

PATENT SPECIFICATION

(11) 1 387 656

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- (21) Application No. 33395/72 (22) Filed 17 July 1972
 (61) Patent of Addition to No. 1,300,692 dated 19 April 1971
 (31) Convention Application No. 53466/71 (32) Filed 17 July 1971
 (31) Convention Application No. 84130/71 (32) Filed 22 Oct. 1971 in
 (33) Japan (JA)
 (44) Complete Specification published 19 March 1975
 (51) INT CL² C07D 501/44; A61K 31/545; C07D 501/18
 (52) Index at acceptance
 C2C 1314 1384 1410 1510 1530 1534 1535 1620 213 214
 215 220 226 227 22Y 246 247 250 251 252 254 256
 25Y 28X 30Y 313 314 316 31Y 321 327 32Y 332
 337 339 342 34Y 351 352 354 360 361 366
 367 36Y 394 39Y 440 57Y 602 603 604 60Y 620
 62X 63X 652 660 661 680 738 QG RC RD RF RV

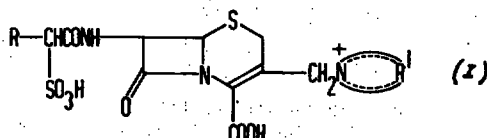


(54) CEPHALOSPORIN COMPOUNDS

(71) We, TAKEDA YAKUHI KOGYO KABUSHIKI KAISHA also known as TAKEDA CHEMICAL INDUSTRIES LTD., a Corporation organised under the laws of Japan, of 27 Doshomachi, 2 chome, Higashi-ku, Osaka, Japan, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to novel cephalosporin compounds, to their preparation and to pharmaceutical compositions containing them; the invention is an improvement in, or modification of, the invention of Patent Specification No. 1,300,692.

The compounds have the general formula (I)



wherein R is a hydrogen atom or an alkyl or cycloalkyl group or a substituted or unsubstituted aryl or thienyl group (which can be monocyclic or polycyclic) and R' forms together with the nitrogen atom a substituted or unsubstituted mono- or polycyclic heterocyclic ring comprising at least one double bond (indicated by a dotted line) and 1 or 2 nitrogen atoms, or a pharmaceutically acceptable salt thereof.

Ever since the discovery of cephalosporin C, there have been developed various derivatives of cephalosporin type antibiotics which have been substituted at the 7- and 3-positions. However, compounds which are effective at practical concentrations against *Pseudomonas aeruginosa* have not been reported yet.

It has been found that the above cephalosporins of general formula (I) are not only effective for the therapy of various infectious diseases derived from Gram-positive and Gram-negative pathogenic bacteria but also show marked activities at low concentrations against *Pseudomonas aeruginosa*.

In the cephalosporins of the invention, when R is other than hydrogen, the carbon atom to which the sulphuric acid group is attached is asymmetric. It should therefore be understood that the present invention includes the D-form, L-form and DL-form of the stereoisomers of such cephalosporin compounds.

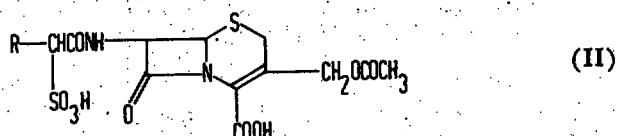
The cephalosporin compounds of the present invention can be prepared by methods known *per se*, for example by subjecting cephalosporin C or a derivative thereof to a treatment which replaces the 5-amino-5-carboxyvaleryl group at the 7-position with the desired α -sulphoacyl group and the acetoxy group at the 3-position with the nitrogen-containing heterocyclic group. Replacement at either the 3- or 7-position may be conducted first, followed by the replacement at the other position.

A) Procedures for conducting the replacement first at the 7-position and then at the 3-position:

7-Aminocephalosporanic acid (hereinafter referred to as "7-ACA") is reacted with an α -sulphocarboxylic acid of general formula,



or with an N-acylating functional derivative thereof, to give a sulphocephalosporanic acid of the formula



(Belgian Patent Specification No. 762,725). In the compound of general formula (II) R is preferably a hydrogen atom; an alkyl group (e.g. a methyl, ethyl, isopropyl, butyl, cyclohexyl or dodecyl group); or an aryl group (e.g. a phenyl, nitrophenyl, aminophenyl, tolyl, naphthyl) or a thienyl group. Further, provided that no detrimental effect is brought about in the subsequent 3-position replacement reaction, the carboxyl group at the 4-position and/or the sulpho group in the 7-position side chain may be in the form of a salt with, for example, sodium, potassium, magnesium, calcium, aluminium or triethylamine. Alternatively, the carboxyl group at the 4-position may be in the form of an ester with a group which is easily replaced by hydrogen, for example a benzyl, β -methylsulphonyl ethyl, benzhydryl or trimethylsilyl group.

Subsequently, the sulphocephalosporanic acid of general formula (II) is reacted with a nitrogen-containing heterocyclic compound of general formula (III)

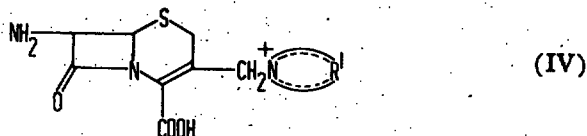
(III)

which is a 5- or 6-membered ring compound containing 1 or 2 nitrogen atoms and which contains at least 1 double bond in the ring. Desirably, the ring is aromatic in character. The compound of general formula (III) may be a condensed ring having 2 adjacent carbon atoms in the rings. These rings may have as a substituent an alkyl group such as a methyl or ethyl group; an amino, carboxyl, carbamoyl, hydrazinocarbonyl, sulpho, hydroxymethyl or aldehyde group; or a halogen such as bromine or chlorine. Examples of the compound of general formula (III) include pyridine derivatives (e.g. picoline, nicotinic acid, nicotinic acid amide, isonicotinic acid amide, isonicotinic acid hydrazide, m-bromopyridine, pyridinesulphonic acid, 3-hydroxymethylpyridine and pyridinealdehyde); and also quinoline; isoquinoline; pyrazine; pyrazinic acid amide (2-carbamoylpyrazine); pyridazine; pyrimidine; imidazole and 1-methylimidazole.

