The α-oximino compounds, Y=H, are
obtained by the mild base hydrolysis of the
α-acetyloximino compounds. For example, p-
nitrobenzyl 7 - (α - acetyloximinophenylgly-
oxamido) - 3 - chloro - 3 - cephem - 4 - 65
carboxylate is reacted in aqueous acetone for
12 hours at room temperature with one mole
of sodium hydroxide to yield p - nitrobenzyl
7 - (α - oximinophenylglyoxamido) - 3 - 70
chloro - 3 - cephem - 4 - carboxylate.

The foregoing is illustrated by the follow-
ing reaction scheme which depicts arbitrarily
the *syn* form



wherein P, R₁ and X have the same meanings
as previously defined.

Illustrative of the 7 - α - oximino, α-
acetyloximino- and α - methoximinoarylgy-
oxamido - 3 - halo cephalosporins provided by
this invention are

7 - (α - oximinophenylglyoxamido) - 3 -
chloro - 3 - cephem - 4 - carboxylic acid,

7 - (α - methoxyimino - 4 - chlorophenyl-
glyoxamido) - 3 - chloro - 3 - cephem - 4 - 85
carboxylic acid,

7 - (α - oximino - 4 - hydroxyphenylgly-
oxamido) - 3 - chloro - 3 - cephem - 4 -
carboxylic acid,

7 - [α - oximino - 2 - (2 - thienyl)glyox-
amido] - 3 - chloro - 3 - cephem - 4 - carb- 90
oxylic acid,

7 - (α - oximinophenylglyoxamido - 3 -
fluoro - 3 - cephem - 4 - carboxylic acid,

7 - (α - methoxyiminophenylglyoxamido)- 95
3 - bromo - 3 - cephem - 4 - carboxylic acid,

7 - [α - oximino - 2 - (3 - thienyl)glyox-
amido] - 3 - chloro - 3 - cephem - 4 - carb-
oxylic acid,

7 - [α - oximino - 2 - (2 - thienyl)glyox- 100
amido] - 3 - fluoro - 3 - cephem - 4 - carb-
oxylic acid,

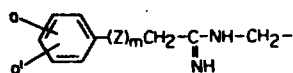
7 - (α - acetyloximinophenylglyoxamido)-
3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - (α - acetyloximinophenylglyoxamido)- 105
3 - fluoro - 3 - cephem - 4 - carboxylic acid,

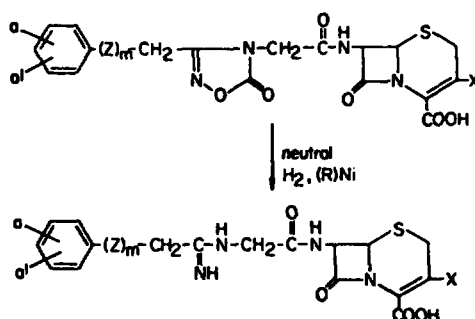
7 - [α - acetyloximino - 2 - (2 - thienyl)-
glyoxamido] - 3 - chloro - 3 - cephem - 4 -
carboxylic acid, and the benzyl, diphenyl-

methyl, p - methoxybenzyl, p - nitrobenzyl, 2,2,2 - trichloroethyl and tert-butyl esters and the pharmaceutically acceptable esters and non-toxic base addition salts thereof.

- 5 The antibiotic compounds of the Formula I, wherein R' is a substituted imidoyl aminoacetamido group of the formula



- 10 are prepared with a 7 - (3 - benzoyl, 3 - phenoxyethyl, or 3 - phenylmercaptomethyl-1,2,4 - oxadiazole - 5 - one - 4 - acetamido)-3 - halo - 3 - cephem - 4 - carboxylic acid by hydrogenation in the presence of Raney nickel catalyst at neutral pH. The following reaction scheme is illustrative



wherein a, a' Z, m, and X are, as previously defined.

- 20 The substituted oxadiazole 3 - halocephalosporin is prepared by acylation of a 3-halo nucleus acid or ester, for example 7 - amino-3 - chloro - 3 - cephem - 4 - carboxylic acid, with a 3-substituted 1,2,4 - oxadiazole - 5-one - 4 - acetyl chloride under conventional acylation conditions.

- 25 Alternatively these intermediates can be prepared by reacting a 7 - haloacetamido - 3-halo - 3 - cephem - 4 - carboxylic acid or ester (Formula I, R=chloro or bromoacetyl) with the 3-substituted 1,2,4 - oxadiazole - 5-one in the presence of a hydrogen halide acceptor such as pyridine.

- 30 Illustrative of the 7-substituted - imidoyl-aminoacetamido - 3 - halo cephalosporin antibiotics represented by the Formula are,

7 - [N - phenoxyacetimidoyl)aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

- 40 7 - [N - (phenylacetamidoyl)aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - [N - (phenylmercaptoacetimidoyl)-aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

- 45 7 - [N - (4 - chlorophenylmercaptoacetimidoyl)aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - [N - phenylacetimidoyl)aminoacet -

amido] - 3 - fluoro - 3 - cephem - 4 - carboxylic acid,

7 - [N - (phenoxyacetimidoyl)aminoacetamido] - 3 - bromo - 3 - cephem - 4 - carboxylic acid,

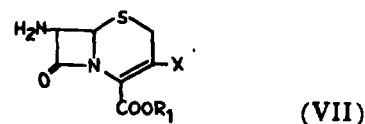
7 - [N - (4 - hydroxyphenoxyacetimidoyl)aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - [N - (4 - chlorophenoxyacetimidoyl)-aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

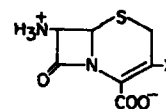
7 - [N - (4 - nitrophenylacetimidoyl)-aminoacetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid, and the benzyl, diphenylmethyl, p - methoxybenzyl, p - nitrobenzyl,

2,2,2 - trichloroethyl and tert - butyl esters and the pharmaceutically acceptable esters and non-toxic base addition salts thereof.

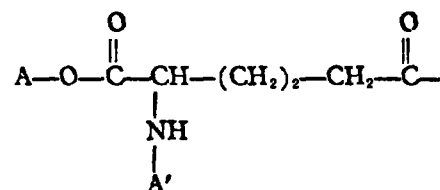
Especially useful compounds of this invention are the 3 - halo nucleus esters and acids of the Formula I wherein R is hydrogen. The 3-halo nucleus esters and acids are represented by the following formula



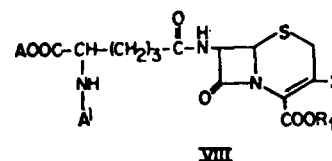
wherein X and R₁ are as previously defined. When R₁ is hydrogen, the zwitterionic form of the compound can exist as illustrated below.



Another especially useful group of compounds provided herein are represented by the Formula I wherein R is a 5 - amino - 5-carboxyvaleryl or an esterified amino-protected 5 - amino - carboxyvaleryl group represented by the formula



wherein A and A' are as previously defined. Such compounds are represented by the following formula



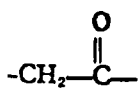
VIII

hem - 4 - carb- 50
 idoyl) acet-
 - cephem - 4-
 phenoxacetimid- 5
 chloro - cep- 55
 oxacetimidoyl)-
 - 3 - cephem- 10
 nylacetimidoyl)- 60
 - 3 - cephem-
 enzy, diphenyl-
 p - nitrobenzyl,
 t - butyl esters
 stable esters and 65
 thereof.
 s of this inven-
 esters and acids
 R is hydrogen.
 acids are repre- 70
 a

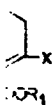
(VII)

viously defined.
 witterionic form
 s illustrated be- 75

group of com-
 represented by
 5 - amino - 5-
 ed amino-pro-
 lieryl group re- 80



iously defined.
 ed by the fol- 85



wherein X and R₁ are as defined previously.

The above represented compounds are valuable intermediates for the preparation of the 3-halo nucleus acids and esters in that they can undergo the well known 7 - acylamino side-chain cleavage reaction with phosphorus pentachloride and pyridine by following the cleavage procedures by which 7 - aminocephalosporanic acid esters are prepared with cephalosporin C.

They are prepared with cephalosporin C in the following manner. Initially, the side-chain amino group and both carboxyl groups of cephalosporin C are protected with such groups as A and A' and then the protected molecule is reacted with a sulfur containing nucleophile, for example, potassium ethyl xanthate or thiourea to form the 3 - thio-substituted methyl derivative by nucleophilic displacement of the acetoxymethyl group of the 3 - acetoxymethyl group. Thereafter the 3 - thio-substituted methyl - 3 - cephem ester is reduced with either Raney nickel in the presence of hydrogen or zinc/formic acid in the presence of DMF to effect the reductive displacement of the 3 - thio substituent and provide the 3 - exomethylenecephem ester.

The 3 - exomethylenecephem ester is then reacted with ozone and the ozonide decomposed to form the 3 - hydroxy - 3 - cephem ester according to the method described previously. The 3-hydroxy ester is then halogenated according to the method of this invention to provide a compound of the above formula VIII.

Cleavage of the protected 7-acyl side-chain with PCl₅/pyridine/isobutanol or methanol affords the 3-halo nucleus ester of the formula VII. Cleavage of the 7-acyl group can also be carried out with nitrosyl chloride when the amino adipoyl group is unprotected.

Illustrative of the compounds represented by the above formula VIII are

diphenylmethyl 7 - [5 - diphenylmethyl-oxycarbonyl - 5 - (2,4 - dichlorobenzamido)-valeramido] - 3 - chloro - 3 - cephem - 4 - carboxylate,

diphenylmethyl 7 - (5 - diphenylmethoxy-carbonyl - 5 - chloroacetamidovaleramido) - 3 - chloro - 3 - cephem - 4 - carboxylate,

p - nitrobenzyl 7 - (5 - p - nitrobenzyl-oxycarbonyl - 5 - propionamido - valeramido) - 3 - chloro - 3 - cephem - 4 - carboxylate,

diphenylmethyl 7 - [5 - diphenylmethyl-oxycarbonyl - 5 - (4 - chlorobenzamido)-valeramido] - 3 - chloro - 3 - cephem - 4 - carboxylate,

p - nitrobenzyl 7 - (5 - p - nitrobenzyl-oxycarbonyl - 5 - acetamido - valeramido) - 3 - chloro - 3 - cephem - 4 - carboxylate and

diphenylmethyl 7 - [5 - diphenylmethyl-oxycarbonyl - 5 - (2,4 - dichlorobenzamido)-valeramido] - 3 - bromo - 3 - cephem - 4 - carboxylate.

Examples of the 7 - amino - 3 - halo - 3 -

cephem acids and esters which are provided are the following.

