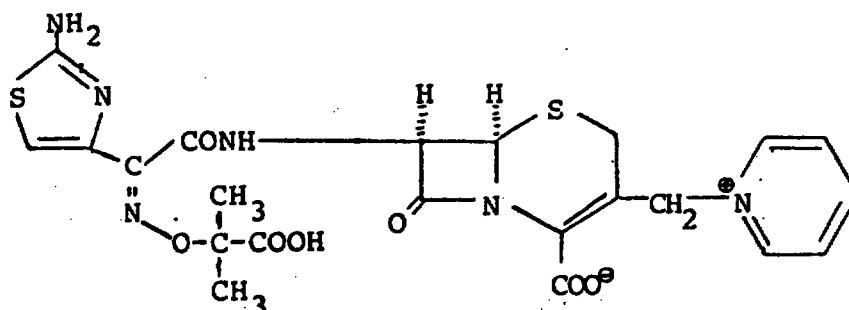


SPECIFICATION

Improvements in or relating to a cephalosporin antibiotic

5 This invention relates to improvements in or relating to (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridinium-methyl)ceph-3-em-4-carboxylate of formula

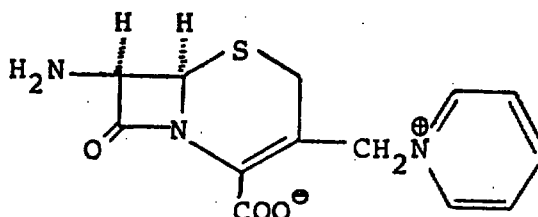


20 This compound, which is variously referred to as 'ceftazidime' and 'GR 20263', has been found to have broad spectrum antibiotic activity and, in particular, unusually high activity against gram-negative organisms, including many β -lactamase-producing gram-negative strains, as described in our UK Patent Specification No. 2025398. The compound possesses excellent activity against organisms normally difficult to combat with β -lactam antibiotics, such as indole-positive *Proteus*, *Serratia*, *Providencia* and especially *Pseudomonas* organisms, and its antibacterial properties are not impaired by human serum. Moreover, the effect of increased inocula against the compound is low and the compound is rapidly bactericidal at concentrations close to the minimum inhibitory concentration. It is well distributed in the bodies of small rodents giving useful therapeutic levels after subcutaneous injection. Experimental infections in mice with gram-negative bacteria have been successfully treated using the compound and, in particular, excellent protection has been obtained against strains of *Pseudomonas aeruginosa*, an organism normally not susceptible to treatment with cephalosporin antibiotics. This protection was comparable with the treatment with an aminoglycoside such as amikacin. Acute toxicity tests with the compound in mice gave LD₅₀ values in excess of 6 g/kg. No nephrotoxicity has been observed in rats at dosages of 2.0 g/kg. In studies in human volunteers the compound has shown good pharmacokinetic properties, giving high and long lasting serum levels after injection. The long serum half-life suggests that less frequent dosages might be required for less serious infections. Early clinical results suggest that the compound reproduces in the clinic the excellent antibiotic properties demonstrated *in vitro* and in experimental animals.

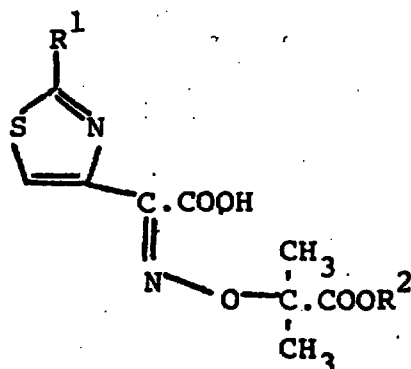
UK Patent Specification No. 2025398 also discloses solvates and non-toxic salts, e.g. base salts and acid addition salts, of the above-mentioned cephalosporin compound.

UK Patent Specification No. 2025398 discloses, *inter alia*, a method for the preparation of the above-mentioned cephalosporin compound (I) as well as solvates and non-toxic salts thereof, which comprises:

acylating a compound of formula



55 preferably as the bishydrochloride acid addition salt, with an acid of formula



(wherein R¹ represents an amino or protected amino group; and R² represents a carboxyl blocking group) or
 15 with an acylating agent corresponding thereto; whereafter the following reactions may be carried out
 i) removal of any amino-protecting group and the carboxyl blocking group R², and, if desired,
 ii) conversion of a carboxyl group into a non-toxic salt.

The desired compound of formula (I) above of UK Patent Specification No. 2025398 was obtained as an
 amorphous solid and its stability was not particularly satisfactory, especially at elevated temperatures.

20 It has now been found that (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-
 oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate (I) can be advantageously prepared
 and isolated in the form of a crystalline hydrate, which by analysis is a pentahydrate, which pentahydrate
 forms one aspect of the invention.

The new hydrate of the cephalosporin compound (I) is of superior quality in terms of its crystallinity and
 25 stability, as well as being of increased purity. In particular, the new hydrate has been found to have a
 well-defined crystalline structure and it has been found to be remarkably stable even when stored at a
 temperature of 50°C for an extended period. These properties render the hydrate of value in pharmaceutical
 use.