The reaction of the sulphocephalosporanic acid of general formula (II) with the compound of general formula (III) is effected according to a conventional procedure. Generally, it is advantageous to carry out the reaction in water or another strongly polar solvent. The reaction is conducted at about neutral pH, preferably a pH of 5 to 8. Ordinarily, the nitrogen-containing compound is used in a proportion of 1 to 10 moles per mole of the sulphocephalosporanic acid. Further, if desired, the reaction may be conducted as is known in the presence of a compound such as an isocyanate, thiocyanate or thiol compound in a proportion of 1 to 40 moles, preferably 5 to 20 moles, per mole of the sulphocephalosporanic acid, whereby the end product can be obtained in a higher yield. The above-mentioned compound reacts at the 3-position of the sulphocephalosporanic acid to give an intermediate compound. This intermediate, which need not be isolated, then reacts with the compound of general formula (III). The reaction temperature and time vary depending on the reactants and on the solvent used. In most cases, however, the reaction is effected at room temperature to 100° C., preferably 40° to 70° C. for 1 to 48 hours. The thus obtained reaction product of general formula (I) can be purified and recovered according to conventional means, e.g. solvent extraction, concentration, chromatography, freeze-drying and recrystallisation.

B) Procedures for conducting the replacement first at the 3-position and then at the 7-position:

The replacement of the acetoxy group at the 3-position of cephalosporin C or its derivatives can be effected according to the process disclosed in, for example, United States Patent Specification No. 3,225,038 or 3,217,000 or German Patent Specification No. 1,817,121. The resulting cephalosporin having the nitrogen-containing heterocyclic group at the 3-position can be easily converted, according to the process disclosed in, for example Dutch Patent Specification 6,401,421, British Patent Specification 1,041,985 or United States Patent Specification 3,575,970, into a corresponding aminocephalosporanic acid derivative of general formula (IV)



Alternatively, the aminocephalosporanic acid derivative of general formula (IV) may be prepared by N-deacylating cephalosporin C and then effecting replacement at the 3-position of the resulting 7-ACA. It is needless to say that the aminocephalosporanic acid derivative of general formula (IV) may be in the form of a salt or readily decomposable ester, as in the case of the aforesaid sulphocephalosporanic acid.

The reaction of the aminocephalosporanic acid derivative of general formula (IV) with the α -sulphocarboxylic acid of general formula (V) or an N-acylating functional derivative thereof may also be accomplished according to a conventional procedure. When the α -sulphocarboxylic acid is used in the free form, it is preferable to carry out the reaction in the presence of a condensing agent. The condensing agent is, for example, an N,N'-disubstituted carbodiimide, e.g. N,N'-dicyclohexylcarbodiimide; an azolide compound, e.g. N,N'-trionylimidazole; or N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, phosphorus oxychloride or alkoxyacetylene. As the N-acylating derivative of the α -sulphocarboxylic acid, there may be used carboxylic acid halides, anhydrides, azides or active esters. The above acylation reaction proceeds advantageously and smoothly in a reaction-inert solvent. As the solvent, a conventional solvent such as water, acetone, tetrahydrofuran, dioxan, acetonitrile, chloroform, dichloromethane, dichloroethylene, pyridine, dimethylaniline, dimethylformamide, dimethylacetamide or dimethyl sulphoxide is conveniently used. The reaction temperature is not particularly critical. Ordinarily, however, the reaction is effected under cooling or at room temperature. The reaction product can be purified and recovered, utilizing the properties of the end product cephalosporin of general formula (I) by, for example column chromatography, solvent extraction, isoelectric point precipitation, countercurrent distribution or recrystallization.

The cephalosporins of general formula (I) can be put to the same uses as the conventional cephalosporins, although their particular applications vary more or less depending on the kind of nitrogen containing heterocyclic group at the 3-position and/or the acyl group at the 7-position. They are useful as pharmaceuticals since they have strong antibacterial activities against a wide scope of pathogenic bacteria including *Pseudomonas aeruginosa*, against which the conventional cephalosporins have been substantially ineffective.

The cephalosporins of the present invention can be administered in for example an injectable form in a similar manner to known cephalosporins, but their dosages and dosage forms may vary according to their substituent groups at the 3-position and acyl groups at the 7-position.

The invention therefore also provides a pharmaceutical composition comprising a compound of general formula (I) and a pharmaceutically acceptable carrier or diluent.

The invention is illustrated by the following Examples wherein all the temperatures are uncorrected.

0.292 Gram of N-(7-aminoceph-3-em-3-ylmethyl)pyridinium-4-carboxylate and 0.17 g. of sodium hydrogencarbonate were dissolved in 7 ml. of water. Into the resulting solution was added dropwise under cooling 3 ml. of a chloroform solution containing 0.234 g. of α -sulphophenylacetyl chloride. After completion of the addition, the stirring was further continued under cooling for 40 minutes to complete the reaction. After removing the organic layer, the aqueous layer was adjusted to pH 6 and subjected to chromatography using Amberlite XAD-2, and the fractions containing the desired product were freeze-dried to obtain sodium N-[7-(α -sulphophenylacetamido)ceph-3-em-3-ylmethyl]pyridinium-4-carboxylate.

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Example 1.

