1,028,563

PATENT SPECIFICATION

NO DRAWINGS

1,028,563

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Date of filing Complete Specification: Dec. 2, 1963.

Application Date: Dec. 14, 1962. No. 47316/62.

Complete Specification Published: May 4, 1966.

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Index at acceptance:—C2 A(1C2C, 2E); A5 B(1R2, 2R2)

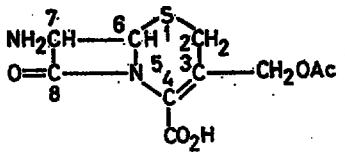
Int. Cl.:—C 07 d // A 61 k

COMPLETE SPECIFICATION

Antibacterial Derivative of 7-Aminocephalosporanic Acid

We, GLAXO LABORATORIES LIMITED, a British Company, of Greenford, Middlesex, 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:—

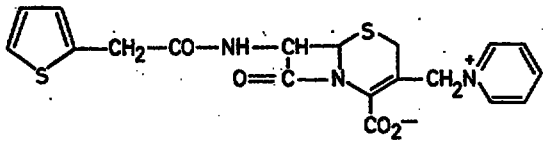
This invention is concerned with a novel antibacterial derivative of 7-aminocephalosporanic acid (7—ACA). 7—ACA has the following structure:—



(I)

The specific cephalosporin compounds (except 7—ACA) disclosed herein are named with reference to the substance cepham (see J.A.C.S. 1962, *84*, 3400).

The present invention provides as a new compound N-(7-2′-thienylacetamido-ceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate:—



(II)

The new compound according to the invention is an excellent broad spectrum antibiotic and in particular exhibits an outstanding combination of desirable properties.

In general terms the new compound possesses the following basic properties:—

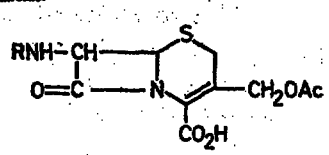
1. It has a good antibiotic spectrum *in vivo* against both gram positive and gram negative organisms.
2. It is effective against a range of penicillin-resistant staphylococci.
3. It has good stability *in vivo*.
4. It has low toxicity.
5. It has good solubility in water facilitating the formulation of and administration of preparations containing it.

The new compound thus has a broad spectrum of *in vivo* activity, and sensitive organisms include strains of *Staph. aureus* (including penicillin resistant strains), *Streptococcus pneumoniae, Strept. pyogenes, Strept. viridans, Corynebacterium diphtheriae, Neisserea catarrhalis* and *N.gonorrheas, Clostridium septicum, Cl.welchii, E.coli, Salmonella typhi, S. typhimurium, S. paratyphi*, various *Shigella sp., Proteus mirabilis, H.pertussis, Leptospira icterohaemorrhagiae, L.pomona* and *L.canicola*. It was also found to be highly resistant to staphylococcal penicillinase, to maintain high serum levels *in vivo* and to have relatively high water solubility (about 20% w/w at about 20°C). It was found to possess much greater *in vivo* stability than the compound of formula (III) below (as evidenced by high urinary levels). Injections of the compound were substantially painless.

[*Price 4s. 6d.*]

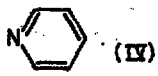
N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate has a low order of acute systemic toxicity in laboratory animals; the intravenous and subcutaneous LD_{50} values determined in mice were 1.3 and 8.5 g/kg and the subcutaneous LD_{50} in rats was 5.5 g/kg. Single intramuscular doses produced no signs of systemic or local toxicity when administered to rabbits (0.2 g/kg), cats (0.2 or 1 g/kg), dogs (0.1, 0.2 or 0.5 g/kg) and monkeys (0.2 g/kg), and mice, rats and cats accepted without incidental oral doses of 15, 3 and 0.1 g/kg respectively. Histological examination of the injection sites showed that the compound was less damaging than sodium penicillin G. No evidence of nephrotoxicity was encountered in rabbits, mice, guinea pigs, monkeys and rats given the compound in single injections of 50, 200, 200, 200 and 1,000 mg/kg, respectively. Kidney function tests on anaesthetised cats which received the compound by intravenous injection showed that the substance was eliminated solely by glomerular filtration and that it had no effect on renal plasma flow, urea clearance or glucose reabsorptive capacity. Subacute toxicity tests on mice, rats, rabbits and cats confirmed the very low toxicity of the compound.

