

SPECIFICATION

A crystalline salt of a cephalosporin derivative

5 This invention relates to a crystalline salt of a cephalosporin derivative. 5

The invention relates, in one aspect, to a crystalline hemi-acid salt comprising one molecule of 7 β -[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid [hereinafter referred to as Compound (I)] and a half-molecule of HCl or HBr, and, in another aspect, to a method of producing the said hemi-acid salt.

10 The compound (I) is a highly antibiotic compound [Ochiai, Aki, Morimoto, Okada and Matsushita: Chemical and Pharmaceutical Bulletin 25, 3115 (1977)]. This compound and other cephalosporin derivatives in general are unstable against heat, light, acid, alkali and so on, and it is generally accepted that the utmost care must be taken in handling (isolating, purifying, storing etc.) these compounds. 10

We have found that when Compound (I) is reacted with HCl or HBr, a crystalline hemi-acid salt comprising 15 one molecule of Compound (I) and a half-molecule of HCl or HBr is unexpectedly obtained, and that the crystalline hemi-acid salt thus formed is endowed with a high stability. This invention has been conceived and developed on the basis of the latter findings. 15

The crystalline hemi-acid salt comprising one molecule of Compound (I) and a half-molecule of HCl or HBr is produced by reacting Compound (I), or a salt or an ester thereof, with HCl or HBr.

20 The Compound (I) may be used as it is, i.e. in the free form, or after conversion by a routine means into the form of a salt, for example with an alkali metal (e.g. sodium, potassium, lithium, etc.) an alkaline earth metal (e.g. calcium, magnesium, etc.), an organic base (e.g. triethylamine, diethylamine, etc.), or an easily cleavable ester (e.g. silyl ester, etc.). The sodium salt is particularly expedient to use. The other starting material HCl or HBr, may be used in gaseous form but, usually, is more conveniently used in a solution in a 25 solvent such as water. Thus, hydrochloric acid and hydrobromic acid are usually employed. The reaction is generally conducted in a solvent. The most preferred solvent is water, but a mixture of water and one or more organic solvents may likewise be employed. The organic solvents may for example be C₁₋₅ alcohols such as methanol, ethanol, *n*-propanol, isopropanol, etc.; C₃₋₉ ketones such as acetone, methyl ethyl ketone, etc.; ethers such as tetrahydrofuran, dioxane etc.; acetonitrile; and other water-soluble solvents. Ethanol and acetone are particularly preferred. The proportion of HCl or HBr depends on the form of the other 30 starting material, the type of solvent, etc. but is at least a half mole relative to one mole of Compound (I), preferably up to 10 moles per mole of Compound (I). Thus, when Compound (I) is employed in its free form, HCl or HBr is used in a proportion of 0.5 to 10 moles, preferably 1 to 5 moles, relative to one mole of Compound (I). When an alkali metal salt, an alkaline earth metal salt or an organic base salt of Compound (I) 35 is employed, it is preferable to employ an additional 1 to 2 moles of HCl or HBr in order to convert such salt to the free form. The reaction is carried out usually at 0 to 40°C and preferably at 10 to 30°C. The reaction time should vary with the purity of the starting materials and the kind of impurities. When the purity of Compound (I), or a salt or an ester thereof, is for example 80 percent or above, the reaction time is from 30 minutes to 24 hours, and preferably from 30 minutes to 16 hours. The reaction product can be isolated, for example by 40 such procedures as filtration or centrifuging. Depending on the proportion of HCl or HBr and the kind or proportion of solvent used, there are cases in which the crystalline salt obtained comprises one molecule of Compound (I) and one molecule of HCl or HBr. In such cases, the desired product can be easily obtained by contacting the crystalline salt with water, for example, by washing it with water or a mixture of water and one or more organic solvents. More particularly, the crystalline salt comprising one molecule of Compound 45 (I) and one molecule of HCl or HBr, is washed with 5 to 50 times as much volume of water or a mixture of water and one or more organic solvents, or allowed to stand or stirred in a similar amount of water or a mixture of water and one or more organic solvents at 0 to 30°C for from 5 minutes to 2 hours to obtain the desired compound. It is thought that, since the crystalline salt comprising one molecule of Compound (I) and one molecule of HCl or HBr is a salt of a weak base contacting the salt with an excess of water or a mixture of 50 water and one or more organic solvents may result in a dissociation of the salt into Compound (I) and HCl or HBr, followed by a further transformation into the desired stable compound. 50