It has been found that the new crystalline hydrate can be conveniently prepared from a solution of a salt of
 30 the above cephalosporin compound. Thus, in another aspect the invention provides a process for the
 preparation of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-
 pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate which comprises adjusting the pH of a solution of
 an acid or base salt of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-
 pyridiniummethyl)ceph-3-em-4-carboxylate in an aqueous medium to 2.7 to 4.8, conveniently 3.0 to 4.0,
 35 preferably 3.3 to 4.0, e.g. about 3.7, and crystallising the desired pentahydrate. For example, it has been found
 that the desired crystalline hydrate may be precipitated from an aqueous solution of acid addition salt on
 addition of an organic or inorganic base to a pH in the above ranges, preferably 3.0 to 4.0.

Bases which may be used in the precipitation include, for example, inorganic bases such as alkali or
 alkaline earth metal hydroxides, carbonates or bicarbonates e.g. sodium carbonate, sodium bicarbonate and
 40 sodium hydroxide. A starting acid addition salt may be formed with an organic or inorganic acid. Examples
 of organic acids which may be used include carboxylic and sulphonic acids such as formic, trifluoroacetic,
 toluene-p-sulphonic or methanesulphonic acids. Examples of inorganic acids which may be used include
 mineral acids such as hydrochloric, hydrobromic, sulphuric, nitric or phosphoric acid. A particularly suitable
 acid addition salt is the bishydrochloride of compound (I), which may be obtained in highly pure form.

45 Alternatively the new pentahydrate may be prepared by addition of an acid to a solution of a base salt of
 compound (I) in an aqueous medium to a pH in the above ranges, preferably 3.5 to 4.2. Acids which may be
 used for this purpose include organic and inorganic acids such as, for example, hydrochloric acid and
 sulphuric acid. Starting base salts include, for example, alkali metal salts, e.g. sodium and potassium salts;
 alkaline earth metal salts, e.g. a calcium salt, amino acid salts e.g. lysine and arginine salts; and organic base
 50 salts e.g. ammonium, triethylamine, procaine, phenethylbenzylamine, dibenzylethylenediamine, ethanolamine,
 diethanolamine and N-methyl-glucosamine salts.

The aqueous medium may contain a water-miscible organic solvent e.g. in an amount up to 60% by
 volume. Examples of organic solvents include alcohols such as ethanol or isopropanol, ethers such as
 tetrahydrofuran, dioxan or diethyl ether, amides such as N,N-dimethylformamide or N,N-dimethylacetamide,
 55 sulphoxides such as dimethylsulphoxide, and nitriles such as acetonitrile.

The precipitation is preferably effected at a temperature of from 0 to 50°C, e.g. 15° to 40°C, conveniently at
 about 24°C, followed where necessary by a step of cooling to enhance crystal yield to a temperature in the
 range from 0° to 10°C.

After precipitation, the pentahydrate product may be recovered by filtration and washed and dried in
 60 conventional manner. For example the hydrate may conveniently be dried by air drying, careful drying under
 reduced pressure or, preferably, in a sterile inert gas atmosphere such as sterile nitrogen.

The salts for use in the preparation of the new pentahydrate according to the invention may be prepared
 by a process disclosed in the above-mentioned UK Patent Specification No. 2025398, i.e. by a process
 corresponding to the method above mentioned. The hydrochloride may be prepared in the form of a highly
 65 stable crystalline bishydrochloride product by crystallisation from a medium comprising acetone and formic

12

acid e.g. under the conditions described in the Preparation 1 below.

The new crystalline pentahydrate according to the invention has been subjected to X-ray powder diffraction studies. The product of the following Example 2 was used to obtain a Debye Scherrer powder diffraction photograph by exposure for 12 hours to CoK α radiation and a second photograph by exposure for 5 3 hours to CuK α radiation. The line intensities were compared against a set of standards to give the relative intensities shown in the following Table:-

TABLE

10	'd' value (Å)	Intensity*	'd' value (Å)	Intensity*	10
	15.9	m	3.24	2vw	
	9.9	s	3.18	w	
15	8.7	s	3.13	w	15
	7.9	tr	3.01	m	
	6.7	md	2.87	m	
	6.3	w	2.77	m	
	5.95	m	2.72	w	
20	5.74	w	2.69	wd	20
	5.42	w	2.52	m	
	5.18	m	2.43	w	
	4.71	m	2.33	w	
	4.50	m	2.29	vw	
25	4.37	tr	2.26	vw	25
	4.15	tr	2.23	vw	
	4.10	s	2.19	w	
	4.01	m	2.13	vw	
	3.93	w	2.07	w	
30	3.86	s	2.03	w	30
	3.68	s	1.97	vw	
	3.41	w	1.94	vw	
	3.33	2vw			
	3.29	2vw			

* s=strong, m=medium, w=weak, v=very, 2v=vv, d=diffuse, tr=trace

The new pentahydrate according to the invention has also been characterised by its infrared spectrum.