The compound according to the invention may be prepared by reacting a compound of the general formula



(IIa)

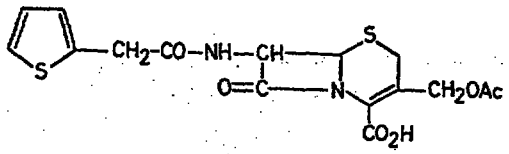
or a water soluble salt thereof, where R is hydrogen or a 2-thienylacetyl group, with pyridine:—



(IV)

as a nucleophile in a strongly polar medium, the resulting compound where R is hydrogen being 2-thienylacetylated.

The preferred process according to the invention comprises reacting the compound of the formula



(III)

or a water soluble salt thereof, with pyridine (IV) in a strongly polar medium. Where salts of compounds of formula (III) are employed suitable salts are for example sodium, potassium or ammonium salts. Water is the most generally suitable medium but a water miscible organic solvent such as N,N-dimethylformamide or acetone may be added although this may tend to lower the yield.

The temperature of reaction is conveniently at least 15°C, but in general less than 70°C.

The time for which the reaction should be allowed to proceed depends on the temperature used but can be determined by preliminary trial.

Th reactants are advantageously employed in a ratio of about 1 molar equivalent of compound (III) to 1—10 molar equivalents of pyridine. The pH of the solution is advantageously maintained within the limits 5 to 8, preferably 6 to 7. If necessary, the pH of the solution should be adjusted to the desired value by the addition of a buffering agent such as ammonium acetate or when employing an alkali metal salt of the compound of formula (III) by the addition of, for example, acetic acid.

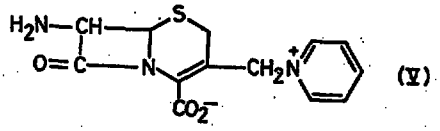
The reaction product may be separated from the reaction mixture which may contain, for example, unchanged compound of formula (III) and other substances, by conventional processes including crystallisation, electrophoresis, paper chromatography or by treatment with an ion-exchange resin.

A convenient procedure is to react excess pyridine (IV) with the compound of formula (III) in water until an optimum yield of the desired compound (II) is obtained. Excess pyridine (IV) and some of the unreacted compound (III) are then extracted with

a water-immiscible organic solvent e.g. methylene chloride, and the residual solution percolated through an anion-exchange resin e.g. in an acetate form to remove any free carboxylic compounds including the rest of the unreacted compound (III). The residual solution is then concentrated e.g. in a rotary evaporator and freeze-dried. The residue may then be crystallised from a suitable solvent e.g. methanol or purified by precipitation, for instance, from a methanolic solution by the addition of acetone.

The compound of formula (III) given above may be prepared by reacting 7—ACA with a 2-thienylacetylating agent. Convenient 2-thienylacetylating agents are in general those of the type suitable for 2-thienylacetylating aminoamides for example peptides, and include particularly 2-thienylacetyl halides, especially the choride or bromide, and mixed anhydrides derived for example from 2-thienylacetic acid and an alkyl haloformate. The acylation is conveniently carried out in an aqueous medium, for example an aqueous water-miscible ketone such as acetone, or aqueous tetrahydrofuran, preferably also in the presence of an acid binding agent for example sodium bicarbonate. The pH is preferably maintained at from 5 to 7 during the reaction, which may be carried out at temperatures of from 0 to 25°C. The acylation may also be carried out in an organic solvent medium e.g. ethyl acetate, for example by simple refluxing.

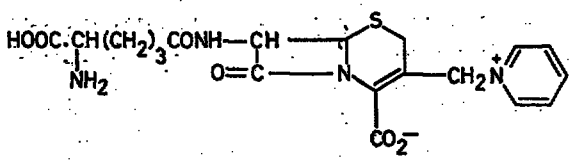
As will be seen, the compound according to the invention can be made by first reacting 7—ACA with pyridine (IV) to form a compound having the formula



(V)

followed by acylation of the resulting compound with a 2-thienylacetylating agent. These reactions can be carried out generally under the conditions described above for the nucleophilic and acylation reactions when carried out in the preferred sequence.

Alternatively the compound of formula (V) can be prepared from a compound of the formula



(VI)

by methods known for the conversion of cephalosporin C to 7—ACA. The compound of formula (VI) can be prepared from cephalosporin C by reaction with pyridine (IV) by methods analogous to those described above for reaction of compounds of formula (III) with pyridine (IV).