Preparation of sodium N-[7-(α -sulphophenylacetamido)ceph-3-em-3-ylmethyl]-4'-carbamoyl-pyridinium-4-carboxylate:

0.514 Gram (1×10^{-3} mole) of 7-(α -sulphophenylacetamido)cephalosporanic acid, 0.366 g. (3×10^{-3} mole) of isonicotinamide and 2.0 g. (2.06×10^{-2} mole) of potassium thiocyanate were dissolved in 2.5 ml. of water. The resulting solution was allowed to stand and heated for 20 hours in a thermostat kept at 50° C., and then directly purified by chromatography on an Amberlite XAD-2 column ("Amberlite" is a Registered Trade Mark) (16×880 mm.). Subsequently, the fractions containing the cephalosporins were collected and subjected to freeze-drying to obtain 270 g. of the title product in the form of a pale yellowish white powder. IR. ν KBr max (cm^{-1}): 3330 (CH, NH), 3200 (OH, broad), 1765 (C=O, β -lactam), 1680 (—CONH—), 1610 (—COO—), 1552 (—NH₂, deformation), 1455, 1390 (C=C, SO₂), 1200 (SO₂, broad), 1120, 1040 (—SO₃—), 850, 810, 700.

NMR (60Mc, D₂O): 2.93 (1H, doublet, $J=18$ c/s, , 3.52 (1H, doublet, $J=18$ c/s, , 5.08 (1H, singlet, , 5.35 (1H, , 5.50 (1H, doublet, $J=5$ c/s, , 5.09 (1H, doublet, $J=6$ c/s, 6-position proton),

5.71 (1H, doublet, $J=4.5$ c/s, 7-position proton), 7.25—7.6 (5H, multiplet, phenyl), 8.25 (2H, doublet, $J=6.5$ c/s, pyridine proton), 9.03 (2H, doublet, $J=6.5$ c/s, pyridine proton).

Minimum inhibitory concentration:

<i>Pseudomonas aeruginosa</i>	(Pd 1)	1	($\mu\text{g./ml.}$)
"	(NCTC)	0.5	"

Example 2.

Example 1 was repeated, except that each of quinoline, nicotinic acid, isonicotinic acid hydrazide, nicotinamide, 2-methylimidazole and isoquinoline was used in place of the isonicotinamide. The minimum inhibitory concentrations of the resulting compounds were as set forth in the following Table:

Substituent at the 3-position	MIC ($\mu\text{g./ml.}$) against <i>Pseudomonas aeruginosa</i> (Pd 1)
	5
	5
	10
	1
	0.5
	10
	5

Example 3.

Preparation of sodium N-[7-(α -sulphophenylacetamido)ceph-3-em-3-ylmethyl]-2'-methylpyridinium-4-carboxylate:

0.355 Gram (0.9×10^{-4} mole) of disodium 7-(α -sulphophenylacetamido)cephalosporanic acid, 0.175 g. (1.88×10^{-3} mole) of α -picoline and 0.15 g. (1.55×10^{-3} mole) of potassium thiocyanate were dissolved in 0.8 ml. of water. The resulting solution was heated at 50°C. for 20 hours and washed twice with 0.5 ml. of methylene chloride. The aqueous layer was loaded on an Amberlite XAD-2 column ($16 \times 900 \text{ mm.}$) and then chromatographically purified using water as a developer. The fractions containing the cephalosporin were collected and subjected to freeze-drying to obtain 220 mg of the title product in the form of a pale yellowish powder. IR $\nu_{\text{max}}^{\text{KBr}}$ (cm.^{-1}): 3390 (OH), 3025 (CH), 1759 (β -lactam), 1670 ($-\text{CONH}-$), 1610 (COO), 1525 (C=C), 1490, 1450 (C=C), 1390-1340 (SO_2), 1220 (SO_2), 1038 (SO_2).

Minimum inhibitory concentration:

Pseudomonas aeruginosa (Pd 1) 3 ($\mu\text{g./ml.}$)
 " (NCTC 10490) 2 "

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Example 4.

Preparation of sodium N-[7-(α -sulphopropionamido)ceph-3-em-3-ylmethyl]-pyridinium-4-carboxylate:

436 Mg. (1×10^{-3} mole) of disodium 7-(α -sulphopropionamido)cephalosporanate, 395 mg. (5×10^{-3} mole) of pyridine and 485 mg. (5×10^{-3} mole) of KSCN were dissolved in 1.0 ml. of water, and the resulting solution was allowed to stand at 50° C. for 5 hours. Subsequently, the reaction solution was purified by column chromatography on Amberlite XAD-2 to obtain 70 mg. of the title product, IR ν $\overset{\text{KBr}}{\text{max}}$ (cm^{-1}): 1760 (β -lactam), 1670 ($-\text{CONH}-$), 1610 ($-\text{COONa}$), 1040 ($-\text{SO}_3\text{Na}$).

NMR δ (D_2O): 1.58 (3H, doublet, $J=6.5$ c/s, CH_3-), 3.54 (2H, $\text{S}-\text{CH}_2$), 3.95 (1H, quartet, $J=6.5$ c/s, $-\text{CH}-$), 5.20 (1H, $-\text{CH}-\text{S}$), 5.55 (2H, $-\text{CH}_2-\text{N}$), 5.73 (1H, $\text{O}-\text{CH}_2$), 8.15 (2H, quartet, $J=1.5$, 6.5 c/s, $-\text{N}^+-\text{C}_5\text{H}_4$), 8.54 (1H, $-\text{N}^+-\text{C}_5\text{H}_4$), 8.94 (2H, quartet, $J=1.5$, 6.5 c/s, $-\text{N}^+-\text{C}_5\text{H}_4$).

Example 5.