The infrared spectrum of the product of the following Example 2 in Nujol (registered Trade Mark) was 40 obtained and this is shown in the Figure of the accompanying drawing.

The following Examples serve to illustrate the preparation of the pentahydrate according to the invention. Preparations 1 and 2 illustrate the preparation of acid addition salt starting materials for the preparation of the pentahydrate. In the Preparations and Examples all temperatures are in °C, and TFA is trifluoroacetic acid. Water contents were determined by the Karl Fischer method.

45 Proton magnetic resonance (p.m.r.) spectra were determined at 100 MHz. The integrals are in agreement with the assignments, coupling constants, J, are in Hz, the signs not being determined: s = singlet, t = triplet, d = doublet, dd = double doublet, m = multiplet and ABq = AB quartet. Amberlite (registered Trade Mark) L.A. 2 is a weakly basic high molecular weight secondary amine sold by Rohm and Haas, Philadelphia USA. Hyflo (registered Trade Mark) Super-Cel is a diatomaceous silica filter aid sold by Johns-Manville, USA.

50 PREPARATION 1

(6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate bishydrochloride

Formic acid (84 ml) was added with stirring to (6R,7R)-7-[(Z)-2-(2-tritylaminothiazol-4-yl)-2-(2-t-butoxycarbonylprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate N,N-dimethylformamide solvate (41.8 g), water cooling being employed to maintain the temperature below 28°. The resulting solution was cooled to 20°, and concentrated hydrochloric acid (17.0 ml) added with stirring over 5 minutes. The mixture was stirred for 3 hours at room temperature, then filtered to remove triphenylmethanol. The filtrate was added to stirred acetone (800 ml). The triphenylmethanol was washed 60 with formic acid (3 x 7 ml), and the combined washings were added to the filtrate-acetone mixture. The resulting suspension was stirred for 1.25 hours, then filtered. The crystalline solid was washed with acetone and dried *in vacuo* to give the *title compound* (20.2 g), Chlorine, found: 11.0%; calculated for C₂₂H₂₄N₆O₇S₂Cl₂: 11.5%; $\lambda_{\max}$ (pH 6 phosphate buffer) 257 nm (E_{1cm}^{1%} 347), λ_{inf} at 240 (E_{1cm}^{1%} 310) and 290 nm (E_{1cm}^{1%} 150).

PREPARATION 2

(6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxymino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate Hydrosulphate

- a) Formic acid (98-100%, 150 ml) was added to (6R,7R)-7-[(Z)-2-(2-tritylaminothiazol-4-yl)-2-(2-*t*-butoxycarbonyl-prop-2-oxymino)-acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate N,N-dimethyl-formamide solvate (80 g) and the mixture stirred till clear and cooled to 15°C. Water (12.6 ml) and conc. sulphuric acid (12.6 ml) were added to the solution and the temperature rose to 25° and stirring was continued at this temperature for 5 hours.

- The suspension was filtered and the solid washed with formic acid (98-100%, 46 ml in several portions). The combined filtrate and wash were diluted with isopropanol (200 ml) and the resultant solution dripped into stirred isopropanol (1400 ml) over 20 minutes. Isopropyl ether (400 ml) was added to the suspension and stirring was continued for a further 10 minutes before the solid was filtered off. The bed was washed with isopropanol (3 x 200 ml) and dried for 16 hours *in vacuo* at 40° to give the *title compound* (51.02 g) as a cream solid. H₂O (by Karl Fischer) 4.2%; sulphur 15.2%, C₂₂H₂₂N₆O₇S₂ · H₂SO₄ · 2H₂O requires H₂O 5.3%; S, 14.13%.

- b) The product from stage (a) was heated in methanol (10 ml) with gentle swirling. After keeping the suspension at room temperature for 2 hours followed by 30 minutes at 0°C, the solid was collected by filtration, washed with cold methanol and dried *in vacuo* at 40° for 3 hours to give the crystalline *title compound* (0.9 g). Found: C, 38.53; H, 3.70; N, 12.36; S, 14.1; H₂O, 4.5; C₂₂H₂₂N₆O₇S₂ · H₂SO₄ · 2H₂O requires C, 38.8; H, 4.15; N, 12.34; S, 14.13; H₂O, 5.3. λ_{max} (pH 6.0 phosphate buffer) 257 nm (E₁_{cm}^{1%} 325), 241 nm (E₁_{cm}^{1%} 292), 289nm (E₁_{cm}^{1%} 144). τ (TFA) 0.90 (d, J=6 Hz, 2 H), 1.32 (t, J=7 Hz, 1 H) and 1.80 (t, J=7 Hz, 2 H) (pyridyl H), 1.40 (d, J=8 Hz, 1 H; NHCO), 2.60 (s, 1H; thiazolyl H), 3.72 and 4.50 (ABq, J=14 Hz, 2 H; -CH₂N<), 3.84 (m, 1 H; 7-H), 4.54 (d, J=5 Hz, 1H; 6-H), 6.06 and 6.54 (ABq, J=18 Hz, 2 H; 2-H, 8.20 (s, 6 H; C(CH₃)₂).