7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylic acid,

7 - amino - 3 - bromo - 3 - cephem - 4 - carboxylic acid, 70

7 - amino - 3 - fluoro - 3 - cephem - 4 - carboxylic acid,

diphenylmethyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate, 75

p - nitrobenzyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate,

p - nitrobenzyl 7 - amino - 3 - bromo - 3 - cephem - 4 - carboxylate,

p - methoxybenzyl 7 - amino - 3 - fluoro - 3 - cephem - 4 - carboxylate, 80

2,2,2 - trichloroethyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate, or

benzyl 7 - amino - 3 - bromo - 3 - cephem - 4 - carboxylate. 85

The term "pharmaceutically acceptable" esters as used herein refers to the C₁-C₄ straight and branched chained alkanoyloxy-methyl esters such as acetoxymethyl, propionylloxymethyl and pivaloyloxymethyl esters. These esters of the 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acids possess anti-biotic activity at levels akin to those of the free acid form of the cephalosporin compound and can be used in place of the antibiotic in the free acid form. In contrast, the other ester groups within the definition of R₁, namely the benzyl, benzhydryl, p - methoxybenzyl, p - nitrobenzyl, 2,2,2 - trichloroethyl and t-butyl esters, do not possess any significant antibiotic activity. 95

The 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acids

(Formula I, R=R'-C=O, R₁=H)

are useful antibiotic compounds for combating infections attributable to gram-positive and gram-negative organisms. The compounds can be administered by injection (s.c. or i.m.) in the free acid form or in the form of a pharmaceutically acceptable ester or non-toxic acid addition salt. Salts formed with the free acids and inorganic bases such as sodium bicarbonate, potassium carbonate, sodium hydroxide and calcium hydroxide provide the sodium, potassium and calcium salts which can be formulated for administration for example, as isotonic solutions or as liquid suspensions. 105

The pharmaceutically acceptable esters, for example the acetoxymethylesters, are prepared by reacting a 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acid alkali metal salt such as the potassium salt with the alkanoyloxymethyl halide such as acetoxymethyl chloride in aqueous acetone. 110

The invitro antimicrobial activity of the 3 - halo cephalosporins is illustrated by the data listed in Table I. Table I contains the anti- 120 125

microbial activity for 7 - [2 - (2 - thienyl)-
acetamido] - 3 - chloro - 3 - cephem - 4-
carboxylic acid obtained in the standard Disc-

plate method. The numerical values are the
diameter in millimeters of the zones of inhibi-
tion observed with the named organisms.

TABLE I

ANTIBIOTIC ACTIVITY OF
7-[2-(2-Thienyl)acetamido]-3-chloro-3-cephem-4-carboxylic acid

Test Organism	Zone of Inhibition (diameter in mm) Concentration (mg./ml)		
	1.0	0.1	0.01
<i>Staphylococcus aureus</i>	31	29	20
<i>Bacillus subtilis</i>	48	40	24
<i>Sarcina lutea</i>	37	28	21
<i>Mycobacterium avium</i>	14	—	—
<i>Proteus vulgaris</i>	17	12H ¹	—
<i>Salmonella gallinarum</i>	32	22	—
<i>Escherichia coli</i>	24	19	Tr ²
<i>Klebsiella pneumoniae</i>	36	27	13
<i>Pseudomonas solanacearum</i>	28	21	—

¹/ H = hazy zone of inhibition

²/ Tr = trace zone of inhibition

1 values are the
zones inhibi-
d organisms.

5

id

01

The following Table II lists the minimum inhibitory concentration (MIC) for 7 - [2 - (2 - thienyl)acetamido] - 3 - chloro 3 - cephem - 4 - carboxylic acid against penicillin resistant *Staphylococcus* both in the presence of and absence of serum. The MIC values were obtained by the Gradient Plate technique carried out essentially as described by Bryson and Szybalski, *Science*, 116, 45 (1952).

TABLE II

ANTIBIOTIC ACTIVITY vs.
Penicillin Resistant *Staphylococcus*

Clinical Isolate	Minimum Inhibitory Concentration (mcg/ml)	
	No Serum	Serum
V 41	5.0	7.0
V 32	8.4	>20
X400 ¹	>20	>20
V 84	0.8	1.0
X 1.1	0.2	>0.1

¹/ Methicillin resistant *Staphylococcus*

In Table III, below, the MIC values for the test compound of Tables I and II against representative gram-negative organisms is

presented. The data were obtained by the Gradient Plate technique.

TABLE III

Antibiotic Activity
vs.
Gram-Negative Organisms

Test Organism	Minimum Inhibitory Conc. (mcg/ml)
<i>Shigella</i> sp.	25
<i>Escherichia coli</i>	18.5
<i>Klebsiella pneumoniae</i>	0.6
<i>Aerobacter aerogenes</i>	0.8
<i>Salmonella heidelberg</i>	0.8
<i>Pseudomonas aeruginosa</i>	>200
<i>Serratia marcescens</i>	>200

In Table IV below the *in vitro* antimicrobial activity of 7 - [2 - (2 - thienyl)acetamido] - 3 - bromo - 3 - cephem - 4 - carboxylic acid is presented for several illustrative microorganisms. The data presented were obtained in the standard disc-plate method with the numerical values recording the diameter in millimeters of the zones of inhibition observed with the named microorganisms.

20

25

TABLE IV

ANTIBIOTIC ACTIVITY OF
7-[2-(2-thienyl) acetamido]-3-bromo-3-cephem-4-carboxylic acid

Test Organism	Zone of Inhibition 1 (diameter in mm) Concentration (mg ml)		
	1.0	0.1	0.01
<i>Staphylococcus aureus</i>	27	25	22
<i>Bacillus subtilis</i>	37	38	23
<i>Sarcina lutea</i>	32	33	17
<i>Mycobacterium svium</i>	23	h42	h33
<i>Saccharomyces pastorianus</i>	—	—	—
<i>Neurospora</i> sp.	—	—	—
<i>Candida albicans</i>	—	—	—
<i>Bacillus subtilis</i> *	60	50	43
<i>Trichophyton mentagrophytes</i>	—	—	—
<i>Proteus vulgaris</i>	28	20	h11
<i>Salmonella gallinarum</i>	30	15	—
<i>Eschiuchia coli</i>	23	14	—
<i>Pseudmonas aeruginosa</i>	—	—	—
<i>Klebsiella pneumoniae</i>	28	19	11
<i>Serratia marcescans</i>	—	—	—
<i>Pseudomonas solanacearum</i>	31	h20	—
<i>Escherichia coli</i> *	27	18	h11

* Synthetic medium

1. An h = hazy zone

A dash indicates no observed zone of inhibition.

The following Table V lists the minimum inhibitory concentration (MIC) for the antibiotic 7 - [2 - (2 - thienyl)acetamido] - 3-bromo - 3 - cephem - 4 - carboxylic acid

against penicillin resistant strains of *Staphylococcus* in the absence of serum.

The inhibitory concentration were obtained by the Gradient Plate method.

TABLE V
ANTIBIOTIC ACTIVITY VS.
PENICILLIN RESISTANT
STAPHYLOCOCCUS

Clinical Isolate	Minimum Inhibitory Concentration (mcg/ml)
V41	0.5
V32	0.6
X400 ¹	>20
V84	0.4
X1.1	0.3

¹ Methicillin resistant *Staphylococcus*.

The minimum inhibitory concentrations for 7 - [2 - (2 - thienyl)acetamido] - 3 - bromo - 3 - cephem - 4 - carboxylic acid against representative gram-negative bacteria is presented in Table VI. The data were obtained by the Gradient Plate method.

TABLE VI
ANTIBIOTIC ACTIVITY OF
7-[2-(2-thienyl)acetamido]-3-bromo-
3-cephem-4-carboxylic acid
vs.
Gram-Negative Bacteria

Test Organism	Minimum Inhibitory Concentration (mcg/ml)
<i>Shigella</i> sp.	19.8
<i>Escherichia coli</i>	20.8
<i>Klebsiella pneumoniae</i>	1.0
<i>Aerobacter aerogenes</i>	1.0
<i>Salmonella heidelberg</i>	1.0
<i>Pseudomonas aeruginosa</i>	>200
<i>Serratia marcescens</i>	>200

The 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acid esters

(Formula I, R=R'-C=O, R₁=ester)

are useful as intermediates in the preparation of the free acid antibiotic forms of the compounds. Ester forming groups within the definition of R₁ are all known groups commonly employed to protect the C₄ carboxylic acid group of the cephalosporin molecule while reactions involving other groups in the molecule are performed. These ester forming groups are readily removed to provide the free acid by known reduction or hydrolysis procedures. For example, the p - nitrobenzyl ester group is removed via catalytic hydrogenolysis over palladium on carbon; the diphenylmethyl group (benzhydryl) is removed with trifluoroacetic acid in anisole at about 10°C.; the p-methoxybenzyl group is removed with trifluoroacetic acid at about 10°C. [J. Org. Chem., 36, 1259 (1971)]; the 2,2,2 - trichloroethyl group is removed with zinc and acid [J. Am. Chem. Soc. 88, 852 (1966)]; the benzyl ester group is removed via catalytic hydrogenolysis over palladium catalyst [J. Org. Chem. 27, 1381 (1962)]; and the tert-butyl group is removed as described in J. Org. Chem., 31, 444 (1966).

The 7 - amino - 3 - halo - 3 - cephem - 4 - carboxylic acids and esters (Formula I, R=H) are valuable intermediates useful in preparing the 3 - halo antibiotics. As previously described these 3-halo nucleus acids and esters are acylated by following conventional N-acylation procedures to provide the 7 - acylamido - 3 - halo - 3 - cephem - 4 - carboxylic acids or esters.

The following Examples are provided to further illustrate the compounds of the invention and the methods and procedures employed in their preparation.

A. Preparation of Starting Materials.

Example 1.

p - Nitrobenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate hydrochloride

To a solution of 965 mg. (2 mmole) of p-nitrobenzyl 7-phenoxyacetamido - 3 - methylenecepham - 4 - carboxylate in 10 ml. of methylene chloride were added 175 mg of dry pyridine and 460 mg. of phosphorus pentachloride and the mixture was stirred at room temperature for 6 hours. One ml. of isobutanol was added to the mixture which was then stored at 0°C. overnight. The reaction product, p - nitrobenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate hydrochloride, which formed as a crystalline precipitate was filtered to yield 430 mg. (58% yield).

Elemental Analysis for C₁₅H₁₆N₃O₅SCl₃

Theory: C, 46.69; H, 4.18; N, 10.89

Found: C, 46.40; H, 4.20; N, 10.62

I.R. (Nujol* Mull)

Carbonyl absorption at 5.65 (β -lactam) and 5.75 (ester) microns.

- 5 N.M.R. (DMSO d_6) signals at 6.34 (2d, 2H, C_2-H_2), 4.98 (d, 1H, C_6-H); 4.7—4.4 (m, 6H, C_1-H , ester CH_2 , C_3-CH_2 and C_7-H); and 2.4—1.6 (m, 4H, aromatic H) tau.

Example 2.

- 10 p - Nitrobenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate p - toluenesulfonate salt.

- 15 To a solution of 965 mg. of p - nitrobenzyl 7 - phenoxyacetamido - 3 - methylenecepham 4 - carboxylate in 10 ml. of methylene chloride were added 175 mg. of dry pyridine and 460 mg. of phosphorus pentachloride and the mixture was stirred for five hours at room temperature. Thereafter the reaction mixture was cooled to 0°C. and 50 ml. of cold methanol were added. Following a stirring period of one-hour at room temperature the reaction mixture was evaporated in vacuo to remove the solvents and the residual reaction product
- 20 mixture was dissolved in a mixture of ethyl acetate and water. The pH was adjusted to pH 7 and the ethyl acetate layer was separated and washed with water and dried. One-equivalent of p - toluene sulfonic acid was added to the dried solution and on cooling 600 mg. of p - nitrobenzyl 7 - amino - 3 - methylene - cepham - 4 - carboxylate p - toluenesulfonate formed as a crystalline precipitate. The product was purified by recrystallization from a mixture of 12 ml. of methanol
- 25 30 35 24 ml. of ether and 15 ml. of petroleum ether.

Elemental analysis for $C_{22}H_{23}N_3O_6S_2$:

Theory: C, 50.66; H, 4.45; N, 8.06

Found: C, 50.41; H, 4.51; N, 7.86

- 40 I.R. (Nujol Mull)

carbonyl absorption at 5.65 (β -lactam) and 5.71 (ester) microns

N.M.R. (DMSO d_6)

- 45 Signals at 7.70 (s, 3H, p - methyl); 6.39 (s, 2H, C_2-H_2); 4.98 (d, 1H, C_6-H); 4.7—4.3 (m, 6H, C_1-H , ester CH_2 , C_3-CH_2 , and C_7-H); and 2.93—1.68 (m, 8H, aromatic H) tau.