Preparation of sodium N-[7-(α -sulphocaproamido)ceph-3-em-3-ylmethyl]-pyridinium-4-carboxylate:

450 mg. (1×10^{-3} mole) of disodium N-7-(α -sulphocaproamido)cephalosporanate, 395 mg. (5×10^{-3} mole) of pyridine and 485 mg. (5×10^{-3} mole) of KSCN were dissolved in 1.0 ml. of water, and the resulting solution was allowed to stand at 60° C. for 5 hours. Subsequently, the reaction liquid was purified by column chromatography on Amberlite XAD-2 to obtain 80 mg. of the title product. IR ν $\overset{\text{KBr}}{\text{max}}$ (cm^{-1}): 1763 (β -lactam), 1673 ($-\text{CONH}-$), 1613 (COONa), 1038 ($-\text{SO}_3\text{Na}$).

NMR δ (D_2O): 0.93 (3H, CH_3), 1.37 (4H, $-(\text{CH}_2)_2-$), 1.97 (2H, $-\text{CH}_2-\text{CH}-$), 3.54 (2H, $\text{S}-\text{CH}_2$), 3.83 (1H, $-\text{CH}-\text{SO}_3\text{Na}$), 5.21 (1H, $-\text{CH}-\text{S}$), 5.50 (2H, $-\text{CH}_2-\text{N}$), 5.75 (1H, $\text{O}-\text{CH}_2$), 8.17 (2H, quartet, $J=1.5$, 6.5 c/s, $-\text{N}^+-\text{C}_5\text{H}_4$), 8.56 (1H, $-\text{N}^+-\text{C}_5\text{H}_4$), 8.97 (2H, quartet, $J=1.5$, 6.5 c/s, $-\text{N}^+-\text{C}_5\text{H}_4$).

Minimum inhibitory concentration:

Staphylococcus aureus
Pseudomonas aeruginosa

(No. 87, penicillin-G resistant) 2 (mcg./ml.)
(10490) 2 "

Example 6.

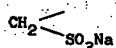
Preparation of sodium 7-sulphoacetamido-3-(pyridinium)methylceph-3-em-4-carboxylate:

638 Mg. of disodium 7-sulphoacetamidoccephalosporanic acid, 1.75 g. of KSCN and 240 mg. of pyridine were dissolved in 2 ml. of water, and the resulting solution was heated at 60° C. for 7 hours. Subsequently, the reaction liquid was purified by column chromatography on Amberlite XAD-2 to obtain 400 mg. of the title product, yield

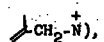
57% IR ν $\frac{\text{KBr}}{\text{max}}$ (cm.⁻¹): 1766 (β -lactam), 1670 ($-\text{CONH}-$), 1620 ($-\text{CO}_2^-$), 1550, 1485, 1395, 1370, 1330, 1210, 1047, ($-\text{SO}_3^-$).

NMR δ (D₂O): 3.30 (1H, doublet, J=18 c/s,

, 3.70 (1H, doublet, J=18 c/s, , 3.92 (2H,

, 5.20 (1H, doublet, J=5 c/s, C₆-H), 5.80 (1H,

doublet, J=5 c/s, C₇-H), 5.4, 5.7 (2H, AB_q, J=15 c/s,

, 7.9-9.2 (5H, multiplet, pyridine).

Example 7.

(1) Preparation of α -sulpho- α -(*p*-nitrophenyl)acetic acid chloride:
6.58 Grams (5.7×10^{-2} M) of thionyl chloride, 2 ml. of ether and 2 drops of dimethylformamide were added to 1.5 g. (5.7×10^{-3} M) of α -sulpho- α -(*p*-nitrophenyl)acetic acid to allow to react for 14.5 hours at room temperature. The reaction product was subjected to the evaporation to dryness under reduced pressure to obtain the crude title product. Yield 81.3%.

NMR (60Mc, CDCl₃): 5.93 (CH, singlet), 8.06 (phenyl, quartet, J=8 c/s, J=14 c/s).

(2) Preparation of disodium 7-[α -sulpho- α -(*p*-nitrophenyl)acetamido]cephalosporanate:

4.45 mg. of 1N—NaOH and 24 ml. of water were added to 1.2 g. (4.45×10^{-3} M) of 7-aminocephalosporanic acid to prepare a solution. 0.87 Gram (1.04×10^{-2} M) of NaHCO₃ was added to the solution under cooling with ice and then a solution of 1.4 g. ($4.45 \times 10^{-3} \times 1.13$ times M) of the crude α -sulpho- α -(*p*-nitrophenyl)acetic acid chloride obtained in (1) above in 8 ml. of acetone was slowly added dropwise over 10 minutes drop. Stirring was continued at 0°—2° C. for 20 minutes and at 5° C. for 1.5 hours, after the addition was completed. The solution was concentrated to 8 ml. under reduced pressure at room temperature. The solution was passed through a column (6×100 cm.) packed with 1.8 l. of Amberlite XAD—2 resin and elution was performed with 8 ml. of water. Fractions No. 1—15 obtained were freeze-dried to obtain 1.2 g. of the title product.

IR ν $\frac{\text{KBr}}{\text{max}}$ (cm.⁻¹): 3450 ($-\text{OH}$), 1760 (lactam), 1685 (acid amide), 1620 (carboxylate), 1520, 1350 ($-\text{NO}_2$), 1230, 1045 ($-\text{SO}_3\text{Na}$).

(3) Preparation of sodium 7-[α -sulpho- α -(*p*-nitrophenyl)acetamido]-3-(pyridinium-methyl)ceph-3-em-4-carboxylate:

0.306 ml. (3.87×10^{-3} M) of pyridine, 2.5 g. (2.58×10^{-2} M) of KSCN and 1.2 ml. of water were added to 0.8 g. (1.43×10^{-3} M) of disodium 7-[α -sulpho- α -(*p*-nitrophenyl)acetamido]cephalosporanate. The solution, after its pH had been brought to 6.5, was left to stand for six hours in a thermostat kept at 60° C., and then overnight under cooling.