- All the following Examples illustrate the preparation of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxymino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate.

Example 1

- The bishydrochloride (2.56 g), prepared as in Preparation 1, dissolved in distilled water (8 ml) was stirred for 2 to 3 minutes until clear, whereafter 98% formic acid (1.0 ml) was added. This mixture was stirred with a liquid anion exchange resin, 'Amberlite' L.A.2, (4 ml) in diisopropyl ether (8 ml), allowed to settle and separated. The aqueous layer was extracted with diisopropyl ether (2 x 5 ml) and the resin layer was back-washed with distilled water (5 ml), which in turn was extracted with the two diisopropyl ether extracts. The combined aqueous layers (pH ca. 2.4), which contained the formate salt, were stirred during the addition of ammonia solution (8 to 10 drops) to pH 3.7 and the clear solution allowed to crystallise slowly at ambient temperature for one hour and then at 0° overnight.

- The *title compound* was collected by filtration, washed with chilled distilled water (2 x 6 ml), and with acetone (2 x 10 ml) and dried at ambient temperature in an air oven for 2 hours to yield a crystalline solid (2.0 g). λ_{max} (pH 6 buffer) 257 nm (E₁_{cm}^{1%} 348); τ(D₂O + TFA) 0.98, 1.36 and 1.84 (pyridinium protons), 2.80 (-thiazole), 4.05 (C₇-H), 4.15 and 4.58 (-CH₂-), 4.64 (C₆-H), 6.21 and 6.67 (C₂-H) and 8.40 (-CH₃)₂; ν_{max} (Nujol) 1760 (β-lactam), 1710 (CO₂H), 1645 and 1538 (CONH) and 1620 cm⁻¹ (CO₂⁻); water (Karl Fischer) 13.6%; residual chlorine, 0.1%; C₂₂H₂₂N₆O₇S₂ · 5 H₂O requires water, 14.1%.

Example 2

- The bishydrochloride, prepared as in Preparation 1, (2.0 g) was dissolved in distilled water (12 ml) and stirred during the addition of ammonia solution to pH 3.5. The clear solution was allowed to crystallise at ambient temperature for 0.5 hour; the pH was adjusted to 3.8 with ammonia solution and the suspension was then kept at 0° for 1 hour.

- The *title compound* was collected by filtration, washed with chilled water (10 ml) and with acetone and dried at ambient temperature *in vacuo* for 3 hours to yield 1.54 g of crystalline solid; λ_{max} (pH 6 buffer) 257 nm (E₁_{cm}^{1%} 356); τ(D₂O + TFA), resembles Example 1 above; ν_{max} (Nujol) see figure; water (Karl Fischer method) 13.8%; residual chlorine <0.1%; found C, 41.5; H, 4.73; N, 13.17%, C₂₂H₂₂N₆O₇S₂ · 5 H₂O requires C, 41.5; H, 5.05; N, 13.2; H₂O, 14.1%. A Debye Scherrer X-ray powder diffraction photograph was obtained on the product; the results are given above.

Example 3

- The bishydrochloride prepared as in Preparation 1 (2.0 g) was dissolved in distilled water (6 ml) and stirred during the slow addition of 2N-sodium hydroxide solution to a pH of 3.8. When crystallisation was well established, the pH which had risen to 4.5, was brought back to 3.8 with 2N-hydrochloric acid (a few drops). The suspension was cooled in ice and the *title compound* was collected by filtration, washed with ice water (10 ml) and with acetone and dried at room temperature in an air oven for 2 hours to yield 1.56 g of crystalline solid; λ_{max} (pH 6 buffer) 257 nm (E₁_{cm}^{1%} 354); τ(D₂O + TFA) resembles Example 1 above; water (Karl Fischer) 14.4%; residual chlorine, <0.1%.

Example 4

The bishydrochloride prepared as in Preparation 1 (4.0 g) was dissolved in distilled water (14 ml) and stirred during the addition of saturated aqueous sodium bicarbonate solution (13 ml) to pH 3.8. The product crystallised rapidly and the suspension was cooled in ice. The *title compound* was collected by filtration, washed with ice-cold water (20 ml) and with acetone and dried at room temperature in an air oven for 2 hours to yield 3.1 g of crystalline solid $\lambda_{\max}$ (pH 6 buffer) 257 nm ($E_{1\%}^{1\text{cm}}$ 358), 241 nm ($E_{1\%}^{1\text{cm}}$ 322), 290 nm ($E_{1\%}^{1\text{cm}}$ 157). Water (Karl Fischer) 13.9%, $\nu_{\max}$ (Nujol) resembles Example 1 above.