The compound according to the invention may be formulated for administration in any convenient way by analogy with other antibiotic substances, such as penicillin and neomycin, and the invention thus includes within its scope a pharmaceutical composition for use in human or veterinary medicine comprising the compound of general formula II in association with a pharmaceutical carrier or excipient.

The compound may thus be made up into injectable preparations either in solution or suspension in suitable media e.g. sterile, pyrogen-free water or as dry preparations suitable for the extempore preparation of injectable preparations. The compositions may further take the form of preparations for topical use e.g. lotions, ointments or creams, formulated with suitable excipients for such preparations.

For veterinary medicine the compound may be formulated in a manner conventional in veterinary medicine particularly for injection as veterinary cerates with a veterinary cerate base.

In general the dosages employed in human medicine on adults will range from 200 mg of the compound per dose upwards, administered for example four times a day.

The compound according to the invention may be administered in combination with other antibacterial antibiotics especially the penicillins such as penicillin G and/or the tetracyclines.

In order that the invention may be well understood the following examples are given by way of illustration only. Ultra-violet absorption pertains to solutions in water or aqueous phosphate buffer at pH 6.0. Melting points were measured with the compounds in capillary tubes.

Paper chromatography was carried out on Whatman No. 1 paper buffered with 0.1M-sodium acetate (pH 5).

Electrophoresis was performed on Whatman 3MM papers at a gradient of 30 v. per cm. Buffer solutions were (parts by volume in brackets): (a) pH 1.9, acetic acid (84), formic acid (17), acetone (105), water (495); (b) pH 7, 0.05M-disodium hydrogen phosphate, the pH being adjusted with phosphoric acid.

Fractions on paper chromatograms and electrophoretograms were detected as dark spots when the papers were irradiated with ultra-violet light. Zwitter-ions ran as bases in electrophoresis at pH 1.9, but were not moved at pH 7.

EXAMPLE 1

(a) *7-2'-Thienylacetamidocephalosporanic acid.*

7-Aminocephalosporanic acid (5.00 g.) which passed through a 100-mesh sieve was suspended in boiling ethyl acetate (200 ml.), and 2-thienylacetyl chloride (Cagniant, *Bull soc. chim. France*, 1949, 847) (4.42 g., 1.5 equiv.) was added in ethyl acetate (20 ml.). The mixture was boiled under reflux for 40 min., cooled, and filtered. Aniline (5.03 ml.) was added, and after 1 hr. the mixture was extracted with 3%-sodium hydrogen carbonate solution (1 × 150 ml., 2 × 100 ml., 1 × 50 ml.) and the alkaline extracts washed with ethyl acetate (3 × 100 ml.). The aqueous solution was acidified to pH 1.2, and extracted with ethyl acetate (2 × 150 ml.). The ethyl acetate extract was washed with water (4 × 40 ml.), dried ($MgSO_4$), and concentrated *in vacuo* to low volume. The crude 7-2'-thienylacetamidocephalosporanic acid (2.5 g.) which separated was collected by filtration. Evaporation of the filtrate gave a further 2.68 g. (71%) of the product, which was purified by crystallisation from ethyl acetate, then aqueous acetone, m.p. 156—157° (decomp.); λ max. 237 mμ (ε 14,400), 261 mμ (ε 8,900; $[\alpha]_D$ + 84° (c, 0.5, dioxan); the sodium salt, m.p. 204—205° (decomp.), $[\alpha]_D$ + 135° (c, 1.0 H_2O), was prepared by treating the acid in ethyl acetate solution with 10%-sodium ethylhexanoate in *n*-butanol (Found: C, 46.2; H, 3.6; N, 6.3; S, 15.2. $C_{16}H_{15}N_2NaO_6S$ requires C, 45.9; H, 3.6; N, 6.7; S, 15.3%).