(4) Preparation of sodium 7-[α -sulpho- α -(*p*-aminophenyl)acetamido]-3-(pyridinium-methyl)ceph-3-em-4-carboxylate:

The solution obtained in (3) above was dissolved in 100 ml. of water and then hydrogenated for 50 minutes at 25° C. under initial hydrogen pressure of 100 kg./cm.² in the presence of 1 g. of Raney nickel. Hydrogen absorption: 333%. The reaction solution was filtered and then the filtrate was concentrated to 10 ml. under reduced pressure. The condensate was passed through a column (4.0×70 cm.) packed with 0.5 l. of Amberlite XAD—2 resin, and eluted with 1.6 l. of water. Fractions No. 1—9 were freeze-dried to obtain the title compound above.

IR ν $\frac{\text{KBr}}{\text{max}}$ (cm.⁻¹): 3400 ($-\text{OH}$), 1760 (lactam), 1675 (acid amide), 1620 ($-\text{COONa}$), 1220, 1040 ($-\text{SO}_3\text{Na}$).

Minimum inhibitory concentration:

Staphylococcus aureus 209 P 5 mcg./ml.
Pseudomonas aeruginosa 5 "

Example 8.

Preparation of sodium 7-(α -sulpho- α -phenyl)acetamido-3-(p -acetaminopyridinium-methyl)ceph-3-em-4-carboxylate:

200 Mg. (3.87×10^{-4} M) of disodium 7-(α -sulpho- α -phenylacetamido)cephalosporanate, 120 mg. (8.82×10^{-4} M) of 4-acetaminopyridine and 570 mg. (59×10^{-3} M) of KSCN were mixed with 4 ml. of water, although some 4-acetaminopyridine was left undissolved. This solution containing some undissolved compound was allowed to react at 50° C. for 18 hours in a glass vessel (8 ml.). After completion of the reaction, the 4-acetaminopyridine had completely dissolved to obtain a transparent solution. The solution was purified by means of chromatography and freeze-dried to obtain 143 mg. of the title product. Yield: 60%.

IR ν $\frac{\text{KBr}}{\text{max}}$ (cm^{-1}): 3500, 1760, 1720, 1670, 1510, 1455, 1360, 1315, 1225, 1200, 1040, 845, 700.

NMR (60Mc, D₂O): 2.308 (—NHCOCH₃).

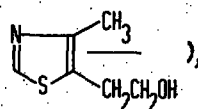
Example 9.

Preparation of sodium 7-(α -sulpho- α -phenyl)acetamido-3-(4'-methyl-5'-hydroxyethyl-thiazolinium)ceph-3-em-4-carboxylate:

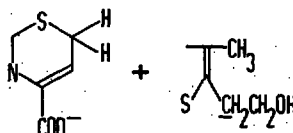
500 Milligrams (9.69×10^{-4} M) of disodium 7-(α -sulfo- α -phenylacetamido)-cephalosporanate, 1.68 g. (1.73×10^{-3} M) of KSCN and 415 mg. (2.9×10^{-3} M) of 4- β -hydroxyethyl-5-methylthiazole were dissolved in 2 ml. of water and allowed to react for 18 hours in a thermostat kept at 50° C. The solution thus obtained was chromatographed using Amberlite XAD-2 resin and freeze-dried to obtain 264 mg. of the title product. Yield: 42.8%.

IR ν $\frac{\text{KBr}}{\text{max}}$ (cm^{-1}): 3400, 3050, 1770, 1680, 1615, 1530, 1350, 1210, 1110, 1040, 700.

NMR (60Mc, D₂O): 2.43 (3H, singlet,



3.11—3.31 (4H,



3.84 (2H, triplet, J = 4.5 c/s), 5.09

(1H, singlet, —CH—SO₃Na), 5.2 (3H, CH₃N⁺, —N—S—),

5.72 (1H, doublet, J=5.0 c/s, —CH—S—), 7.44 (5H, quartet),

9.12 (1H, —N—S—).

Example 10.

514 Mg. of disodium N-7-(α -sulphophenylacetamido)cephalosporanate and 1.2 g. of KSCN were dissolved in 1.5 ml. of water and to the solution was added 1.58 g. of 3-bromo-pyridine. A small amount of dimethylformamide was added to the mixture and then the resulting solution was left standing at 60° C. for 10 hours. Subsequently, the reaction solution was purified by column chromatography on Amberlite XAD-2. The desired product-containing fractions were freeze-dried to obtain sodium N-[7-(α -sulphophenylacetamido)ceph-3-em-3-(3'-bromopyridinium)methyl]-4-carboxylate.

IR ν $\frac{\text{KBr}}{\text{max}}$ (cm^{-1}): 1778 shoulder, 1765 (β -lactam), 1725 shoulder, 1670 (—CONH—), 1615 (—CO₂[⊖]), 1210, 1038 (—SO₃[⊖]).

NMR δ (D_2O): 3.5 (2H, broad, C-2), 5.17 (1H, C₆-H), 5.26 (1H, s, -CH<), 5.4 (2H, -CH₂-), 5.72 (1H, C₇-H), 7.6 (5H, phenyl), 8.0 (1H, -N<), 8.7-9.1 (2H, -N<), 9.33 (1H, -N<).

Example 11.

(1) With cooling and stirring, 1.84 g. (0.0068 mole) of 7-ACA was suspended in 25 ml. of water and to the suspension was added dropwise 6.8 ml. of a 1N-sodium hydroxide to dissolve the 7-ACA. Meanwhile to a suspension of 1.5 g. (0.0068 mole) of α -sulpho-3-thienylacetic acid in 10 ml. of ether were added 5.5 ml. of thionyl chloride dropwise and 3 drops of dimethylformamide, and then the resulting solution was stirred for 5 hours at room temperature. The reaction solution was evaporated to dryness under reduced pressure to give α -sulpho-3-thienylacetyl chloride as a residue. The residue was dissolved in 10 ml. of ethyl acetate and the solution was added dropwise to the 7-ACA solution mentioned above, followed by stirring for 30 minutes. The aqueous layer was separated from the reaction solution and its pH adjusted to 6.5 by adding saturated aqueous NaHCO₃. The resulting solution was freeze-dried to obtain 3.6 g. of a crude product. The product was purified by column chromatography on Amberlite XAD-2 to obtain 1.6 g. (42%) of 7-(α -sulpho-3'-thienylacetamido)-cephalosporanic acid.