Example 5

10 The hydrosulphate (10 g) prepared as in Preparation 2(a) was dissolved in water (20 ml) and cooled with stirring to 15°C. Ammonia solution (SG 0.88) was added to the stirred solution adjusting to pH 3.75 with the temperature rising to 25°C. Seeds of the *title compound* were added and the mixture chilled for 16 hours at ca. 0°. The suspension was readjusted to pH 3.75 with a little more ammonia solution, then filtered. The bed was washed with cold water (2 x 15 ml) and acetone (50 ml). The product was dried for 6 hours at ambient temperature in an air oven to afford the *title pentahydrate* (3.8 g). Water 14.2%, τ resembles that of Example 1 above.

Example 6

20 The crystalline hydrosulphate (9.4 g), prepared as in Preparation 2(b) was dissolved in water (19 ml) with the addition of a few drops of ammonia solution (SG 0.88). The stirred solution was cooled to 15° and further ammonia solution added with stirring to pH 3.75 at 25°. Seeds of the *title compound* were added and the mixture chilled at ca. 0° for 16 hours. The suspension was readjusted to pH 3.75 with a few drops of 2N sulphuric acid and then filtered. The bed was washed with ice cold water (2 x 15 ml) and acetone (50 ml). The product was dried for 6 hours at ambient temperature in an air oven to afford the *title pentahydrate* (5.6 g). Water 14.0%. τ resembles that of Example 1.

Example 7

The bishydrochloride prepared as in Preparation 1 (10 g) was dissolved in distilled water (40 ml) and stirred during the addition of 2N NaOH (20 ml) to pH 6. The solution of the sodium salt thus obtained was kept at ambient temperature for 3 hours then clarified by filtration. The filtrate was stirred whilst 2N HCl (7 ml) was added to pH 3.8. The suspension was refrigerated for 2 hours and the precipitate collected by filtration, washed with ice-cold water (50 ml) and with acetone (50 ml) and dried at room temperature in an air oven for 2 hours to yield crystalline *title compound* (8.01 g); τ ($D_2O/T.F.A.$) resembles Example 1 above; water 14.1%.

Example 8

35 The bishydrochloride prepared as in Preparation 1 (20 g) was dissolved in distilled water (50 ml) and stirred during the addition of 2N NaOH (ca. 40 ml) to pH 6 to give a solution of the sodium salt. Sodium dithionite (20 mg) was added and the solution cooled to 20°. 5N H_2SO_4 was added to the stirred solution to pH 3.8. The suspension was aged at ambient temperature for 1½ hours, cooled to 10° and the *title compound* collected by filtration, washed with ice-cold water (60 ml) and with acetone (60 ml) and dried at room temperature in an air oven to yield crystalline *title compound* (16.6 g); τ (T.F.A.) resembles Example 1 above; water 14.2%.

Example 9

45 A solution of the bishydrochloride prepared as in Preparation 1 (10 g) in ice-cold distilled water (20 ml) was brought to pH 6 by the addition of 2N KOH (ca. 24 ml). The solution of the potassium salt thus produced was clarified by filtration with 'Hyflo Super-Cel' (1 g) with a water wash (10 ml). The filtrate was acidified to pH 3.5 by the addition of 2N H_2SO_4 (ca. 8 ml) was seeded with the *title compound* (0.05 g). Crystallisation was effected without stirring for 1 hour. The pH was then readjusted to 3.5 and the suspension cooled to 5°. The *title compound* was collected by filtration, washed with ice-cold water (40 ml) followed by acetone (40 ml) and dried in an air oven at ambient temperature to yield 7.84 g; H_2O 14.2%, τ (T.F.A.) resembles Example 1 above.

Example 10

55 A solution of the bishydrochloride prepared as in Preparation 1 (10 g) in ice-cold distilled water (20 ml) was brought to pH 7.2 by the addition of 2N-ammonium hydroxide solution (ca. 24 ml). The solution of the ammonium salt thus produced was clarified and the filtrate treated with 2N H_2SO_4 as in Example 9 above to yield the *title compound* (5.93 g); H_2O 14.2%, τ (T.F.A.) resembles Example 1 above.

Example 11

60 A solution of the bishydrochloride prepared as in Preparation 1 (10 g) in ice-cold water (40 ml) was brought to pH 6.5 by the dropwise addition of triethylamine (6.55 ml). The solution of the triethylamine salt thus obtained was clarified as in Example 9 above and the filtrate was acidified to pH 3.5 by the addition of 2N HCl (ca. 8 ml) and the product isolated as above to yield the *title compound* (7.13 g); H_2O 14.1%, τ (T.F.A.)

resembles Example 1 above.

Example 12

A solution of the bishydrochloride prepared as in Preparation 1 (10 g) in ice-cold water (20 ml) was brought to pH 6 by the dropwise addition of 2N NaOH (ca. 24 ml). The solution of the sodium salt thus obtained was clarified by filtration with 'Hyflo Super-Cel' (1 g) with a water wash (10 ml). The solution was cooled to -2° and acidified to pH 3.5 by the addition of 2N H_2SO_4 (ca 8 ml) and seeded with the title compound. The solution was stirred at 0° for 3 hours. The *title compound* was collected by filtration, washed with ice-cold water (40 ml) followed by acetone (40 ml) and dried in an air oven at ambient temperature to yield 7.84 g; H_2O 14.2%; τ (T.F.A.) resembles Example 1 above.