(b) *N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate.*

Pyridine (10 ml., 10 equiv.) was added with stirring to a suspension of 7-2'-thienylacetamidocephalosporanic acid (5 g.) in water (45 ml.); the acid dissolved to give a solution pH 6.5. This solution, under nitrogen, was left at 46° for 16 hr. and was then extracted with methylene chloride (4 × 20 ml.). The solution, after concentration in a rotary evaporator at <40°, was passed down a column of Dowex—1 ("Dowex" is a registered Trade Mark) in the acetate cycle. Eluates that contained the desired pyridine derivative (as judged polarimetrically) were combined and freeze-dried. Trituration of the freeze-dried solid (1.75 g.) with methanol gave the pyridine derivative (994 mg., 19%) as a white solid, λ max. 237 mμ ($E^{1\%}_{1\,cm.}$ 365), λ (inflection) 256 mμ ($E^{1\%}_{1\,cm.}$ 328), $[\alpha]_D$ + 45° (c, 0.64, water) (Found: C, 52.3; H, 4.4; N, 9.8; S, 14.4. $C_{19}H_{17}N_3O_4S_2.1H_2O$ requires C, 52.6; H, 4.4; N, 9.7; S, 14.8%). The methanol was concentrated in a rotary evaporator at not greater than 40° and was then added slowly to a large volume of magnetically stirred acetone (ca. 500 ml.). By this procedure more of the required pyridine derivative was obtained as a pale yellow solid (301 mg., 6%), λ max. 237 mμ ($E^{1\%}_{1\,cm.}$ 228), λ (inflection) 255 mμ ($E^{1\%}_{1\,cm.}$ 207).

The biological properties of the compound according to the invention are shown in the following table. The Staph.aureus Strains A and C were penicillin resistant while the Strain B was penicillin sensitive.

Tube Dilution Assay (γ/ml.)				Mouse Protection (ED_{50}/mg./kg./dose) subcutaneous administration	
Gram positive organisms		Gram negative organisms			
Staph. aureus Strain A	0.62	E. coli	8	S. aureus Strain B	2
Staph. aureus Strain B	0.02	S. typhimurium	4	E. coli	<6.25
Staph. aureus Strain C	<0.5	Pr. mirabilis	8		

EXAMPLE 2(a)

N-7-Aminoceph-3-em-3-ylmethylpyridinium-4-carboxylate.

7-Aminocephalosporanic acid (10.0 g.) was suspended in water (1 litre) and pyridine (40 ml., 10 equiv.) was added. The solution (pH 6.4) was kept at 35° for 60 hrs. and then allowed to stand in a refrigerator for 2 days. The mixture was extracted with methylene chloride (3 × 1 litre) and the aqueous layer was passed through a column (40 × 3.3 cm.) of Dowex—1 resin in the acetate form (pH 4.5). The resin was eluted with water (700 ml.) until the eluate had zero optical rotation. The eluate was then stirred with Dowex—50 resin (excess) until the pH of the solution was 2.5. The resin was filtered, washed with water until the washings were colourless, then stirred in water (250 ml.) and adjusted to pH 7.0 with 7*N*-ammonium hydroxide solution. The resin was filtered, washed with water, and the washings and filtrate freeze-dried to give a crude specimen of N-7-aminoceph-3-em-3-ylmethylpyridinium-4-carboxylate (1.03 g., 9.6%), λ max. 255—256 mμ ($E^{1\%}_{1\text{ cm.}}$ 349, ε 10,150), $\nu^{\text{nujol}}_{\text{max.}}$ 1760 (β-lactam) and 1605 cm. (—CO_2^-); R_F 0.25 (n-propanol; water = 7:3) relative to 7-phenylacetamidocephalosporanic acid. Electrophoresis at pH 1.9, showed a cation moving 6.3 cms. relative to the neutral marker, at a potential gradient of 15.5 volts per cm.

EXAMPLE 2(b)

N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate.

A solution of N-7-aminoceph-3-em-3-ylmethylpyridinium-4-carboxylate (10 mgs.) in water (10 ml.) was treated with excess (5 equiv.) of thienyl-2-acetyl chloride and the mixture was shaken at room temperature for 1 hr., then examined by paper chromatography (n-propanol-water, 7:3) in an ascending system, and by electrophoresis at pH 1.9. N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate was detected, R_F 0.66 relative to 7-phenylacetamidocephalosporanic acid; it moved as a cation on electrophoresis, 1.8 cms. at 15.5 volts per cm. This was identical behaviour to the material prepared as in Example 1(b), which was used on these papers as a standard.

EXAMPLE 3

N - (7,2' - Thienylacetamidoceph - 3 - em - 3 - ylmethyl) - pyridinium - 4-carboxylate previously freeze-dried, crystallised from methanol and vacuum dried to a moisture content of less than 0.5 per cent w/w, was dry filled into 5 ml. glass vials, each vial containing 250 mg. of antibiotic. The antibiotic tends to be very hygroscopic and the weighing of the antibiotic and filling into the vial were conducted in a sterile atmosphere containing less than 15 per cent relative humidity, the moisture content of the antibiotic after filling not being greater than 1.0 per cent w/w. A moisture-proof closure, such as a butyl rubber disc or plug, was used to maintain the antibiotic at this moisture content during storage. Alternatively the antibiotic may be presented in a glass ampoule sealed by fusion.