IR ν KBr max (cm⁻¹): 1750 (β -lactam, -OCOCH₃), 1677 (-CONH-), 1605 (-COONa), 1225 (-SO₃Na, -OAC), 1043 (SO₃Na).

NMR δ (D_2O): 2.13 (3H, singlet, -CO₂CH₃), 3.54 (2H, broad, S<H), 5.10 (1H, doublet, J=4.7 c/s, -H<S), 5.24 (1H, singlet, -CH<), 5.70 (1H, doublet, J=4.7 c/s, -H<S), 7.38-7.67 (3H, multiplet,).

(2) 260 Mg. (0.5×10^{-3} mole) of 7-(α -sulpho-3-thienylacetamido)cephalosporanic acid and 97 mg. (1×10^{-3} mole) of KSCN were added to a solution of 240 mg. (3×10^{-3} mole) of pyridine dissolved in 2 ml. of water, and the resulting solution left standing for 24 hours at 40° C. The reaction mixture was washed with ether. The aqueous layer separated was purified by column chromatography on Amberlite XAD-2 to obtain sodium N-[7-(α -sulpho-3-thienylacetamido)ceph-3-em-3-ylmethyl]-pyridinium-4-carboxylate.

Minimum inhibitory concentration:

Pseudomonas aeruginosa (Pd 1) 2 (μ g./ml.)
" (NCTC 10490) 1 "

IR ν KBr max (cm⁻¹): 1764 (β -lactam), 1675 (-CONH-), 1614 (-CO₂Na).

NMR δ (D_2O): 3.46 (2H, broad, S<H), 5.14 (1H, doublet, J=4.7 c/s, -H<S), 5.22 (1H, singlet, -CH<SO₃Na), 5.43 (2H, broad, -CH₂-), 5.71 (1H, doublet, J=4.7 c/s, -H<S), 7.35-7.58 (3H, broad,), 8.08 (2H, broad, -N<), 8.54 (1H, broad, -N<), 8.91 (2H, broad, -N<).

Example 12.

A mixture of 1.5 ml. of dimethylformamide, 1 ml. of water and 307 mg. (2.5×10^{-3} mole) of pyrazineamide was heated at 50°C . to obtain a uniform solution. The solution was added to a solution of 260 mg. (0.5×10^{-3} mole) of 7-(α -sulpho-3-thienylacetamido)cephalosporanic acid and 97 mg. (1×10^{-3} mole) of KSCN dissolved in 1 ml. of water. The mixed solution was allowed to stand at 45°C . for 24 hours, followed by cooling. The precipitated crystals were filtered off and the filtrate was purified by column chromatography on Amberlite XAD-2 to give sodium N-[7-(α -sulpho-3'-thienylacetamido)ceph-3-em-3-ylmethyl]-carbamoyl-pyrazinium-4-carboxylate.

IR ν_{max} (KBr) (cm^{-1}): 1757 (β -lactam), 1660 ($-\text{CONH}-$, $-\text{CONH}_2$), 1615 ($-\text{COONa}$), 1040 ($-\text{SO}_3\text{Na}$).

Example 13.

213 Mg. (2.5×10^{-3} mole) of thiazole, 97 mg. (1×10^{-3} mole) of KSCN and 260 mg. (0.5×10^{-3} mole) of 7-(α -sulpho-3-thienylacetamido)cephalosporanic acid were dissolved completely in 0.8 ml. of water. The solution was left standing at 50°C . for 20 hours and then purified by column chromatography on Amberlite XAD-2 to obtain 80 mg. of sodium N-[7-(α -sulpho-3'-thienylacetamido)ceph-3-em-3-ylmethyl]thiazolium-4-carboxylate.

IR ν_{max} (KBr) (cm^{-1}): 1757 (β -lactam), 1670 ($-\text{CONH}-$, $-\text{N}^+=\text{C}$), 1617 ($-\text{COONa}$), 1038 ($-\text{SO}_3\text{Na}$).

NMR δ (D_2O): 3.5 (2H, , 5.1 (1H, , 5.25 (1H, singlet, $-\text{CH}-\text{SO}_3\text{Na}$), 5.3 (2H, $-\text{CH}_2-\text{N}^+$), 5.7 (1H, , 7.37-7.6 (3H, thiophene), 8.15, 8.35, 10.0 (1H each, ).

Example 14.

408 Mg. (3×10^{-3} mole) of 4-acetamidopyridine, 485 mg. (5×10^{-3} mole) of KSCN and 260 mg. (0.5×10^{-3} mole) of 7-(α -sulpho-3-thienylacetamido)cephalosporanic acid were dissolved in 1 ml. of water and the solution was left standing at 50°C . for 10 hours. The reaction solution was purified by column chromatography on Amberlite XAD-2 to obtain sodium N-[7-(α -sulpho-3-thienylacetamido)ceph-3-em-3-ylmethyl]-4'-acetamidopyridinium-4-carboxylate.

IR ν_{max} (KBr) (cm^{-1}): 1760 (β -lactam), 1670 ($-\text{CONH}-$, $-\text{NH}-\text{COCH}_3$), 1610 ($-\text{COONa}$), 1040 ($-\text{SO}_3\text{Na}$).