The resulting crystalline hemi-acid salt comprising one molecule of Compound (I) and a half-molecule of HCl or HBr may contain water or/and organic solvent as a solvent of crystallization or as an adherent solvent, and such salt also falls within the scope of this invention. The amount of water thus contained is 55 usually not more than 3 percent but the less the water thus contained, the higher is the stability of the crystals. The water content can be controlled by drying the crystals in air or by using a desiccant such as silica gel or phosphorus pentoxide under reduced pressure. The preferred water content is 0.05 to 1 percent and, for better results, from 0.1 to 0.5 percent. When one or more organic solvents are present, they may be removed if desired by bringing a humid gas or air into contact with the crystals in order to remove such 60 solvents. 60

The contemplated compound thus obtained has been confirmed by elemental analysis to comprise one molecule of Compound (I) and a half-molecule of HCl or HBr, and by microscopic or polarizing-microscopic observation, X-ray diffraction and the like to be a crystalline substance. Moreover, the infrared absorption spectrum of the crystals shows sharp peaks which are not observed for amorphous powder. This crystalline 65 hemi-acid salt comprising one molecule of Compound (I) and a half-molecule of HCl or HBr is stable, does 65

not change its crystalline form even by drying and besides is sparingly soluble in water, so that it offers many advantages such as a long storage life.

The crystalline hemi-acid salt according to this invention can be administered orally as it is or, alternatively, it can be used parenterally or externally in the form of a solution in distilled water with the aid of a non-toxic alkali metal hydroxide or alkali metal salt, e.g. sodium hydrogen carbonate, sodium carbonate, trisodium phosphate or the like the aqueous solution being adjusted to a desired pH, ion type or ionic strength. For example, an aqueous solution containing 3/4 mole of sodium carbonate per mole of the crystalline hemi-acid salt can be used not only as an external disinfectant or steriliser in the disinfection of surgical equipment, sickrooms, drinking water, etc., but also as a therapeutic agent in the following and other applications. Thus, for the purpose of treating infectious diseases caused by gram-positive bacteria (e.g. *Staphylococcus aureus*, etc.) or gram-negative bacteria (e.g. *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus morganii*, etc.) in warm-blooded animals such as man, mouse, rat or dog, an aqueous solution prepared as above can be administered intramuscularly or intravenously. When such an aqueous solution as above is used as an external disinfectant for surgical instruments, it is prepared to contain 100γ as solvent-free product per milliliter and applied to the surgical instruments. For the treatment of urinary tract infections resulting from *Escherichia coli* in man or mouse, the aqueous solution may be administered by the intravenous route in a daily dose of about 2.5 to 25 mg/kg as a solvent-free product in 3 divided doses daily. Thus, the crystalline hemi-acid salt comprising Compound (I) and HCl or HBr and the non-toxic alkali metal hydroxide or alkali metal salt may be stored, independently or in admixture, under an inert gas or under reduced pressure in a sealed container. These products are extemporaneously dissolved in sterile distilled water or an equivalent thereof.

The following Reference Examples and Examples are intended to illustrate this invention in further detail and should by no means be construed as limiting the scope of the invention. In this specification, the following abbreviations are used: microgram = mcg, milligram = mg, gram = g, milliliter = ml, liter = l, percent = %, minute = min, parts per million = ppm, Kilovolt = KV, millampere = mA, Angstrom = Å, Hertz = Hz, Megahertz = MHz, dimethylsulfoxide = DMSO, Figure = Fig, Infrared spectrum = IR, Nuclear Magnetic Resonance spectrum = NMR, Karl-Fischer Method = K.F.

Reference is also made to the accompanying drawings in which Figure 1 is a graph of "intensity of diffraction" as a function of "angle of diffraction" and Figure 2 is a graph of "percentage transmission(%)" as a fraction of "wave number (cm⁻¹)".

Reference Example 1

Antimicrobial potency of the contemplated product as solvent-free form (Minimum Inhibitory Concentrations)

Antimicrobial spectrum (agar dilution)

Staphylococcus aureus FDA 209P:

1.56 mcg/ml

Staphylococcus aureus 1840:

3.13 mcg/ml

Escherichia coli NIHJ-2:

0.1 mcg/ml

Escherichia coli 0-111:

0.024 mcg/ml

Escherichia coli T-7:

0.39 mcg/ml

Klebsiella pneumoniae DT:

0.024 mcg/ml

Proteus vulgaris IFO 3988:

0.024 mcg/ml

Proteus morganii IFO 3168:

0.1 mcg/ml

Proteus mirabilis GN4359:

0.05 mcg/ml

Proteus rettgeri TN336:

≤ 0.012 mcg/ml

Citrobacter freundii GN1706:

0.20 mcg/ml

Reference Example 2

250 grams (potency) of the crystalline hemi-acid salt (composition: 1 mole of Compound (I) and 1/2 mole of HCl) and 42.7 g of sterile pure sodium carbonate are aseptically admixed and 50 mg (potency) aliquots of the mixture are filled into sterile vials of 12 ml capacity, which are then vacuum-sealed at 50 mmHg. The contents of each vial are dissolved by the addition of 2 ml of distilled water to prepare an injectable solution.