Because the antibiotic is sensitive to light and quickly discolours it was protected from light as much as possible during the whole of its pharmaceutical processing. In addition, the filled vial should also be protected from light.

When required for use a clinician adds approximately 1.5 to 2.0 ml. of sterile water for injection to the vial, shakes for a moment to dissolve, withdraws the solution into a syringe and then injects the solution in the patient.

EXAMPLE 4

Intramuscular Injection

Composition of single injection.

N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate	250	mg.
Sodium chloride	0.8	w/v
Water for injection to produce	2	ml.

The solids were dissolved in water and the resulting solution sterilized by filtration through a No. 5/3 sintered glass filter. The sterilized solution was distributed in 2.2 ml. portions into 2 ml. ampoules and the ampoules sealed hermetically. The ampoules were then subjected to sterility tests in the usual manner.

EXAMPLE 5

Intramuscular Injection

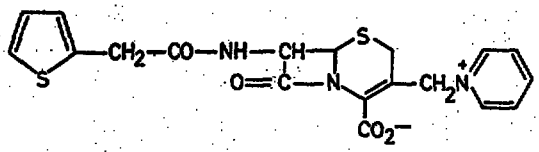
Composition of single injection.

N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate	500	mg.
Water for injection to produce	2	ml.

The solids were dissolved and the resulting solution sterilized and distributed into ampoules as in Example 4.

WHAT WE CLAIM IS:—

1. N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate



2. A pharmaceutical preparation for use in human or veterinary medicine comprising the compounds claimed in claim 1 in association with a pharmaceutical carrier or excipient.

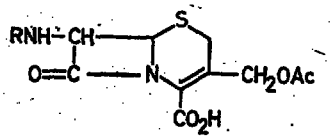
3. A pharmaceutical preparation as claimed in claim 2 comprising said compound in solution in sterile, pyrogen-free water.

4. A pharmaceutical preparation as claimed in claim 2 for topical use, comprising the compound claimed in claim 1 together with a topical base.

5. A pharmaceutical composition as claimed in claim 2 for use as a veterinary cerate comprising the compound claimed in claim 1 together with a veterinary cerate base.

6. A pharmaceutical composition as claimed in claim 2 substantially as herein described in Example 3, 4 or 5.

7. A process for the preparation of the compound claimed in claim 1 in which a compound of general formula



(IIa)

where R represents hydrogen or a 2-thienylacetyl group, is reacted in a strongly polar medium with pyridine



(IV)

the resulting compound where R represents hydrogen being 2-thienylacetylated.

When required for use a clinician adds approximately 1.5 to 2.0 ml. of sterile water for injection to the vial, shakes for a moment to dissolve, withdraws the solution into a syringe and then injects the solution in the patient.

EXAMPLE 4
Intramuscular Injection
Composition of single injection.

N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate	250	mg.
Sodium chloride	0.8	w/v
Water for injection to produce	2	ml.

The solids were dissolved in water and the resulting solution sterilized by filtration through a No. 5/3 sintered glass filter. The sterilized solution was distributed in 2.2 ml. portions into 2 ml. ampoules and the ampoules sealed hermetically. The ampoules were then subjected to sterility tests in the usual manner.

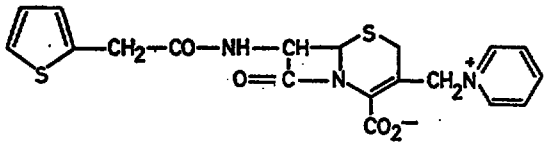
EXAMPLE 5
Intramuscular Injection
Composition of single injection.

N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate	500	mg.
Water for injection to produce	2	ml.

The solids were dissolved and the resulting solution sterilized and distributed into ampoules as in Example 4.

WHAT WE CLAIM IS:—

1. N-(7-2'-Thienylacetamidoceph-3-em-3-ylmethyl)-pyridinium-4-carboxylate



2. A pharmaceutical preparation for use in human or veterinary medicine comprising the compounds claimed in claim 1 in association with a pharmaceutical carrier or excipient.