Example 13

A solution of the bishydrochloride prepared as in Preparation 1 (10 g) in distilled water (20 ml) was brought to pH 6 by the addition of 2N NaOH (ca. 24 ml). The solution of the sodium salt thus obtained was clarified by filtration with 'Hyflo Super-Cel' (1 g) with a water wash (10 ml). The solution was heated to 39° and acidified to pH 3.5 with 2N H_2SO_4 (ca. 8 ml) and seeded with the title compound (50 mg). Crystallisation was effected at 40° ; stirring was continued for one hour and the pH readjusted to 3.5. The suspension was filtered and the residue was washed with ice-cold water (40 ml) followed by acetone (40 ml) and dried in an air oven at ambient temperature to yield *title compound* (2.11 g); H_2O 14.1%; τ (T.F.A.) resembles Example 1 above.

Example 14

After 1 hour at 40° , the suspension obtained as in Example 13 was allowed to cool to 25° during one hour and then cooled to 5° for one hour. The *title compound* was collected by filtration, washed with ice-cold water (40 ml) followed by acetone (40 ml) and dried in an air oven at ambient temperature to yield 6.9 g; H_2O 14.3%; τ (T.F.A.) resembles Example 1 above.

Example 15

A solution of the bishydrochloride prepared as in Preparation 1 (2.0 kg) was dissolved in freshly distilled water (5 l) and basified to pH 6 with 2N sodium hydroxide solution (approximately 4.8 l) keeping the solution at about $14-15^{\circ}\text{C}$. The solution of sodium salt thus obtained was sterilised by passage through a membrane filter (0.22 μm pore size) followed by a freshly distilled water wash (0.5 l). Subsequent operations were carried out aseptically. The filtered solution was adjusted to pH 3.75 with 2N hydrochloric acid (approximately 1.2 l), seeded with sterile title compound (20 mg) at $14-15^{\circ}\text{C}$ and then stirred until crystallisation was established. The mixture was cooled to approximately 5°C and maintained overnight at this temperature (without stirring). The pH was re-adjusted to pH 3.75 as necessary. The title compound was collected by filtration using fibre free nylon mesh and washed with ice-cold water for injections (approximately 5.5 l) and sterile acetone (approximately 5.5 l). The product was dried in a stream of sterile filtered nitrogen until the acetone content was less than 0.2% to yield sterile *title compound* (1.48 kg); H_2O 14.55%; τ (T.F.A.) resembles Example 1 above.

Example 16

Bishydrochloride (5 g) prepared as in Preparation 1 was dissolved in mixtures of distilled water and acetone (20 ml *in toto*) containing various proportions of acetone from 5% to 60% of the mixture, and the solution cooled to 15° . The pH value was adjusted to 3.5 by the addition of 2N NaOH solution and the mixture immediately seeded with authentic title compound (0.015 g). The mixture, now at $21-23^{\circ}$, was kept for 90 minutes without stirring then cooled to 12° with stirring, and the pH re-adjusted to 3.5. After keeping for a further one hour at 5° , the solid product was isolated by filtration, washed with water at 5° (25 ml) and finally with acetone (25 ml). Drying to constant weight in the air oven at ambient temperature afforded the *title compound*.

Following the above procedure, the *title compound* has been obtained from the bishydrochloride by crystallisation from water containing the following: ethanol, isopropanol, tetrahydrofuran, dioxan, diethyl ether and ethyl acetate.

Example 17

Samples of the bishydrochloride (5 g) prepared as in Preparation 1 were dissolved in distilled water (20 ml each) and the resulting solutions chilled to 15° with rapid stirring. The pH values of the solutions were adjusted by the dropwise addition of 2N NaOH. After seeding with authentic title compound (0.015 g), the mixtures were kept for 90 minutes at ambient temperature without stirring. They were then cooled to 5° with stirring and the pH values re-adjusted before keeping for a further hour at 5° without stirring.

Title compound isolated by suction filtration was in each case washed with distilled water (25 ml) at 5° and then with acetone (25 ml) before drying to constant weight in the air oven at ambient temperature.

In the table below are noted yields of the title compound obtained at a variety of pH values, together with water contents. The p.m.r. spectra resembled those of Example 1.

TABLE

	pH	Yield (m/m%)	Water Content (%)	
5	2.7	13.2	14.15	5
	2.9	65.8	13.95	
	3.2	81.6	13.95	
	3.6	80	14.05	
	3.8	77.8	14.1	
10	4.1	57.6	14.2	10
	4.35	15.8	14.2	

Example 18

Samples of the bishydrochloride (5 g) prepared as in Preparation 1, were dissolved in distilled water (10 ml) each) and the resulting solutions chilled to 7° with rapid stirring. After adjusting to pH 6 by the dropwise addition of 2N sodium hydroxide the resulting solutions of sodium salt were kept without stirring for 10 minutes. They were then acidified to pre-determined pH values by the dropwise addition of 2N sulphuric acid and immediately seeded with authentic title compound (0.015 g). After keeping at room temperature for 40 minutes without stirring, the mixtures were cooled to 5° with stirring and the pH values re-adjusted before keeping for a further 2 hours at 5° without stirring. The *title compound* isolated by suction filtration was in each case washed with distilled water (25 ml) at 5° and then with acetone (25 ml) before drying to constant weight in the air oven overnight at ambient temperature.