U.V. (ph 6 buffer)

- 50 Maxima at 219 $m\mu$ ($\epsilon=19,600$) and 268 $m\mu$ ($\epsilon=9,400$).

Example 3.

p - Methoxybenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate hydrochloride.

- 55 To a solution of 4.3 g. of p - methoxybenzyl 7 phenoxyacetamido-3-methylene-cepham-carboxylate in 50 ml. of methylene chloride were added 880 mg. of dry pyridine and 2.3 g of phosphorus pentachloride and the mixture was stirred at the reflux temperature for 3
- 60

* The word "NUJOL" is a trade mark.

hours. The reaction mixture was then cooled in an ice-water bath and 5 ml. of isobutanol were added. The mixture was stirred in the cold for several hours during which time 2.2 g. of the reaction product, p - methoxybenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate hydrochloride, precipitated from the mixture. The product was filtered and washed with cold methylene chloride and was dried in vacuo.

Elemental analysis for $C_{16}H_{19}N_2O_6S$:

Theory: C, 51.82; H, 5.16; N, 7.55

Found: C, 51.65; H, 5.04; N, 7.72

Example 4.

p - Methoxybenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate p - toluenesulfonate.

To a solution of 937 mg. of p - methoxybenzyl 7 - phenoxyacetamido- 3 - methylenecepham - 4 - carboxylate in 10 ml. of methylene chloride was added 0.18 ml. of dry pyridine and 460 mg. of phosphorus pentachloride. The mixture was stirred at room temperature for 2 hours and was then cooled to 5°C. To the cold mixture was added 50 ml. of cold methanol and the mixture was allowed to warm to room temperature. The reaction mixture was evaporated in vacuo and the residue was dissolved in a mixture of ethyl acetate and water. The pH of the solution was adjusted to pH 7 and the ethyl acetate layer was separated, washed with water and dried. To the dried ethyl acetate layer was added one equivalent of p - toluene sulfonic acid. On cooling 600 mg. of p - methoxybenzyl 3 - methylenecepham - 4 - carboxylate p - toluenesulfonate precipitated as a crystalline solid.

Elemental analysis for $C_{22}H_{26}N_2O_6S_2$:

Theory: C, 54.53; H, 5.17; N, 5.53

Found: C, 54.33; H, 5.05; N, 5.47.

I.R. (Nujol Mull):

Carbonyl absorption band at 5.65 (β -lactam) and 5.78 (ester) microns.

N.M.R. (DMSO d_6):

Signals at 7.69 (s, 3H, para methyl)
6.41 (s, 2H, C_2-H_2)
6.23 (s, 3H, para methoxy)
5.0 (d, 1H, C_6-H)
4.82 (s, 2H, ester CH_2)
4.7—4.55 (m, 4H, C_1-H , C_3-CH_2 and C_7-H)
3.2—2.0 (m, 8H, aromatic H) tau.

Example 5.

p - Nitrobenzyl 7 - amino - 3 - hydroxy - 3 - cephem - 4 - carboxylate hydrochloride

A solution of 3.85 g. of p - nitrobenzyl 7-amino - 3 - methylenecepham - 4 - carboxylate hydrochloride, prepared as described by

e was then cooled
ml. of isobutanol
was st: 1 in the
ig which time 2.2
p - methoxybenzyl
ephem - 4 - carb-
ipitated from the
ltered and washed
de and was dried

$\text{H}_{10}\text{N}_2\text{O}_4\text{S}_2\text{Cl}$:
5.16; N, 7.55
5.04; N, 7.72

p - 3 - methylene-
late p - toluene-

of p - methoxy-
p - 3 - methylene-
10 ml. of methyl-
1 ml. of dry pyr-
thorus pentachlor-
ed at room tem-
is then cooled to
as added 50 ml.
mixture was allowed
ure. The reaction
vacuo and the
mixture of ethyl
of the solution
the ethyl acetate
with water and
cetate layer was
toluene sulfonic
of p - methoxy-
- 4 - carboxylate
ted as a crystal-

$\text{N}_2\text{O}_6\text{S}_2$:
5.17; N, 5.53
5.05; N, 5.47.

at 5.65 (β -lac-
microns.

ra methyl)
—H₂)
ra methoxy)
—H)
ter CH₂)
4H, C₄—H,
d C₇—H)
aromatic H

- hydroxy - 3 -
hydrochloride
- nitrobenzyl 7 -
- 4 - carboxyl-
as described by

Example 1, in 600 ml. of methanol was cooled
in an acetone-dry ice bath. Ozone was bubbled
through the reaction mixture for approxi-
mately 20 minutes at which time the reaction
mixture developed a faint blue coloration.
Nitrogen was then passed through the reaction
mixture to expel excess ozone. Next, the in-
termediate ozonide was decomposed by passing
sulfur dioxide gas through the reaction mix-
ture until the mixture gave a negative potas-
sium iodide-starch test.

The reaction mixture was evaporated in
vacuo and the residue was dissolved in 200
ml. of 0.1N hydrogen chloride in methylene
chloride. The solution was evaporated to dry-
ness and the residual reaction product was
dissolved in acetone. On cooling, 3.15 g. of
p - nitrobenzyl 7 - amino - 3 - hydroxy - 3 -
cephem - 4 - carboxylate hydrochloride pre-
cipitated as a crystalline solid.

I.R. (Nujol Mull):

Carbonyl absorption at

5.55 (β -lactam carbonyl) and

5.02 (ester carbonyl hydrogen bonded
hydroxy) microns.

Electrometric titration (66% DMF) pKa
4.0 and 6.3.

Example 6.

p - Nitrobenzyl 7 - amino - 3 - hydroxy - 3 -
cephem - 4 - carboxylate hydrochloride.

A solution of 4 g. of p - nitrobenzyl 7 -
amino - 3 - methylenecephem - 4 - carboxy-
late hydrochloride in 620 ml. of methanol
was cooled in a dry ice-acetone bath and ozone
was bubbled through the cold solution for
about 20 minutes. The reaction mixture was
purged of the remaining ozone by passing
nitrogen through the solution and 10 g. of
sodium bisulfite were added.

The reaction mixture was stirred for one
hour at ice-bath temperature at which time
the mixture gave a negative potassium iodide
starch test.

The mixture was evaporated in vacuo to
yield the reaction product as an amorphous
yellow residue. The residue was crystallized
in acetone to yield 3.4 g. of p - nitro-
benzyl - 7 - amino - 3 - hydroxy - 3 - cephem-
4 - carboxylate hydrochloride as a crystalline
acetone solvate.

I.R. (Nujol Mull):

Carbonyl absorption bands at 5.60 (β -
lactam) and 6.04 (ester carbonyl hydro-
gen bonded to 3 hydroxy) microns.

N.M.R. (DMSO d₆):

signals at 7.92 (s, 3H, 1/2 mole acetone),
6.22 (2d, 2H, C₂—H₂),
5.07 (d, 1H, C₆H),
4.8—4.5 (m, 3H, ester CH₂ and
C₇H),
2.4—1.6 (m, 4H, aromatic H)
tau.

Example 7.

p - Nitrobenzyl 7 - amino - 3 - hydroxy - 3 -
cephem - 4 - carboxylate hydrochloride.

Following the ozonization procedure de-
scribed by Examples 5 and 6, 3.85 g. of p -
nitrobenzyl 7 - amino - 3 - methylenecephem-
4 - carboxylate hydrochloride was ozonized in
methanol and the intermediate ozonide was
decomposed at a temperature of 0°C. with
3.5 ml. of trimethyl phosphite. The reaction
mixture was evaporated and the residue was
dissolved in 100 ml. of 0.1N HCl in methy-
lene chloride. The said solution was evaporated
and the residue was crystallized from acetone
to yield 2.8 g. of p - nitrobenzyl 7 - amino -
3 - hydroxy - 3 - cephem - 4 - carboxylate
hydrochloride.

Example 8.

p - Nitrobenzyl 7 - amino - 3 - hydroxy - 3 -
cephem - 4 - carboxylate.

Four millimole of p - nitrobenzyl 7-amino
3 - hydroxy - 3 - cephem - 4 - carboxylate
hydrochloride, prepared as described in Ex-
ample 5, was dissolved in water and ethyl
acetate was added to the solution. The pH of
the slurry was adjusted from pH 2.2 to pH 5
with 1N sodium hydroxide. The ethyl acetate
layer was separated and was washed with
water and dried over magnesium sulfate. The
dried ethyl acetate layer was evaporated to
dryness to yield 1.2 g. of p - nitrobenzyl 7 -
amino - 3 - hydroxy - 3 - cephem - 4 - carb-
oxylate as a crystalline residue.

Elemental analysis for: C₁₁H₁₃N₃O₆S:

Theory: C, 47.86; H, 3.73; N, 11.96

Found: C, 47.87; H, 4.00; N, 12.11

I.R. (Nujol Mull):

Carbonyl absorption at 5.65 (broad, β -
lactam and ester) and 6.0 amide) microns.

N.M.R. (DMSO d₆):

signals at 6.63 (2d, 2H, C₂H),
5.31 (d, 1H, C₆H),
4.89 (d, 1H, C₇H),
4.62 (s, 2H, ester CH₂),
4.30 (broad s, 2H, 7 N—H),
2.5—1.8 (m, 4H, aromatic H)
and
1.2 (d, 1H, C₃OH) tau.

Example 9.

Methyl 7 - phenoxyacetamido - 3 - hydroxy -
3 - cephem - 4 - carboxylate.

A solution of 1.6 g. of methyl 7 - phen-
oxyacetamido - 3 - methylenecephem - 4 -
carboxylate in 300 ml. of methylene chloride
was cooled in an acetone-dry ice bath. Ozone
was bubbled through the cold solution for
three minutes at which time the reaction mix-
ture developed a slight blue coloration.
Excess ozone was expelled
with a stream of oxygen and 10 g.
of sodium bisulfite were added. The reaction

mixture was stirred and allowed to warm to 0°C. The liquid phase was separated by decantation and was washed successively with a 5% solution of hydrochloric acid, water and a saturated solution of sodium chloride. The washed mixture was dried and evaporated to yield 1.5 g. of crude methyl 7 - phenoxyacetamido - 3 - hydroxy - 3 - cephem - 4 - carboxylate as an amorphous solid.

The crude product was dissolved in ethyl acetate and was extracted with a 5% solution of sodium bicarbonate. Ethyl acetate was added to the extract which was then acidified to pH 2 with 1N hydrochloric acid. The organic phase was separated and washed with a saturated solution of sodium chloride and dried. The dried extract was evaporated to dryness to yield 709 mg. of the reaction product contaminated with a minor amount of the corresponding 3 - hydroxy - 3 - cephem sulfide, an over oxidation product. The product was separated from the sulfoxide impurity and obtained pure by preparative thin layer chromatography on silica gel with chloroform: methanol (9:1).

Elemental analysis for: $C_{16}H_{18}N_2O_5S \cdot H_2O$

Theory: C, 50.26, H, 4.75; N, 7.33;
S, 8.38

Found: C, 51.03; H, 4.62; N, 7.06;
S, 8.37

I.R. (chloroform): absorption peaks at 2.8 (amide NH), 5.6 (β -lactam carbonyl), 5.85 (broad, amide and ester carbonyl) and 6.6 (amide II) microns.

N.M.R. ($CDCl_3$): signals at 6.65 (s, 2H, C_2-H_2),
6.13 (s, 3H, methyl ester),
5.40 (s, 2H, side-chain CH_2),
4.93 (d, 1H, C_6H),
4.32 (q, 1H, C_7H),
3.15—2.38 (m, 6H, aromatic and amide H), and
1.60 (broad s, 1H, 3—OH) tau.