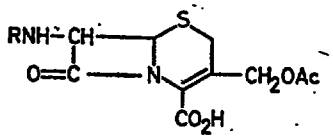
3. A pharmaceutical preparation as claimed in claim 2 comprising said compound in solution in sterile, pyrogen-free water.

4. A pharmaceutical preparation as claimed in claim 2 for topical use, comprising the compound claimed in claim 1 together with a topical base.

5. A pharmaceutical composition as claimed in claim 2 for use as a veterinary cerate comprising the compound claimed in claim 1 together with a veterinary cerate base.

6. A pharmaceutical composition as claimed in claim 2 substantially as herein described in Example 3, 4 or 5.

7. A process for the preparation of the compound claimed in claim 1 in which a compound of general formula



(IIa)

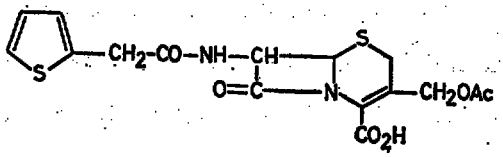
where R represents hydrogen or a 2-thienylacetyl group, is reacted in a strongly polar medium with pyridine



(IV)

the resulting compound where R represents hydrogen being 2-thienylacetylated.

8. A process for the preparation of the compound claimed in claim 1 wherein a compound of the formula:—



(III)

is reacted in a strongly polar medium with pyridine.

9. A process as claimed in claim 8 when carried out at a temperature of at least 15°C.

10. A process as claimed in claim 8 or claim 9 when carried out at a temperature below 70°C.

11. A process as claimed in any of claims 7 to 10 in which the polar medium is water.

12. A process as claimed in any of claims 7 to 11 in which the polar medium is a mixture of water and a water-miscible organic solvent.

13. A process as claimed in claim 12 in which the water-miscible organic solvent is N,N-dimethylformamide or acetone.

14. A process as claimed in any of claims 8 to 13 in which the reaction of compound (III) with pyridine (IV) is effected using 1—10 molar equivalents of the latter.

15. A process as claimed in claim 14 in which any excess pyridine (IV) and some of the unreacted compound (III) are thereafter extracted with a water-immiscible organic solvent, the residual solution is percolated through an anion-exchange resin to remove any carboxylic compounds including the rest of the unreacted compound (III), and is thereafter concentrated and freeze-dried.

16. A process as claimed in claim 15 in which the residue is crystallised from a suitable solvent or purified by precipitation.

17. A process as claimed in any of claims 8 to 16 in which the reaction of compound (III) with compound (IV) is effected at a pH of from 5 to 8.

18. A process as claimed in claim 17 in which the reaction is effected at a pH of from 6 to 7.

19. A process as claimed in any of claims 8—18 in which the compound of formula (III) is prepared by reacting 7—ACA with 2-thienylacetyl chloride or bromide.

20. A process as claimed in claim 19 in which the acylation is carried out in an aqueous medium.

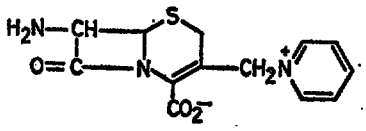
21. A process as claimed in claim 20 in which the reaction is effected in the presence of an acid binding agent.

22. A process as claimed in claim 20 or claim 21 in which the pH is maintained at a pH of from 5 to 7 during the acylation.

23. A process as claimed in claim 19 in which the acylation is carried out in an organic solvent.

24. A process as claimed in claim 23 in which the solvent is ethyl acetate.

25. A process as claimed in claim 7 in which 7—ACA is reacted with pyridine (IV) to form a compound of the formula:—

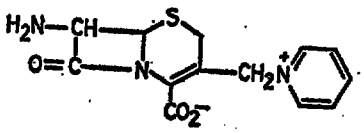


(V)

and the resultant compound is 2-thienylacetylated with a 2-thienylacetylating agent.

26. A process as claimed in claim 25 in which the 2-thienylacetylating agent is 2-thienylacetyl chloride or bromide.

27. A process for the preparation of the compound claimed in claim 1 which comprises 2-thienylacetylating a compound of formula:—



(V)

* C L M S *

28. A process as claimed in any of the preceding claims wherein the compound of formula (IIa) or (III) is used in the form of a water-soluble salt.

29. A process for the preparation of the compound claimed in claim 1 substantially as herein described.

30. A process as claimed in claim 29 substantially as herein described in Example 1.

31. A process as claimed in claim 29 substantially as herein described in Example 2.

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Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press (Leamington) Ltd.—1966. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2, from which copies may be obtained.





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