In the table below are noted yields of the title compound obtained at a variety of pH values, together with water contents. The p.m.r. spectra resembled those of Example 1.

TABLE

	pH	Yield (m/m%)	Water Content %	
30	3.2	18.8	13.6	30
	3.4	52.8	13.65	
	3.8	82.8	13.35	
	4.2	72.0	13.95	
	4.7	21.0	13.7	
35	4.8	8.8	13.85	35

Pharmaceutical formulations

The crystalline pentahydrate of the present invention exhibits the antibiotic properties of the above-mentioned compound (I) and may be used for treating a variety of diseases caused by pathogenic bacteria in human beings and animals, such as respiratory tract infections and urinary tract infections.

In another aspect, the present invention provides pharmaceutical compositions containing the new pentahydrate in association with a pharmaceutical carrier or excipient. Such compositions are adapted for use in human or veterinary medicine and may be presented in conventional manner.

The antibiotic pentahydrate compound according to the invention may be formulated for injection and may be presented in unit dose form in ampoules, or in multi-dose containers if necessary with an added preservative.

The compositions may also take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Desirably the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. Preferably such powder formulations may contain an appropriate non-toxic base in order to improve the water-solubility of the active ingredient and/or to ensure that when the powder is constituted with water, the pH of the resulting aqueous formulation is physiologically acceptable. Alternatively, the base may be present in the water with which the powder is constituted. The base may be, for example, an inorganic base such as sodium carbonate, sodium bicarbonate, trisodium orthophosphate or sodium sulphite or an organic base such as lysine, lysine acetate, tromethamine, arginine or sodium glycinate.

The antibiotic compound may also be formulated as suppositories e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

For medication of the eyes or ears, the preparations may be formulated as individual capsules, in liquid or semi-liquid form, or as drops.

Compositions for veterinary medicine may also, for example, be formulated as intramammary preparations in either long acting or quick-release bases.

The compositions may contain from 0.1% upwards, e.g. 0.1-99%, of the active material, depending on the method of administration. When the compositions comprise dosage units, each unit will preferably contain 50-1500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably



range from 500 to 6000 mg per day, depending on the route and frequency of administration. For example, in adult human treatment 1000 to 3000 mg per day administered intravenously or intramuscularly should normally suffice. In treating *Pseudomonas* infections higher daily doses may be required.

The antibiotic compound according to the invention may be administered in combination with other therapeutic agents such as antibiotics, for example penicillins or other cephalosporins.

The following Formulations illustrate the pharmaceutical compositions:-

FORMULATION A, for injection

Formula per vial

10	(6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-ceph-3-em-4-carboxylate pentahydrate	582 mg	10
	Sodium carbonate (anhydrous)	58 mg	

15 Method

The cephalosporin hydrate was blended with sodium carbonate and filled into a glass vial. The vial headspace was purged with nitrogen and a combination seal applied by crimping. The product was dissolved, as for administration, by addition of 2 ml Water for Injections.

20 FORMULATION B, Injection twin-pack

- a) Fill sterile (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate aseptically into glass vials under a blanket of sterile nitrogen, such that each vial contains an amount equivalent to 500 mg of the anhydrous cephalosporin. Close the vials using rubber disks or plugs, held in position by aluminium overseals, thereby preventing gaseous exchange or ingress of microorganisms.
- b) Prepare a 3.84% w/v solution of sodium bicarbonate, clarify by filtration and fill 2.15 ml into clean ampoules. Pass carbon dioxide into the contents of each ampoule for one minute before sealing. Sterilise the ampoules by autoclaving and check for clarity.
- c) Constitute the cephalosporin antibiotic shortly before administration by dissolving in 2.0 ml of the sodium bicarbonate solution.

FORMULATION C, for injection

Formula per vial

35	(6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate	582 mg	35
	L-Arginine	167 mg	

Method

- 40 The cephalosporin was blended with L-arginine and filled into a glass vial. The vial headspace was purged with nitrogen and a combination seal applied by crimping. The product was dissolved as for administration, by the addition of 1.5 ml Water for Injection.

FORMULATION D, for injection

45 Formula per vial

45	(6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate	582 mg	45
	L-Arginine	167 mg	
50	Sodium dihydrogen phosphate dihydrate	15 mg	50

Method

- 55 Mix the sterile L-arginine and sterile sodium dihydrogen phosphate under aseptic conditions. Blend aseptically the resultant powder mix with the sterile cephalosporin. Fill aseptically into glass vials under a blanket of sterile nitrogen. Close the vials using rubber discs, or plugs, held in position by aluminium overseals, thereby preventing gaseous exchange or ingress of microorganisms. Reconstitute the product by dissolving in Water for Injections or other suitable sterile vehicle shortly before administration.