Electrometric titration (66% aqueous DMF):
pKa 5.6

Example 10.

p - Methoxy 7 - phenoxyacetamido - 3 - hydroxy - 3 - cephem - 4 - carboxylate.

A solution of 2.5 g. of p - methoxybenzyl 1 - phenoxyacetamido - 3 - methylenecepham - 4 - carboxylate in 350 ml. of ethyl acetate was cooled in an acetone-dry ice bath. Ozone was bubbled through the cold solution for 8 minutes and then oxygen was passed through the ozonized reaction mixture to expel excess ozone. The intermediate ozonide was decomposed by adding to the reaction mixture 25 g. of sodium bisulfite with stirring at a temperature of about 0°C. The reaction solution was decanted and was washed successively with water, 5% hydrochloric acid and a saturated

solution of sodium chloride. The washed mixture was dried and evaporated to yield the reaction product, p - methoxybenzyl 7-phenoxyacetamido - 3 - hydroxy - 3 - cephem - 4 - carboxylate as an amorphous solid.

N.M.R. ($CDCl_3$): signals at

6.73 (s, 2H, C_2H_2),
6.23 (s, 3H, p-methoxy),
5.53 (s, 2H, side-chain CH_2),
5.03 (d, 1H, C_6H),
4.87 (s, 2H, ester CH_2),
4.47 (q, 1H, C_7H),
3.40—2.50 (m, 9H, aromatic H),
2.33 (d, 1H, amide NH), and
1.53 (broad s, 1H, 3 OH) tau.

Example 11.

p - Nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate.

To a solution of 1.55 g. of p - nitrobenzyl 7 - amino - 3 - hydroxy - 3 - cephem - 4 - carboxylate hydrochloride in 30 ml. of acetone containing 364 mg. (0.5 ml., 3.6 mmole), of triethylamine was added 962 mg. of urea. With stirring at room temperature, a solution of 730 mg. (4.4 mmole) of 2 - thiophene acetyl chloride in 20 ml. of acetone was added dropwise to the mixture. After 2.5 hours the reaction mixture was filtered and evaporated. The residue was dissolved in ethyl acetate and the solution was washed successively with water, a 5% solution of sodium bicarbonate, 5% hydrochloric acid, and a saturated solution of sodium chloride. The washed solution was dried and then was concentrated by evaporation in vacuo to yield 1.2 g. of the reaction product as a crystalline residue. The product was recrystallized from ethyl acetate to yield pure p - nitrobenzyl 7 - [2 - (2-thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate having the following spectral properties.

I.R. (Nujol Mull): absorption peaks at 3.0 (amide NH), 5.68 (β -lactam carbonyl), and 6.1 (amide, and ester hydrogen bonded to 3 OH) microns.

N.M.R. ($CDCl_3$ /DMSO d_6): signals at 6.54 (2d, 2H, C_2H_2), 6.16 (s, 2H, side-chain CH_2), 4.90 (d, 1H, C_6H), 4.60 (d, 2H, ester CH_2), 4.43 (q, 1H, C_7H), 3.1—1.6 (m, 7H, aromatic H) and 1.30 (d, 1H, amide NH) tau.

Example 12.

p - Nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate.

p - Nitrobenzyl 7 - amino - 3 - methylenecepham - 4 - carboxylate hydrochloride, 3.85 g., was reacted with ozone in methanol as described in Example 5 to provide the ozonide. The ozonide was decomposed with sulfur

The washed mixture yielded the oxybenzyl 7-phenyl-3-cephem-4-ol solid.	65	5	dioxide to produce the 3-hydroxy product which was isolated as crude product. The crude 3-hydroxy-3-cephem nucleus ester was dissolved in 175 ml. of tetrahydrofuran and 50 ml. of water. Sodium bisulfite, 2.1 g., was suspended in the solution and a solution of 4.8 g. of 2-thiophene acetyl chloride in 200 ml. of THF was added dropwise to the suspension.	N.M.R. (CDCl ₃): signals at 7.90 (s, 3H, 7-acetamido (CH ₃), 6.55 (s, 2H, C ₂ H ₂), 4.92 (d, 1H, 6 _a H), 4.63 (m, 2H, ester CH ₂), 4.30 (q, 1H, C ₇ H), 2.81 (d, 1H, amide NH), 2.5—1.8 (m, 4H, aromatic H), and 2.8 (s, 1H, C ₃ OH) tau. Electrometric titration (66% aqueous DMF) pKa 5.9	65
is at sy), CH ₂), H ₂), aromatic H), II), and OH) tau.	70	10	The mixture was stirred for two hours at room temperature and was then evaporated to an aqueous residue. The residue was slurried with ethyl acetate, the organic layer separated and washed with 5% hydrochloric acid and with water. The washed layer was dried and evaporated to dryness to yield the reaction product as a crystalline residue. The residue was triturated three times with diethyl ether to remove contaminating 2-thiophene acetic acid and to provide 2.9 g. of the purified crystalline product, p-nitrobenzyl 7-[2-(2-thienyl)acetamido]-3-hydroxy-3-cephem-4-carboxylate.	Example 14. p-Nitrobenzyl 7-phenylacetamido-3-hydroxy-3-cephem-4-carboxylate. Following the ozonization procedures described in Example 9, a solution of 350 mg. of p-nitrobenzyl 7-phenylacetamido-3-methylenecephem-4-carboxylate in 250 ml. of methylene chloride was cooled to -78°C. and was ozonized. The intermediate ozonide was decomposed in situ with sulfur dioxide and the reaction product was recovered and obtained crystalline by extraction with ethyl acetate.	70
1. (2-thienyl)acet- -3-cephem-4-	80	20	Electrometric titration (66% aqueous DMF) pKa 5.9. N.M.R. (CDCl ₃ /D ₂ O): signals at 6.60 (s, 2H, C ₂ H ₂), 6.13 (s, 2H, side-chain CH ₂), 4.96 (d, 1H, C ₆ H), 4.62 (d, 2H, ester CH ₂), 4.46 (d, 1H, C ₇ H) and 3.1—1.7 m, 7H, aromatic H) tau.	Elemental analysis for C ₂₂ H ₁₉ N ₃ O ₅ S: Theory: C, 56.28; H, 4.80; N, 8.95 Found: C, 56.11; H, 4.15; N, 8.74. N.M.R. (CDCl ₃): signals at 6.68 (2d, 2H, C ₂ H ₂), 6.37 (s, 2H, side-chain CH ₂), 5.03 (d, 1H, C ₆ H), 6.66 (d, 2H, ester CH ₂), 4.40 (q, 1H, C ₇ H), 2.7 (m, 6H, amide NH and aromatic H), 2.53—1.70 (q, 4H, aromatic H) and a singlet in low field integrating for 1H of C ₃ hydroxy group tau.	80
of p-nitrobenzyl 3-cephem-4- 30 ml. of acetone l, 3.6 mmole), of 62 mg. of urea. erature, a solution of 2-thiophene acetone was added fter 2.5 hours the d and evaporated. in ethyl acetate successively with dium bicarbonate, a saturated solu- e washed solution concentrated by ld 1.2 g. of the lline residue. The rom ethyl acetate yl 7-[2-(2- hydroxy-3- aving the follow-	85	25	Example 13. p-Nitrobenzyl 7-acetamido-3-hydroxy-3-cephem-4-carboxylate. A solution of 10 mmole of p-nitrobenzyl 7-amino-3-hydroxy-3-cephem-4-carboxylate hydrochloride in a mixture of 325 ml. of acetone and 125 ml. of water was cooled in an ice-water bath. With stirring a stream of ketene gas was bubbled through the solution for 30 minutes. Thereafter the reaction mixture was evaporated to remove acetone and the aqueous residue was slurried with ethyl acetate. The ethyl acetate layer was separated and was washed with 5% hydrochloric acid and a saturated solution of sodium chloride. The washed extract was dried and evaporated in vacuo to yield the reaction product as a crystalline residue. The residue was triturated with diethyl ether and vacuum dried to yield 3.55 g. of p-nitrobenzyl 7-acetamido-3-hydroxy-3-cephem-4-carboxylate melting at about 146—152°C. with decomposition.	I.R. (Nujol Mull): absorption peaks at 3.04 (amide), 5.60 and 6.0 (β-lactam, ester and amide carbonyl) microns.	85
ion peaks at 3.0 actam carbonyl), hydrogen bonded	105	45	Elemental analysis for: C ₁₆ H ₁₃ N ₃ O ₅ S: Theory: C, 48.85; H, 3.84; N, 10.68 Found: C, 48.97; H, 3.96; N, 10.42.	Example 15. p-Nitrobenzyl 7-(D-α-phenyl-α-formyloxyacetamido)-3-hydroxy-3-cephem-4-carboxylate. To a solution of 1.54 g. of p-nitrobenzyl 7-amino-3-hydroxy-3-cephem-4-carboxylate hydrochloride in 120 ml. of acetone and 40 ml. of water was added 936 mg. of sodium bisulfite. With stirring a solution of 960 mg. of O-formyl-D-mandelic acid chloride in 20 ml. of anhydrous acetone was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 16 hours and was then evaporated to remove acetone. The aqueous residue was slurried with ethyl acetate and the organic layer separated. The extract was washed with water was dried and evaporated. The crystalline residue was triturated with diethyl ether and dried to yield 1 g. of p-nitrobenzyl 7-(D-α-phenyl-α-formyloxyacetamido)-3-hydroxy-3-cephem-4-carboxylate.	95
): signals at 6.54 s, 2H, side-chain l), 4.60 (d, 2H, C ₇ H), 3.1—1.6 nd 1.30 (d, 1H,	110	50	I.R. (CHCl ₃): absorption bands at 2.9 and 3.0 (amide NH and OH), 5.63 (β-lactam carbonyl) and 5.95 (broad, amide, and ester carbonyl hydrogen bonded to 3 OH) microns.	Elemental analysis for C ₂₃ H ₁₉ N ₃ O ₅ S: Theory: C, 53.80; H, 3.73; N, 8.18 Found: C, 53.51; H, 3.81; N, 8.46	110
2-thienyl)acet- -3-cephem-	115	55	I.R. (CHCl ₃): carbonyl absorption peaks at		115
-3-methylene- chloride, 3.85 in methanol as provide the ozon- osed with sulfur	120	60			120

5.55, 5.73, 5.85 and 5.93 microns.
N.M.R. (CDCl_3): signals at 6.61, (s, 2H, C_2H_2), 4.95 (d, 1H, C_6H), 4.61 (d, 2H, ester CH_2), 4.39 (q, 1H, C_7H), 3.70 (s, 1H, $\alpha\text{-CH}$) and 2.80—1.70 (m, 11H, amide NH and aromatic H) tau.

B. Preparation of 3 - halo - 3 - cephem acids and esters.

10 Example 16.
Diphenylmethyl 7 - [2 - (2 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylate.

15 a) To a solution of 34 g. (100 mmole) of 7 - [2 - (2 - thienyl)acetamido] - 3 - methylenecepham - 4 - carboxylic acid in 500 ml. of methylene chloride was added 21.4 g. (110 mmole) of diphenyl diazomethane and the resulting mixture was stirred for 2 hours at
20 room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with a 5% solution of sodium bicarbonate, then with water and was
25 dried over magnesium sulfate. The dried solution was concentrated to a small volume. On standing 40 g. of diphenylmethyl 7 - [2 - (2 - thienyl)acetamido] - 3 - methylenecepham-4-carboxylate melting at about 132—133°C.
30 precipitated as a crystalline solid.

IR (chloroform): absorption peaks at 2.9 (amide N—H, 5.65, 5.75 and 5.93 β -lactam, ester and amide carbonyls respectively) and 6.62 (amide II) microns.