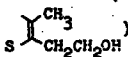
NMR δ (D_2O): 2.3 (3H, singlet, $-\text{COCH}_3$), 3.4 (2H, , 5.1 (1H, , 5.2 ($-\text{CH}-\text{SO}_3\text{Na}$), 5.3 (2H, $-\text{CH}_2-\text{N}^+$), 5.65 (1H, , 7.4-7.6 (3H, thiophene), 8.1 (2H, , 8.6 (2H, ).

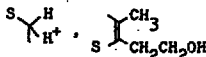
Example 15.

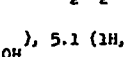
420 Mg. (3×10^{-3} mole) of 4- β -hydroxyethyl-5-methylthiazole, 485 mg. (5×10^{-3} mole) of KSCN and 260 mg. (0.5×10^{-3} mole) of 7-(α -sulpho-3-thienylacetamido)cephalosporanic acid were dissolved in 1 ml. of water and the thus obtained solution was allowed to stand at 50°C . for 10 hours. The reaction solution was purified by column chromatography on Amberlite XAD-2 to obtain sodium N-[7-(α -sulpho-

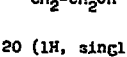
3'-thienylacetamido)ceph-3-em-3-ylmethyl]-4'' - β - hydroxyethyl - 5'' - methyl-thiazolium-4-carboxylate.

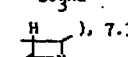
IR ν_{max} (KBr) (cm.⁻¹): 1760 (β -lactam), 1675 ($-\text{CONH}-$, $-\text{N}=\text{C}$), 1615 ($-\text{COONa}$), 1040 ($-\text{SO}_3\text{Na}$).

NMR δ (D₂O): 2.43 (3H, singlet, )

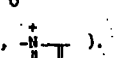
3.2-3.5 (4H, )

3.84 (2H, triplet, )

J=4.5 c/s, )

5.1 (1H, doublet, J=4.9 c/s, )

5.20 (1H, singlet, $-\text{CH}-\text{SO}_3\text{Na}$), 5.3 (2H, $-\text{CH}_2-\text{N}^+$)

5.70 (1H, doublet, J=4.9 c/s, )

7.3-7.6 (3H, thiophene), 9.12 (1H, singlet, $-\text{N}^+-\text{CH}_2-$)

Example 16.

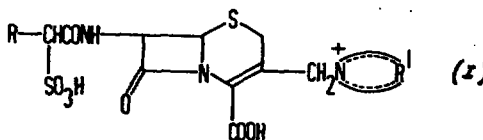
558 Mg. (2×10^{-3} mole) of sodium 7-aminocephalosporanate, 790 mg. (1×10^{-3} mole) of pyridine and 1.94 g. (2×10^{-3} mole) of KSCN were dissolved in 2 ml. of water and the resulting solution was left standing at 50° C. for 8 hours. The reaction solution was purified by column chromatography on Amberlite XAD-2 to obtain 3-pyridinium 7-aminocephalosporanate, which was dissolved in 10 ml. of water and the solution was reacted with 235 mg. of α -sulpho-3-thienylacetyl chloride to obtain sodium N-[7-(α -sulpho-3'-thienylacetamido)ceph-3-em-3-ylmethyl]pyridinium-4-carboxylate. This compound was identified as being the same as the final product obtained in Example 11 by means of IR and NMR.

In this Specification, no claim is made to N-[7-(α -sulphophenylacetamido)ceph-3-em-3-ylmethyl]-pyridinium-4-carboxylate; this compound is claimed in claim 9 of Patent Specification No. 1,310,642.

Subject to the foregoing Disclaimer,

WHAT WE CLAIM IS:—

1. A compound of general formula (I).



wherein R is a hydrogen atom or an alkyl or cycloalkyl group or a substituted or unsubstituted aryl or thienyl group; and R' forms together with the nitrogen atom a substituted or unsubstituted mono- or poly-cyclic heterocyclic ring comprising at least one double bond and 1 or 2 nitrogen atoms, or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, wherein R is a hydrogen atom, an alkyl group or an unsubstituted aryl group.

3. A compound according to claim 1, wherein R is an unsubstituted thienyl group.

4. N - [7 - (α - Sulphopropionamido)ceph - 3 - em - 3 - ylmethyl]pyridinium-4-carboxylate.

5. N - [7 - (α - Sulphocaproamido)ceph - 3 - em - 3 - ylmethyl]pyridinium-4-carboxylate.

6. 7 - Sulphoacetamido - 3 - (pyridinium)methylceph - 3 - em - 4 - carboxylate.

7. N - [7 - (α - Sulphophenylacetamido)ceph - 3 - em - 3 - ylmethyl] - 4'-carbamoylpyridinium-4-carboxylate.

8. N - [7 - (α - Sulphophenylacetamido)ceph - 3 - em - 3 - ylmethyl] - 2'-methylpyridinium-4-carboxylate.

3'-thienylacetamido)ceph-3-em-3-ylmethyl]-4'' - β - hydroxyethyl - 5'' - methyl-thiazolium-4-carboxylate.

IR ν $\frac{\text{KBr}}{\text{max}}$ (cm.⁻¹): 1760 (β -lactam), 1675 ($-\text{CONH}-$, $-\text{N}^+=\text{C}$), 1615 ($-\text{COONa}$), 1040 ($-\text{SO}_3\text{Na}$).

NMR δ (D_2O): 2.43 (3H, singlet, CH_3), 3.2-3.5 (4H, $\text{S}-\text{CH}_2$), 3.84 (2H, triplet, $\text{CH}_2\text{CH}_2\text{OH}$), 4.5 c/s, $\text{CH}_2\text{CH}_2\text{OH}$), 5.1 (1H, doublet, $\text{CH}_2\text{CH}_2\text{OH}$), 5.20 (1H, singlet, $-\text{CH}-\text{SO}_3\text{Na}$), 5.3 (2H, $-\text{CH}_2-\text{N}^+$), 5.70 (1H, doublet, $\text{CH}_2\text{CH}_2\text{OH}$), 7.3-7.6 (3H, thiophene), 9.12 (1H, singlet, $-\text{N}^+=\text{C}$).