CLAIMS

- 60 1. (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-ceph-3-em-4-carboxylate pentahydrate.
2. A process for the preparation of the pentahydrate according to claim 1 which comprises adjusting the pH of a solution of an acid or base salt of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-ceph-3-em-4-carboxylate in an aqueous medium to 2.7 to 4.8



range from 500 to 6000 mg per day, depending on the route and frequency of administration. For example, in adult human treatment 1000 to 3000 mg per day administered intravenously or intramuscularly should normally suffice. In treating *Pseudomonas* infections higher daily doses may be required.

The antibiotic compound according to the invention may be administered in combination with other therapeutic agents such as antibiotics, for example penicillins or other cephalosporins.

The following Formulations illustrate the pharmaceutical compositions:-

FORMULATION A, for injection

Formula per vial

- | | | | |
|----|---|--------|----|
| 10 | (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-ceph-3-em-4-carboxylate pentahydrate | 582 mg | 10 |
| | Sodium carbonate (anhydrous) | 58 mg | |

15 Method

The cephalosporin hydrate was blended with sodium carbonate and filled into a glass vial. The vial headspace was purged with nitrogen and a combination seal applied by crimping. The product was dissolved, as for administration, by addition of 2 ml Water for Injections.

20 FORMULATION B, Injection twin-pack

- | | | | |
|----|--|----|----|
| | a) Fill sterile (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate aseptically into glass vials under a blanket of sterile nitrogen, such that each vial contains an amount equivalent to 500 mg of the anhydrous cephalosporin. Close the vials using rubber disks or plugs, held in position by aluminium overseals, thereby preventing gaseous exchange or ingress of microorganisms. | 25 | 20 |
| 25 | b) Prepare a 3.84% w/v solution of sodium bicarbonate, clarify by filtration and fill 2.15 ml into clean ampoules. Pass carbon dioxide into the contents of each ampoule for one minute before sealing. Sterilise the ampoules by autoclaving and check for clarity. | | |
| 30 | c) Constitute the cephalosporin antibiotic shortly before administration by dissolving in 2.0 ml of the sodium bicarbonate solution. | | 30 |

FORMULATION C, for injection

Formula per vial

- | | | | |
|----|--|--------|----|
| 35 | (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate | 582 mg | 35 |
| | L-Arginine | 167 mg | |

Method

- | | | |
|----|---|----|
| 40 | The cephalosporin was blended with L-arginine and filled into a glass vial. The vial headspace was purged with nitrogen and a combination seal applied by crimping. The product was dissolved as for administration, by the addition of 1.5 ml Water for Injection. | 40 |
|----|---|----|

FORMULATION D, for injection

45 Formula per vial

- | | | | |
|----|--|--------|----|
| | (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate | 582 mg | 45 |
| | L-Arginine | 167 mg | |
| 50 | Sodium dihydrogen phosphate dihydrate | 15 mg | 50 |

Method

- | | | |
|----|---|----|
| 55 | Mix the sterile L-arginine and sterile sodium dihydrogen phosphate under aseptic conditions. Blend aseptically the resultant powder mix with the sterile cephalosporin. Fill aseptically into glass vials under a blanket of sterile nitrogen. Close the vials using rubber discs, or plugs, held in position by aluminium overseals, thereby preventing gaseous exchange or ingress of microorganisms. Reconstitute the product by dissolving in Water for Injections or other suitable sterile vehicle shortly before administration. | 55 |
|----|---|----|

CLAIMS

- | | | |
|----|---|----|
| 60 | 1. (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-ceph-3-em-4-carboxylate pentahydrate. | 60 |
| | 2. A process for the preparation of the pentahydrate according to claim 1 which comprises adjusting the pH of a solution of an acid or base salt of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)-ceph-3-em-4-carboxylate in an aqueous medium to 2.7 to 4.8 | 65 |



(19)

and crystallising the desired pentahydrate.

3. A process according to claim 2 wherein an acid salt of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate is used and the pH adjusted to 3.0 to 4.0.

5 4. A process according to claim 2 wherein a base salt of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate is used and the pH adjusted to 3.5 to 4.2.

5. A process for the preparation of the pentahydrate according to claim 1 which comprises adjusting the pH of a solution of an acid or base salt of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate in an aqueous medium to 3.3 to 4.0 and crystallising the desired pentahydrate.

6. A pharmaceutical composition which comprises as active ingredient (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate in association with a pharmaceutical carrier or excipient.

15 7. A process according to any one of claims 2 to 5 substantially as herein described.

8. A process for the preparation of (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate substantially as herein described in any one of Examples 1 to 18.

9. A composition according to claim 6 substantially as herein described.

20 10. A pharmaceutical composition which comprises as active ingredient (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(2-carboxyprop-2-oxyimino)acetamido]-3-(1-pyridiniummethyl)ceph-3-em-4-carboxylate pentahydrate substantially as herein described in any one of Formulations A to D.