35 NMR (CDCl_3): signals at 6.72 (ABq, 2H, C_2H_2), 6.21 (s, 2H, $\alpha\text{-CH}_2$), 4.83—4.65 (m, 4H, C_1H , C_6H and $\text{C}_3\text{-CH}_2$), 4.39 (q, 1H, C_7H), 3.4—2.65 (m, 15H, $\text{C}_7\text{-NH}$, ester CH and aromatic H) tau.

40 b) To the solution of 8.1 g. (16 mmole) of the above ester in 80 ml. of methylene chloride were added 1.57 g. (1.6 ml, 19.6 mmole) of dry pyridine and 3.8 g. (18.1 mmole) of phosphorus pentachloride. The reaction mixture was stirred for 2 hours at room temperature and was thereafter cooled in an ice-water bath. The cold mixture was treated with 8 ml. of isobutanol with stirring. Stirring was continued for 2 hours during which time 3 g. of diphenylmethyl 7 - amino - 3 - methylenecepham - 4 - carboxylate hydrochloride formed as a crystalline precipitate. The product was filtered and washed with methylene
45 chlorine and vacuum dried.

Elemental analysis (percent) for $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_2\text{SCl}$:

60 Theory:
C, 60.50; H, 5.08; N, 6.72; Cl, 8.50;
Found:
C, 60.70; H, 5.02; N, 6.71; Cl, 8.80.

NMR ($\text{DMSO } d_6$): signals at 6.45 (ABq, 2H, C_2H_2), 5.00 (d, 1H, C_6H), 4.68 (d, 1H, C_7H), 4.60 (s, 2H, 3— CH_2), 4.44, (s, 1H, C_αH) 3.10 (s, 1H, ester CH), and 2.61 (s, 10H, aromatic H) tau.

c) The 7 - amino - 3 - exomethylenecepham ester hydrochloride salt product, 2.1 g. (5 mmole) was dissolved in 200 ml. of methanol and the solution was cooled in an acetone-dry ice bath. Ozone was bubbled into the cold solution for 7 minutes to form the intermediate ozonide. The ozonide was decomposed by passing a stream of sulfur dioxide gas through the reaction mixture for 2 minutes. Thereafter the reaction mixture was evaporated and the residue was triturated with diethyl ether to yield 1.6 g of diphenylmethyl 7 - amino - 3 - hydroxy - 3 - cephem - 4 - carboxylate hydrochloride as a crystalline solid.

NMR (CDCl_3): signals at 6.4 (ABq, 2H, C_2H_2), 5.0—4.5 (m, 2H, C_6H and C_7H), 3.2—2.4 (m, 11H, ester CH and aromatic H) tau.

IR: (chloroform): carbonyl absorption peaks at 5.57 and 5.70 (β -lactam and ester carbonyl respectively) microns.

UV (pH7 buffer): λ max 275 m μ , ϵ =7550.
Electrometric titration (60% aq. DMF): titratable groups at 4.5 and 6.5.

d) To a solution of 840 mg. of diphenylmethyl 7 - amino - 3 - hydroxy - 3 - cephem-4 - carboxylate in 10 ml. of water and 10 ml. of acetone was added one gram of sodium bisulfite. The mixture was stirred and 800 mg. of thiophene - 2 - acetyl chloride in 10 ml. of acetone were added dropwise. The mixture was stirred for 4.5 hours at room temperature and was then evaporated under reduced pressure. The residue was dissolved in a mixture of ethyl acetate and an aqueous 5% solution of sodium bicarbonate. The ethyl layer was separated, washed with water and dried. The dried solution was evaporated and the residue triturated with ether to yield 500 mg. of diphenylmethyl 7 - [2 - (2 - thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate.

NMR (CDCl_3): signals at 6.79 (s, 2H, C_2H_2), 6.16 (s, 2H, $\alpha\text{-CH}_2$), 5.0 (d, 1H, C_6H), 4.32 (q, 1H, C_7H), 3.05—2.46 (m, 15H, $\text{C}_7\text{-NH}$, ester CH and aromatic H) tau.

I.R. (chloroform): absorption peaks at 2.9 (amide NH), 5.6, 5.73, and 5.95 (β -lactam, ester and amide carbonyls respectively) and 6.65 (amide II) microns.

e) To a solution of 4.2 g. of diphenylmethyl 7 - [2 - (2 - thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate in 44

als at 6.45 (ABq, 1H, C₆-H), 4.68 (s, 2H, —CH₂), 3.10 (s, 1H, ester aromatic H) tau.

3 - ex ethylene-salt product, 2.1 g. 200 ml. of meth-cooled in an acet-was bubbled into nutes to form the zonide was decom-of sulfur dioxide mixture for 2 min-mixture was evaporated with di-diphenylmethyl 3 - cephem - 4-as a crystalline

t 6.4 (ABq, 2H, 2H, C₆-H and 1H, ester CH and

absorption peaks m and ester carb-

ns. 275 mμ, ε=7550. 5% aq. DMF): nd 6.5.

mg. of diphenyl-oxy - 3 - cephem-of water and 10 e gram of sodium stirred and 800 yl chloride in 10 opwise. The mix-ers at room tem-orated under re-was dissolved in d an aqueous 5% ate. The ethyl l with water and s evaporated and ther to yield 500 2 - (2 - thienyl)- 3 - cephem - 4-

t 6.79 (s, 2H, —CH₂), 5.0 (d, C₆-H), 3.05— , ester CH and

on peaks at 2.9 d 5.95 (β-lactam, respectively) and

g. of diphenyl-acetamido] - 3-carboxylate in 44

ml. of dry dimethylformamide was added 865 mg. of phosphorus trichloride. The mixture was stirred for 1.5 hours at room temperature and was poured into an ethyl acetate 5% aqueous hydrochloric acid mixture. The ethyl acetate layer was evaporated, was washed with 5% hydrochloric acid, water and was dried. The dried solution was concentrated in vacuo and the product crystallized. The 3 - chloro ester was filtered, washed with cold ethyl acetate and dried to yield 2.2 g.

Elemental analysis (percent) for C₁₆H₂₁N₂O₄S₂Cl:

Theory:

C, 59.48; H, 4.03; N, 5.34; Cl 6.75

Found:

C, 59.77; H, 4.25; N, 5.40; Cl, 6.91.

NMR. (CDCl₃): signals at 6.49 (ABq, 2H, C₂-H₂), 6.22 (s, 2H, α-CH₂), 5.08 (d, 1H, C₆-H), 4.19 (q, 1H, C₇-H), 3.13—2.5 (m, 15H, C₇-NH, ester CH, and aromatic H) tau.

I.R. (CHCl₃): absorption peaks at 2.9 (amide NH), 5.55, 5.72 and 5.90 (β-lactam, ester and amide carbonyls) and 6.60 (amide II) microns.

U.V. (dioxane): λ max, 275 mμ, ε=8700.

Example 17.

p - Nitrobenzyl 7 - [2 - (2 - thienyl)acet-amido] - 3 - chloro - 3 - cephem - 4 - carboxylate (via thionyl chloride).

To a solution of 1.9 g. (4 mmole) of p-nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate in 10 ml of DMF (dried over a molecular sieve) was added 950 mg. (0.58 ml., 8 mmole) of freshly distilled thionyl chloride. The mixture was stirred at room temperature for 6.5 hours and was then poured into 100 ml. of ethyl acetate. The mixture was extracted three times with 30 ml. portions of 5% hydrochloric acid and with a saturated solution of sodium chloride. The washed ethyl acetate solution was filtered and evaporated to dryness in vacuo. The residue was triturated with ether to yield 1.2 g of p - nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylate as a brown crystalline solid melting at about 164—166°C.

Elemental analysis (percent) for C₂₆H₁₆N₂O₆S₂Cl:

Theory:

C, 48.63; H, 3.27; N, 8.51; Cl, 7.18

Found:

C, 48.47; H, 3.29; N, 8.78; Cl, 6.96

IR (Chloroform) showed absorption bands at 2.9 (amide NH), 5.59 (β-lactam carbonyl), 5.75 (ester carbonyl) and 5.92 microns (amide carbonyl).

UV absorption spectrum (acetonitrile) showed maxima at

λ max 235 mμ, ε=12,100

and

λ max 286 mμ, ε=15,800.

The mass spectrum of the product showed a molecular ion of 493 m/e.

NMR (CDCl₃) showed signals at 6.39 (ABq, 2H, C₂-H₂), 6.17 (s, 2H, α-CH₂), 4.99 (d, 1H, C₆-H), 4.64 (s, 2H, ester CH₂), 4.19 (q, 1H, C₇-H), 3.45 (d, 1H, C₇-NH), 3.1—1.67 (m, 7H, aromatic H) tau.

Example 18.

7 - [2 - (2 - Thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

To a solution of 995 mg. (2 mmole) of p - nitrobenzyl 7 - [2 - (2 - thienyl)acet-amido] - 3 - chloro - 3 - cephem - 4 - carboxylate, prepared as described in Example 17, in 60 ml. of tetrahydrofuran and 100 ml. of methanol containing 5 drops of 1N hydrochloric acid was added one gram of 5% palladium on carbon catalyst. The catalyst was pre-reduced as a suspension in 40 ml. of ethanol at room temperature under 50 psi hydrogen pressure prior to use.

The suspension was hydrogenated at room temperature for 2.5 hours under a hydrogen pressure of 50 psi. The catalyst was filtered and washed on the filter with THF and with water. The combined filtrate and catalyst washes were evaporated to dryness and the reaction product residue was dissolved in a mixture of ethyl acetate and water. The pH of the solution was adjusted to pH 2.5 and the ethyl acetate layer was separated. The acid reaction product was extracted with water from the ethyl acetate solution at pH 7. The aqueous phase was separated, layered with ethyl acetate and acidified to pH 2.5. The ethyl acetate layer was separated, washed with water, dried over sodium sulfate and dried in vacuo. The amorphous residue was triturated with ether to yield 165 mg. of 7 - [2 - (2 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid as a crystalline solid melting at about 114—120°C. with decomposition and a softening at about 110°C.

The reduction product had the following physical characteristics.

I.R. (nujol mull) showed absorption bands at 3.1 (amide NH), 5.64 and 5.75 (β-lactam and carboxylic acid carbonyl respectively) and 6.1 (amide II) microns.

U.V. (acetonitrile): absorption maxima at

λ max 235, ε=10,700

λ max 268, ε=7,200

NMR (CDCl₃) shows signals at 6.38 (ABq, 2H, C₂-H₂), 6.16 (s, 2H, α-CH₂), 4.98 (d, 1H, C₆-H), 4.20 (q, 1H, C₇-H)

and 3.1—2.5 (m, 4H, aromatic H and C₇—NH) tau.

Percent Elemental Composition for
C₁₃H₁₁N₂O₄SCl

- 5 Theory:
C, 43.52; H, 3.09; N, 7.81; Cl, 9.88
Found:
C, 43.55; H, 3.79; N, 7.27; Cl, 9.28.

Example 19.

- 10 p - Nitrobenzyl 7 - [2 - (2 - Thienyl)acet-
amido] - 3 - chloro - 3 - cephem - 4 -
carboxylate (via phosphorus trichloride.
To a cooled solution of 439 mg. (0.93
mmole) of p - nitrobenzyl 7 - [2 - (2 - thi-
15 enyl)acetamido] - 3 - hydroxy - 3 - cephem -
4 - carboxylate in 4.4 ml. of DMF was slowly
added 85 mg. (0.05 ml., 0.63 mmole) of
phosphorus trichloride. The reaction mixture
20 was allowed to stand for 4 hours at room
temperature and thereafter the reaction pro-
duct mixture was worked-up by following the
recovery procedures described in Example 17
to provide 374 mg. of p - nitrobenzyl 7 - [2 -
25 (2 - thienyl)acetamido] - 3 - chloro - 3 -
cephem - 4 - carboxylate. The NMR spec-
trum of the product was consistent with the
expected product and with that of the com-
pound of Example 17.