Example 16.

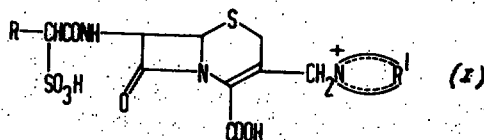
558 Mg. (2×10^{-3} mole) of sodium 7-aminocephalosporanate, 790 mg. (1×10^{-3} mole) of pyridine and 1.94 g. (2×10^{-3} mole) of KSCN were dissolved in 2 ml. of water and the resulting solution was left standing at 50° C. for 8 hours. The reaction solution was purified by column chromatography on Amberlite XAD-2 to obtain 3-pyridinium 7-aminocephalosporanate, which was dissolved in 10 ml. of water and the solution was reacted with 235 mg. of α -sulpho-3-thienylacetyl chloride to obtain sodium N-[7-(α -sulpho-3'-thienylacetamido)ceph-3-em-3-ylmethyl]pyridinium-4-carboxylate. This compound was identified as being the same as the final product obtained in Example 11 by means of IR and NMR.

In this Specification, no claim is made to N-[7-(α -sulphophenylacetamido)ceph-3-em-3-ylmethyl]-pyridinium-4-carboxylate; this compound is claimed in claim 9 of Patent Specification No. 1,310,642.

Subject to the foregoing Disclaimer,

WHAT WE CLAIM IS:—

1. A compound of general formula (I)



wherein R is a hydrogen atom or an alkyl or cycloalkyl group or a substituted or unsubstituted aryl or thienyl group; and R' forms together with the nitrogen atom a substituted or unsubstituted mono- or poly-cyclic heterocyclic ring comprising at least one double bond and 1 or 2 nitrogen atoms, or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, wherein R is a hydrogen atom, an alkyl group or an unsubstituted aryl group.

3. A compound according to claim 1, wherein R is an unsubstituted thienyl group.

4. N - [7 - (α - Sulphopropionamido)ceph - 3 - em - 3 - ylmethyl]pyridinium-4-carboxylate.

5. N - [7 - (α - Sulphocaproamido)ceph - 3 - em - 3 - ylmethyl]pyridinium-4-carboxylate.

6. 7 - Sulphoacetamido - 3 - (pyridinium)methylceph - 3 - em - 4 - carboxylate.

7. N - [7 - (α - Sulphophenylacetamido)ceph - 3 - em - 3 - ylmethyl] - 4'-carbamoylpyridinium-4-carboxylate.

8. N - [7 - (α - Sulphophenylacetamido)ceph - 3 - em - 3 - ylmethyl] - 2'-methylpyridinium-4-carboxylate.



9. 7 - (α - Sulpho - p - nitrophenylacetamido)ceph - 3 - em - 3 - ylmethyl - 2'-methylpyridinium-4-carboxylate.

10. 7 - (α - Sulpho - p - aminophenylacetamido)ceph - 3 - em - 3 - ylmethyl-2'-methylpyridinium-4-carboxylate.

5 11. N - [7 - (α - Sulpho - 3' - thienylacetamido)ceph - 3 - em - 3 - ylmethyl]-pyridinium-4-carboxylate. 5

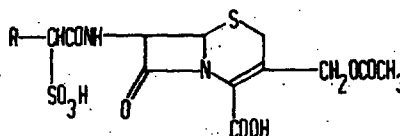
12. N - [7 - (α - Sulpho - 3' - thienylacetamido)ceph - 3 - em - 3 - ylmethyl]-carbamoyl-pyrazinium-4-carboxylate.

10 13. N - [7 - (α - Sulpho - 3' - thienylacetamido)ceph - 3 - em - 3 - ylmethyl]-thiazolium-4-carboxylate. 10

14. N - [7 - (α - Sulpho - 3' - thienylacetamido)ceph - 3 - em - 3 - ylmethyl]-4''-acetamido-pyridinium-4-carboxylate.

15. N - [7 - (α - Sulpho - 3' - thienylacetamido)ceph - 3 - em - 3 - ylmethyl]-4''- β -hydroxyethyl-5''-methyl-thiazolium-4-carboxylate.

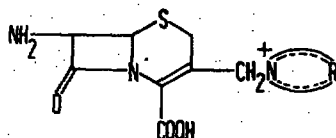
15 16. A process for preparing the compounds according to any one of the preceding claims, which process comprises (1) reacting a sulphocephalosporanic acid of general formula



20 wherein R is as defined in claim 1 or a salt or ester thereof, with a compound of general formula



wherein R' is as defined in claim 1; or (2) reacting an aminocephalosporanic acid of general formula



25 25 wherein R' is as defined in claim 1, or a salt or readily decomposable ester thereof, with an α -sulphocarboxylic acid of general formula



30 30 wherein R is as defined in claim 1, or an N-acylating functional derivative thereof; and if desired converting the reaction product into a pharmaceutically acceptable salt.

17. A process according to claim 16 substantially as described in any one of the Examples.

18. A compound of general formula (I) prepared by a process according to claim 16 or 17.

35 19. A pharmaceutical composition comprising a compound according to any one of claims 1 and 4 to 15 and a pharmaceutically acceptable carrier or diluent. 35

20. A pharmaceutical composition comprising a compound according to claim 2 and a pharmaceutically acceptable carrier or diluent.

21. A pharmaceutical composition comprising a compound according to claim 3 and a pharmaceutically acceptable carrier or diluent.

J. A. KEMP & CO.,
Chartered Patent Agents,
14, South Square, Gray's Inn, London, WC1R 5EU.