Example 20.

- 30 7 - Phenoxyacetamido - 3 - chloro - 3 -
cephem - 4 - carboxylic acid.
Following the chlorination procedure of Ex-
ample 19 p - nitrobenzyl 7 - phenoxyacetamido -
3 - chloro - 3 - cephem - 4 - carboxylate was
35 prepared with phosphorus trichloride. The p-
nitrobenzyl ester group was removed by the
acidic hydrogenolysis procedure by Example
18 to provide the 3 - chlorocephalosporin acid
antibiotic compound.

Example 21.

- 40 p - Nitrobenzyl 7 - [2 - (2 - thienyl)acet-
amido] - 3 - chloro - 3 - cephem - 4 -
carboxylate (via Phosphorus oxychloride).
To a solution of 325 mg. (0.7 mmole) of
45 p - nitrobenzyl 7 - [2 - (2 - thienyl)acet-
amido] - 3 - hydroxy - 3 - cephem - 4 - carb-
oxylate in 3.3 ml. of DMF cooled in an ice-
water bath, was slowly added 212 mg. (0.13
50 ml., 1.4 mmole). The mixture was allowed to
stand for 4 hours room temperature and the
product, 225 mg. of recovered by following
the work-up procedures described in Example
17. The nuclear magnetic resonance spectrum
55 of the product was consistent with the spec-
trum of the previously characterized com-
pound.

Example 22.

p - Nitrobenzyl 7 - [2 - (2 - thienyl)acet-
amido] - 3 - chloro - 3 - cephem - 4 -
carboxylate (via Oxalyl chloride).

To a solution of 439 mg. (0.93 mmole) of
p - nitrobenzyl 7 - [2 - (2 - thienyl)acet-
amido] - 3 - hydroxy - 3 - cephem - 4 -
carboxylate in 4.4 ml. of DMF cooled in an
ice bath was added dropwise 118 mg. (0.07
ml., 0.93 mmole) of oxalyl chloride. The re-
action mixture was allowed to stand for 4
hours at room temperature and was then
poured into a mixture of aqueous 5% hydro-
chloric acid and ethyl acetate. The organic
layer was separated and was washed sequen-
tially with 5% hydrochloric acid, water and
a saturated solution of sodium chloride. The
washed layer was dried and evaporated to
dryness to yield the reaction product, p-nitro-
benzyl 7 - [2 - (2 - thienyl)acetamido] - 3 -
chloro - 3 - cephem - 4 - carboxylate as an
amorphous solid. The product was obtained
crystalline by triturating the amorphous res-
idue with ester. Yield 360 mg. The infrared
spectrum and NMR spectrum of the crystal-
line product were consistent with the spectra
of authentic material.

Example 23.

7 - [2 - (2 - Thienyl)acetamido] - 3 -
bromo - 3 - cephem - 4 - carboxylic acid.

To a solution of 19 g (40 mmole) of p-
nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] -
3 - hydroxy - 3 - cephem - 4 - carboxylate in
300 ml of dry DMF was added 15 g. (56
mmole) of phosphorus tribromide and the re-
action mixture was stirred at room tempera-
ture overnight. The reaction mixture was
poured into a mixture of ethyl acetate and
water and the organic phase was separated
and washed repeatedly with water and dried
over magnesium sulfate. The dried organic
phase was evaporated in vacuo to dryness.
The crude reaction product residue weigh-
ing about 9 g. was purified by chromato-
graphy over 500 g of silica gel using ethyl
acetate-hexane (55:45 v:v) as eluent. The
eluate was evaporated to dryness under re-
duced pressure and the product, p - nitro-
benzyl 7 - [2 - (2 - thienyl)acetamido] - 3 -
bromo - 3 - cephem - 4 - carboxylate was
obtained crystalline by triturating the dry
residue with diethyl ester.

U.V. (ethanol) λ max. 270 m μ ($\epsilon=13,300$)
and λ max. 243 m μ ($\epsilon=12,700$)

Elemental analysis calculated for

C₂₀H₁₄BrN₂O₅S₂:

Theory:

C, 44.61; H, 3.00; N, 7.81; Br, 14.84.

Found:

C, 44.78; H, 3.03; N, 7.65; Br, 14.91.

2. (2 - thienyl)acet-
3 - c em - 4 -
1 chloride). 60
(0.93 mmole) of
(2 - thienyl)acet-
- cep' m - 4 -
DMF cooled in an
se 118 mg. (0.07 65
chloride. The re-
d to stand for 4
e and was then
queous 5% hydro-
tate. The organic
is washed sequen-
c acid, water and
um chloride. The 15
nd evaporated to
product, p-nitro-
l)acetamido] - 3 -
carboxylate as an
lact was obtained
e amorphous resi-
mg. The infrared
um of the crystal-
; with the spectra
- Nuclear magnetic resonance spectrum
(DMSO_d) showed signals at 6.21 (s, 2H,
α-CH₂), 5.98 (ABq, 2H, C₂-H₂),
4.72 (d, 1HC-H, 451 (s, 2H, ester-
CH₂), 4.20 (q, 1H, C₇H),
3.04-1.74 (m, 7H; aromatic H) and 0.66
(d, 1H, C₇-CH) tau.
- The above 3 - bromo ester was de-esterified
in the following manner. The ester, 545 mg
(1.0 mmole) was hydrogenated at room tem-
perature in ethanol in the presence of pre-
reduced 5 percent palladium-on-carbon cata-
lyst. The catalyst was filtered and the filtrate
evaporated under reduced pressure to dry-
ness. The residual product was triturated with
diethyl ether to yield 180 mg (44 percent)
of crystalline product, 7 - [2 - (2 - thienyl)-
acetamido] - 3 - bromo - 3 - cephem - 4 -
carboxylic acid.
- Electrometric titration (66 percent
aqueous DMF) showed a pK_a of 4.4 and
an apparent molecular weight of 393.
The calculated molecular weight=403.
- Elemental analysis calculated for
C₁₃H₁₁BrN₂O₄S₂. 1/2 diethyl etherate
Theory:
C, 40.91; H, 3.66; N, 6.36; Br, 1815.
Found:
C, 41.29; H, 3.20; N, 6.29; Br, 1850.
- Nuclear magnetic resonance spectrum
(CDCl₃) showed signals at 8.8 (t, diethyl
ether-CH₃), 6.68-5.86 (m, C₂-H₂,
α-CH₂ and diethyl ether-CH₂), 4.90 (d,
1H, C₇-H), 3.0-2.63 (m, 3H, aromatic-
H), and 1.9 (d, 1H, amide NH) tau.
- Example 24.
Following the bromination method of Ex-
ample 23, p - nitrobenzyl 7 - phenoxyacet-
amido - 3 - bromo - 3 - cephem - 4 - carb-
oxylate is prepared with phosphorus tribro-
mide.
- Example 25.
Following the bromination method of Ex-
ample 23, 2,2,2 - trichloroethyl 7 - acetamido-
3 - bromo - 3 - cephem - 4 - carboxylate is
prepared with phosphorus tribromide.
- Example 26.
7 - [2 - (2 - Thienyl)acetamido] - 3 - fluoro-
3 - cephem - 4 - carboxylic acid.
To a solution of 325 mg. (0.7 mmole) of
p - nitrobenzyl 7 - [2 - (2 - thienyl)acet-
amido] - 3 - hydroxy - 3 - cephem - 4 - carb-
oxylate in 10 ml. of methylene chloride was
slowly added an equivalent amount of N-
(2 - chloro - 1,1,2 - trifluoroethyl) diethyl-
amine. The reaction mixture was heated for
30 minutes under gentle reflux and was then
evaporated to dryness in vacuo. The residue
was dissolved in a mixture of ethyl acetate-
water and the organic layer was separated. 60
The organic layer was washed with 5%
hydrochloric acid, water and brine and was
then dried. The dried reaction product solu-
tion was concentrated to a small volume and
n-hexane was added to precipitate, p - nitro-
benzyl 7 - [2 - (2 - thienyl)acetamido] - 3 -
fluoro - 3 - cephem - 4 - carboxylate. 65
The ester group was removed by catalytic
hydrogenolysis by the method described in
Example 18 to yield the 3 - fluoro cephalo-
sporin free acid antibiotic. 70
- Example 27.
p - Nitrobenzyl 7 - amino - 3 - chloro - 3 -
cephem - 4 - carboxylate.
To a solution of 500 mg. of p - nitro-
benzyl 7 - [2 - (2 - thienyl)acetamido] - 3 -
chloro - 3 - cephem - 4 - carboxylate in 6
ml. of methylene chloride was added 95 mg.
of dry pyridine and 237 mg. of phosphorus
pentachloride. The reaction mixture was
stirred at room temperature for 1.5 hours, was
thereafter cooled in an ice-water bath to
about 5°C. and 0.6 ml. of isobutyl alcohol
were added. On continued cooling and stir-
ring the reaction product, p - nitrobenzyl 7 -
amino - 3 - chloro - 3 - cephem - 4 - carb-
oxylate hydrochloride, crystallized from the
reaction mixture. The product was filtered,
washed with cold methylene chloride and dried
to yield 200 mg. of the crystalline product
melting with decomposition at about 168°C. 75
- Percent elemental composition for
C₁₄H₁₃ClN₃O₄S.HCl
Theory:
C, 41.39; H, 3.20; N, 10.34; Cl, 17.45. 80
Found:
C, 41.14; H, 3.31; N, 10.44; Cl, 17.29.
- I.R. (Nujol mull): showed absorption bands
at 5.55 (β-lactam carbonyl) and
at 5.78 (ester carbonyl) microns. 85
UV (pH 7 buffer): showed absorption maxi-
mum λ max 268 mμ (ε=13,800)
N.M.R. (DMSO_d): signals at 5.97 (s, 2H,
C₂-H₂), 4.8-4.5 (m, 4H, C₆-H, C₇-H
and ester CH₂), and 2.35-1.6 (g, 4H, 90
aromatic H) tau.
- Example 28.
7 - Amino - 3 - chloro - 3-cephem - 4 -
carboxylic acid.
To a solution of 750 mg. (1.85 mmole) 110
of p - nitrobenzyl 7 - amino - chloro - 3 -
cephem - 4 - carboxylate hydrochloride in 20
ml. of tetrahydrofuran and 40 ml. of meth-
anol was added a suspension of 750 mg. of
pre-reduced 5 / palladium on carbon catalyst 115
in 20 ml. of ethanol and the suspension was
hydrogenated under 50 psi of hydrogen at
room temperature for 45 minutes. The cata-
lyst was filtered and washed with THF and
water. The filtrate and catalyst washes were 120
- mu (ε=13,300)
12,700) 110
for
81; Br, 14.84.
85; Br, 14.91. 115

combined and evaporated to dryness. The residue was dissolved in a water-ethyl acetate mixture and the pH adjusted to pH 3. The insoluble product was filtered and triturated with acetone. The product was then dried to yield 115 mg. of 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

I.R. (mull): absorption peaks at 5.61 (β -lactam carbonyl) and 6.2 (carboxylic acid).

NMR (D_2O - $NaHCO_3$) signals at 6.25 (ABq, 2H, C_2-H_2) 4.88 (d, 1H, C_6-H) and 4.54 (d, 1H, C_7-H) tau.

U.V. (pH 7 buffer): absorption maximum at λ max 265 m μ , $\epsilon=7550$

Example 29.

Diphenylmethyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate.

To a solution of 525 mg. of diphenylmethyl 7 - [2 - (2 - thienyl)acetamido] - 3 - chloro - 3 - cephem - 4 - carboxylate in 20 ml. of methylene chloride was added 0.1 ml. of dry pyridine and 237 mg. of phosphorous pentachloride. The reaction mixture was stirred for 2 hours at room temperature and was then cooled in an ice-water mixture. To the cold mixture was added 0.6 ml. of isobutanol and after 30 minutes the reaction mixture was evaporated. The residue was dissolved in ethyl acetate and the solution was washed with 5% sodium bicarbonate and with water and was dried. The dried solution was evaporated to dryness and the residue was triturated with ether to yield 190 mg. of 3 - chloro nucleus ester, diphenylmethyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylate.

I.R. (mull): absorption peaks at 5.7 and 5.9 (β -lactam and ester carbonyl) microns.

NMR ($CDCl_3$): signals at 6.35 (ABq, 2H, C_2-H_2), 4.78 (2d, 2H, C_6-H and C_7-H), 3.05 (s, 1H, ester CH) and 2.65 (s, 10H, aromatic H).

Example 30.

Following the 7-acyl side chain cleavage reaction conditions described in Example 29 p - nitrobenzyl 7 - amino - 3 - fluoro - 3 - cephem - 4 - carboxylate was prepared with the intermediate of Example 26, p - nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylate.

Example 31.

Following the 7-acyl side chain cleavage reaction procedure described by Example 29 diphenylmethyl 7 - amino - 3 - bromo - 3 - cephem - 4 - carboxylate is prepared with diphenylmethyl 7 - phenoxyacetamido - 3 - bromo - 3 - cephem - 4 - carboxylate.

Example 32.

7 - (D - Mandelamido) - 3 - chloro - cephem - 4 - carboxylic acid.

To a suspension of 812 mg. (2 mmole) of p - nitrobenzyl 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylic acid hydrochloride in 40 ml. of ethyl acetate was added a solution of 520 mg. (5 mmole) of sodium bisulfite in 40 ml. of water. The mixture was vigorously stirred while 395 mg. (2.2 mmole) of D-mandelic acid O-carboxy anhydride were added. The mixture was stirred for 1.5 hours at room temperature and the aqueous layer was separated from the ethyl acetate layer and washed with ethyl acetate. The ethyl acetate wash was combined with the ethyl acetate layer and the combined wash and ethyl acetate layer were washed several times with water and then dried and evaporated to yield the reaction product as a dry residue. The residue was triturated with ether to yield 685 mg. of p - nitrobenzyl 7 - (D - mandelamido) - 3 - chloro - 3 - cephem - 4 - carboxylate melting at about 158-164°C. with decomposition.

Elemental analysis for $C_{22}H_{19}N_3O_5 \cdot SCl$:

Theory:

C, 52.44; H, 3.60; N, 8.34; Cl, 7.04%

Found:

C, 52.25; H, 3.45; N, 8.58; Cl, 6.82%.

NMR ($CDCl_3$): signals at 6.24 (ABq, 2H, C_2-H_2), 5.0-4.7 (m, 2H, C_6-H and $\alpha-H$), 4.57 (s, 2H, ester CH_2), 6.23 (q, 1H, C_7-H), and 2.8-1.2 (m, 10H, aromatic H and C_7-NH) tau.

UV acetonitrile: λ max 265 m μ ($\epsilon=18,600$).

The reaction product, 200 mg., was reacted with hydrogen in the presence of 5% Palladium on carbon to effect removal of the p-nitrobenzyl ester group and provide 75 mg. of 7 - (D - mandelamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

NMR (D_2O -sodium bicarbonate): signals at 6.42 (ABq, 2H, C_2-H_2), 4.90 (d, 1H, C_6-H), 4.68 (s, 1H, $\alpha-CH$), 4.37, (d, 1H, C_7-H) and 2.49 (s, 5H, aromatic H) tau.

Example 33.

a) p - Nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - methylsulfonyloxy - 3 - cephem - 4 - carboxylate.

To a solution of 4.75 g. (10 mmole) of p-nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - hydroxy - 3 - cephem - 4 - carboxylate in 50 ml. of dry dimethylacetamide were added 2 ml. of propylene oxide. To the solution was added with stirring one equivalent of methanesulfonyl chloride and stirring was continued for 3 hours. The re-

- chloro - cephem- 60
acid.
ng. (2 mmole) of
- 3 - chloro - 3-
d hydrochloride in
; added a solution
ium bicarbonate in 40
e was vigorously
2 mmole) of D-
anhydride were
rred for 1.5 hours
the aqueous layer
thyl acetate layer
te. The ethyl acet-
h the ethyl acetate
h and ethyl acetate
imes with water
rated to yield the
sidue. The residue
yield 685 mg. of
andelamido) - 3-
arboxylate melting
h decomposition.

$H_{11}N_3O_7SCl$:

.34; Cl, 7.04%

.58; Cl, 6.82%.

6.24 (ABq, 2H,
2H, C_6-H and
r CH_2), 6.23 (q,
.2 (m, 10H, aro-
au.
mu ($\epsilon=18,600$).

1 mg., was reacted
ice of 5% Pallad-
removal of the p-
provide 75 mg.
- 3 - chloro - 3-
id.

onate): signals at
4.90 (d, 1H,
-CH), 4.37, (d,
(s, 5H, aromatic

- (2 - thienyl)-
sulfonyloxy - 3-
te.

(10 mmole) of
- thienyl)acet-
- cephem - 4-
try dimethylacet-
propylene oxide.
with stirring one
of chloride and
5 hours.. The re-

action mixture was then taken up in ethyl
acetate and the solution was washed with a
saturated solution of sodium chloride. The
washed organic phase was evaporated *in vacuo*
to dryness to obtain the reaction product mix-
ture as a residue. The reaction product was
purified by preparative thin layer chromatog-
raphy on silica gel using for elution 65%
ethyl acetate/hexane.

The purified product gave the following
percent elemental composition on micro-
analysis.

Calculated for $C_{21}H_{11}N_3O_7S$,

Theory:

C, 45.56; H, 3.46; N, 7.59; S, 17.38.

Found:

C, 45.74; H, 3.56; N, 7.30; S, 17.06.

The nuclear magnetic resonance spectrum
and the infrared absorption spectrum were in
agreement with the structure of the product
formed.

b) p - Nitrobenzyl 7 - [2 - (2 - thienyl)acet-
amido] - 3 - fluoro - 3 - cephem - 4 -
carboxylate.

To 93 mg. of dicyclohexyl - 18 - crown-
6 - ether in 15 ml. of acetonitrile dried over
molecular sieves were added 25 mg. of potas-
sium fluoride which had been dried *in vacuo*
at 90°C. The mixture was stirred for ten
minutes, and 138 mg. of p - nitrobenzyl 7 -
[2 - (2 - thienyl)acetamido] - 3 - methyl-
sulfonyloxy - 3 - cephem - 4 - carboxylate
in 4 ml. of acetonitrile were then added. The
mixture was stirred for 1 hour. The mixture
was acidified by addition of dilute (5%) HCl,
and the resulting acidified mixture was ex-
tracted with ethyl acetate. Pure product was
obtained from the ethyl acetate extract by
preparative thin layer chromatography on
silica gel using ethyl acetate:benzene (1:1).
Ten mg. of p - nitrobenzyl 7 - [2 - (2-
thienyl)acetamido] - 3 - fluoro - 3 - cephem-
4 - carboxylate were obtained.

I.R.: absorption peaks at 1792, 1740, and
1685 cm^{-1} .

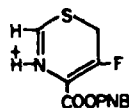
NMR ($CDCl_3$) showed signals at 6.15 (s, 2H,
 α CH_2), 4.97 (d, 1H, $J=4Hz$, C_6-H),
4.20 (q, 1H, C_7-H), 3.52 (d, 1H, C_7-H ,
NH), 2.32-1.7 (m, 2H, C_2-H_2) tau.

Fluorine NMR - - (d, $J=10Hz$).

M.S.: Calculated - - 477.0465

Found - - 477.0455

Fragment



Calculated - - 297.0345

Found - - 297.0344

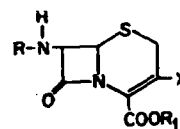
Example 34.

7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro-
3 - cephem - 4 - carboxylic acid.

To 110 ml. of methanol and 83 mg. of
pre-reduced 5 percent palladium on carbon
were added 83 mg. of p - nitrobenzyl 7 - [2-
(2 - thienyl)acetamido] - 3 - fluoro - 3-
cephem - 4 - carboxylate. The mixture was
hydrogenated at 53 psig. for one hour. The
resulting mixture was filtered, the catalyst was
washed with methanol, and the methanol wash-
ings were added to the filtrate. The filtrate was
then evaporated *in vacuo*. The residue was
dissolved in ethyl acetate, and the ethyl
acetate solution was extracted with dilute
aqueous sodium bicarbonate. The sodium bi-
carbonate solution was washed with ethyl
acetate, layered with ethyl acetate, and dilute
aqueous HCl was added. The layered ethyl
acetate was separated and evaporated to re-
cover 7 - [2 - (2 - thienyl)acetamido] - 3-
fluoro - 3 - cephem - 4 - carboxylic acid,
shown by bioautogram to be biologically active.

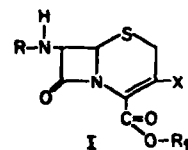
WHAT WE CLAIM IS:—

1. A compound of the formula:

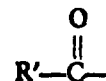


wherein R is hydrogen or an acyl group
derived from a carboxylic acid, R_1 is
hydrogen, a carboxylic acid protecting ester
group or a pharmaceutically-acceptable ester
group, and X represents fluoro, chloro or
bromo; or, when R_1 is hydrogen, a pharma-
ceutically-acceptable salt thereof.

2. A compound of the formula:



wherein R is a hydrogen or an acyl group
of the formula:



wherein R' is C_1-C_6 alkyl, C_1-C_6 haloalkyl,
 C_1-C_6 cyanoalkyl, phenyl, methylphenyl,
hydroxyphenyl, halophenyl, nitrophenyl,
aminophenyl, methoxyphenyl, 5 - amino - 5-
carboxybutyl, or a 5 - substituted - amino-
5 - carboxybutyl ester group of the formula:

cephem- 60
 (mole) of 5
 fluoro - 3- 65
 chloride in a solution
 of 40
 vigorously
 e) of D- 10
 ide were 70
 1.5 hours
 ous layer
 ate layer
 ethyl acet-
 yl acetate
 yl acetate 75
 with water
 yield the
 he residue
 85 mg. of
 ido) - 3- 80
 te melting
 position.

SCI:

7.04% 85
 6.82%.

ABq, 2H,
 —H and 90
 6.23 (q,
 10H, aro-
 =18,600).

as reacted 35
 % Palla- 95
 of the p-
 e 75 mg.
 fluoro - 3- 40

signals at 100
 (d, 1H,
 4.37, (d,
 aromatic 45

thienyl)- 105
 yloxy - 3- 40

mole) of 110
 yl) acet-
 hem - 4-
 methylacet-
 one oxide.
 rring one
 oride and 115
 The re- 45

action mixture was then taken up in ethyl acetate and the solution was washed with a saturated solution of sodium chloride. The washed organic phase was evaporated *in vacuo* to dryness to obtain the reaction product mixture as a residue. The reaction product was purified by preparative thin layer chromatography on silica gel using for elution 65% ethyl acetate/hexane.

The purified product gave the following percent elemental composition on microanalysis.

Calculated for $C_{21}H_{19}N_3O_5S$,

Theory:

C, 45.56; H, 3.46; N, 7.59; S, 17.38.

Found:

C, 45.74; H, 3.56; N, 7.30; S, 17.06.

The nuclear magnetic resonance spectrum and the infrared absorption spectrum were in agreement with the structure of the product formed.

b) p - Nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylate.

To 93 mg. of dicyclohexyl - 18 - crown-6 - ether in 15 ml. of acetonitrile dried over molecular sieves were added 25 mg. of potassium fluoride which had been dried *in vacuo* at 90°C. The mixture was stirred for ten minutes, and 138 mg. of p - nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - methylsulfonyloxy - 3 - cephem - 4 - carboxylate in 4 ml. of acetonitrile were then added. The mixture was stirred for 1 hour. The mixture was acidified by addition of dilute (5%) HCl, and the resulting acidified mixture was extracted with ethyl acetate. Pure product was obtained from the ethyl acetate extract by preparative thin layer chromatography on silica gel using ethyl acetate:benzene (1:1). Ten mg. of p - nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylate were obtained.

I.R.: absorption peaks at 1792, 1740, and 1685 cm^{-1} .

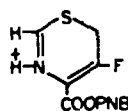
NMR (CDCl_3) showed signals at 6.15 (s, 2H, α CH_2), 4.97 (d, 1H, $J=4\text{Hz}$, $\text{C}_5\text{—H}$), 4.20 (q, 1H, $\text{C}_4\text{—H}$), 3.52 (d, 1H, $\text{C}_7\text{—NH}$), 2.32—1.7 (m, 2H, $\text{C}_2\text{—H}_2$) tau.

Fluorine NMR - - (d, $J=10\text{Hz}$).

M.S.: Calculated - - 477.0465

Found - - 477.0455

Fragment



Calculated - - 297.0345

Found - - 297.0344

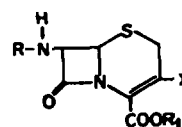
Example 34.

7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro-3 - cephem - 4 - carboxylic acid.

To 110 ml. of methanol and 83 mg. of pre-reduced 5 percent palladium on carbon were added 83 mg. of p - nitrobenzyl 7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylate. The mixture was hydrogenated at 53 psig. for one hour. The resulting mixture was filtered, the catalyst was washed with methanol, and the methanol washings were added to the filtrate. The filtrate was then evaporated *in vacuo*. The residue was dissolved in ethyl acetate, and the ethyl acetate solution was extracted with dilute aqueous sodium bicarbonate. The sodium bicarbonate solution was washed with ethyl acetate, layered with ethyl acetate, and dilute aqueous HCl was added. The layered ethyl acetate was separated and evaporated to recover 7 - [2 - (2 - thienyl)acetamido] - 3 - fluoro - 3 - cephem - 4 - carboxylic acid, shown by bioautogram to be biologically active.

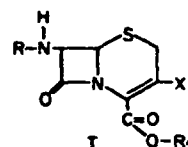
WHAT WE CLAIM IS:—

1. A compound of the formula:

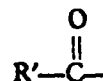


wherein R is hydrogen or an acyl group derived from a carboxylic acid, R_1 is hydrogen, a carboxylic acid protecting ester group or a pharmaceutically-acceptable ester group, and X represents fluoro, chloro or bromo; or, when R_1 is hydrogen, a pharmaceutically-acceptable salt thereof.

2. A compound of the formula:

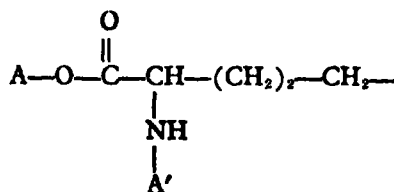


wherein R is a hydrogen or an acyl group of the formula:

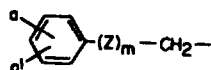


wherein R' is $\text{C}_1\text{—C}_6$ alkyl, $\text{C}_1\text{—C}_3$ haloalkyl, $\text{C}_1\text{—C}_3$ cyanoalkyl, phenyl, methylphenyl, hydroxyphenyl, halophenyl, nitrophenyl, aminophenyl, methoxyphenyl, 5 - amino - 5 - carboxybutyl, or a 5 - substituted - amino - 5 - carboxybutyl ester group of the formula:





wherein A is diphenylmethyl, p - nitrobenzyl, p-methoxybenzyl, benzyl, t-butyl or 2,2,2 - trichloroethyl, and A' is C₂-C₄ alkanoyl, C₂-C₄ haloalkanoyl, benzoyl, halobenzoyl, 2,4-dinitrophenyl, or phthaloyl; or R' is a group of the formula:



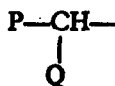
wherein

10 a and a' independently are hydrogen, C₁-C₄ alkyl, C₁-C₄ alkoxy, halogen, hydroxy, nitro, amino or carboxy;

Z is O or S; and

m is 0 or 1; or

15 R' is a group of the formula:



wherein P is 2 - thienyl, 3 - thienyl, phenyl or a substituted phenyl group of the formula:

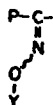


20 wherein

a and a' are as defined above,

Q is hydroxy, formyloxy, acetoxy, carboxy, or sulfo; or

R' is a group of the formula:



wherein

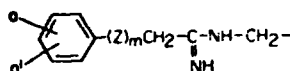
P has the same meanings as defined above and Y is hydrogen, methyl or acetyl; or

R' is a group of the formula:

30



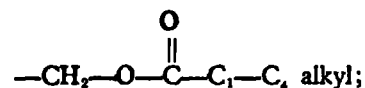
wherein R'' is 2-thienyl, 3-thienyl, 2-furyl, 2-oxazolyl, 2-thiazolyl, or 1-tetrazoyl; or R' is a group of the formula:



wherein

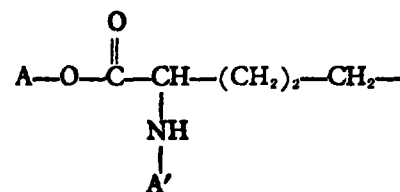
a, a', Z and m have the same meanings as defined above;

R₁ is hydrogen, benzyl, p-methoxybenzyl, p - nitrobenzyl, diphenylmethyl, 2,2,2 - trichloroethyl, t - butyl, or a pharmaceutically acceptable ester of the formula:



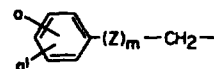
and X is fluoro, chloro, or bromo; or, when R₁ is hydrogen, a pharmaceutically-acceptable salt thereof.

3. A compound as claimed in Claim 2, wherein R' is a 5 - amino - 5 - carboxybutyl group or a 5-substituted - amino - 5 - carboxybutyl ester group of the formula:



4. A compound as claimed in any one of Claims 1 to 3, wherein X is chloro.

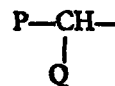
5. A compound as claimed in Claim 2 or Claim 4, wherein R' is a group of the formula:



Z, m, a and a' being as defined in Claim 2.

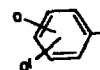
6. A compound as claimed in Claim 5, wherein m is 0 or m is 1 and Z is O.

7. A compound as claimed in Claim 2 or Claim 4, wherein R' is the group of the formula:



P and Q being as defined in Claim 2.

8. A compound as claimed in Claim 7, wherein P is phenyl or a substituted phenyl group of the formula:



a and a' being as defined in claim 2.

9. A compound as claimed in Claim 7 or Claim 8, wherein Q is hydroxy, formyloxy or acetoxy.

10. 7 - (D - Mandelamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

11. A compound as claimed in Claim 2 or Claim 4, wherein R' is a group of the formula:



(39)

same meanings as

p-methoxybenzyl,
nethyl, 2,2,2-tri-
a pharmaceutically
mula:

—C₄ alkyl;

r bromo; or, when
eutically-acceptable

imed in Claim 2,
- 5 - carboxybutyl
amino - 5 - carb-
e formula:

(Z)₂—CH₂—

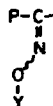
ed in any one of
is chloro.
ed in Claim 2 or
up of the formula:

2—

efined in Claim 2.
med in Claim 5,
and Z is 0.
ed in Claim 2 or
group of the for-

n Claim 2.
ned in Claim 7,
substituted phenyl

claim 2.
ed in Claim 7 or
oxy, formyloxy or
do) - 3 - chloro-
ic acid.
ed in Claim 2 or
up of the formula:



P and Y being as defined in Claim 2.

12. A compound as claimed in Claim 11,
wherein Y is hydrogen or acetyl.

13. A compound as claimed in Claim 2
or Claim 4, wherein R' is a group of the
formula:



R'' being as defined in Claim 2.

14. 7 - [2 - (2 - Thienyl)acetamido] - 3 -
chloro - 3 - cephem - 4 - carboxylic acid.

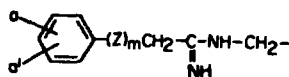
15. A compound as claimed in any one of
Claims 1 to 3, 5 to 9 or 11 to 13, wherein X
is bromo.

16. 7 - [2 - (2 - Thienyl)acetamido] - 3 -
bromo - 3 - cephem - 4 - carboxylic acid.

17. A compound as claimed in any one of
Claims 1 to 3, 5 to 9 or 11 to 13, wherein
X is fluoro.

18. 7 - [2 - (2 - Thienyl)acetamido] - 3 -
fluoro - 3 - cephem - 4 - carboxylic acid.

19. A compound as claimed in any one of
Claims 2, 4, 15 or 17, wherein R' is a group
of the formula:



Z, m, a and a' being as defined in Claim 2.

20. A compound as claimed in Claim 19,
wherein m is 0.

21. A compound as claimed in Claim 19,
wherein m is 1 and Z is 0.

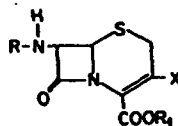
22. A compound as claimed in any one of
Claims 1 to 9, 11 to 13, 15, 17 and 19 to 21,
wherein R is hydrogen.

23. 7 - Amino - 3 - chloro - 3 - cephem -
4 - carboxylic acid.

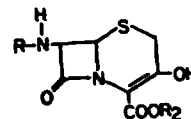
24. p - Nitrobenzyl 7 - amino - 3 - chloro -
3 - cephem - 4 - carboxylate.

25. Diphenylmethyl 7 - amino - 3 - chloro -
3 - cephem - 4 - carboxylate.

26. A process for preparing compounds of
the formula:



wherein R and R₁ are as defined in any one
of Claims 1 to 3, 5 to 9, 11 to 13, or 19 to
22, and X is chloro or bromo, which com-
prises reacting a compound of the formula:



wherein R is as defined above and R₂ is a
carboxylic acid protecting ester forming group,
with a halogenating agent in a solvent cap-
able of forming an iminium halide; optionally
acylating the 3 - halo - 3 - cephem ester
wherein R is hydrogen to provide the corres-
ponding 7 - acylamido compound wherein R
is other than hydrogen; and optionally re-
moving the carboxylic acid protecting ester
forming group to give the corresponding acid.

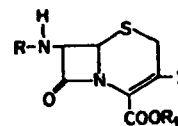
27. A process according to Claim 26, where-
in the solvent is dimethylformamide.

28. A process according to Claim 26 or
Claim 27, wherein the halogenating agent is
phosphorus trichloride.

29. A process according to Claim 26 or
Claim 27, wherein the halogenating agent is
thionyl chloride.

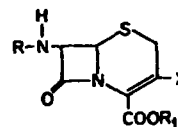
30. A process according to Claim 26 or
Claim 27, where the halogenating agent is
phosphorus tribromide.

31. A process for preparing a compound
of the formula:



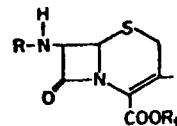
wherein R, R₁ and X are as defined in Claim
1, substantially as hereinbefore described with
particular reference to any one of Examples
16 to 32.

32. A compound of formula:



wherein R, R₁ and X are as defined in Claim
1, substantially as hereinbefore described with
particular reference to any one of Examples
16 to 34.

33. A compound of the formula:



whenever prepared by a process as claimed
in any one of Claims 26 to 31.



(17)

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