Reference Example 3.

500 grams (potency) of the crystalline hemi-acid salt (composition: 1 mole of Compound (I) and 1/2 mole of HCl) and 116.6 g of sterile pure sodium carbonate and 1,000 mg (potency) aliquots are dispensed into sterile vials of 28 ml capacity which are then vacuum-sealed at 50 mmHg. The contents of each vial are dissolved by the addition of 2 ml of distilled water to prepare an injectable solution.

It is considered that the crystalline hemi-acid salt comprising one molecule of Compound (I) and 1/2 molecule of HCl or HBr may assume tautomeric structures with respect to its 2-aminothiazol-4-yl group, i.e. the 2-aminothiazole form and the 2-iminothiazoline form. While this invention encompasses both of these tautomers, the 2-aminothiazole form is indicated in all references to this compound in this specification.

Example 1

In 3.3l of water there are dissolved 165.0 g of sodium salt of 7β-[2-aminothiazol-4-yl]-(Z)-2-methoxyiminoacetamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, and, while the solution is stirred at 25.5°C 0.3l of a 10% aqueous solution of HCl is added dropwise. In the course of the dropwise addition, the above-mentioned carboxylic acid separates out as a colourless precipitate and, then, dissolves. As this solution is stirred as it is for one hour, colourless crystals are formed. The crystals are collected by filtration (pH of filtrate 1.1), washed with water, drained well, dried over silica gel (blue) at 25°C in a desiccator evacuated with a vacuum pump (about 1 mmHg) for 5 hours, and allowed to stand for 24 hours under reduced pressure, whereby 154.5 g of the crystals of the said carboxylic acid-1/2 HCl are obtained.

Elemental analysis (C₁₆H₁₇N₉O₅S₃ · 1/2 HCl · %H₂O)

Calcd.: C, 35.67; H, 3.46; N, 23.40; S, 17.85; Cl, 3.29

Found : C, 35.78; H, 3.41; N, 23.46; S, 17.88; Cl, 3.46

Water content (K.F.): 2.1% (calcd. 1.7%)

NMR (90 MHz, DMSO-d₆)δ : 3.54, 3.79 (each 1H, doublet, J=18Hz), 3.89(3H, singlet), 3.91(3H, singlet), 4.18, 4.36 (each 1H, doublet, J=13Hz), 5.09 (each 1H, doublet, J=5Hz), 5.71(1H, quartet, J=5, 9Hz), 6.80(1H, singlet), 9.65(1H, doublet, J=9Hz).

IR(KBr)cm⁻¹: 1780(β-lactam) (see Figure 2 of the accompanying drawings)

The powder X-ray diffraction pattern (see Figure 1 of the accompanying drawings; CuKα, 40KV, 45mA) of this product attests to its crystallinity, with interplanar spacings being 9.1Å, 6.7Å, 6.5Å, 5.0Å, 4.1Å, 3.9Å, 3.7Å, 3.5Å, 3.4Å, 3.3Å, 3.2Å.

A portion of the above product is dried over P₂O₅ at 25°C in a desiccator evacuated with a vacuum pump (about 1 mm Hg) for 5 hours, and allowed to stand for 16 hours under reduced pressure, whereby the water content is reduced to 0.3% (K.F.). The powder X-ray diffraction pattern and the IR value of this compound are in agreement with the pattern and spectrum of the above-mentioned compound having a water content of 2.1%. The undecomposed portion of this compound after standing at 40°C for a month was not less than 98%.

Example 2

In 18 ml of water there is suspended 1.00 g of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, and, under stirring at 25°C, 2 ml of a 10% aqueous solution of HCl are added. The resulting solution is stirred at that temperature for 30 minutes, whereupon colourless crystals separate out. The crystals are collected by filtration, washed with water and dried in air. 0.73 g of the said carboxylic acid-1/2 HCl salt [water content 3.2% (K.F.)] is obtained by the above procedure.

In powder X-ray diffraction pattern and IR values, this compound is in agreement with the compound according to Example 1.

Example 3

0.50 g of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid is dissolved in a mixture of 5 ml of acetone and 1 ml of water. While the solution is stirred at 23°C, 0.15 ml of a 10% aqueous solution of HCl is added. The mixture is stirred as it is for 1.5 hours, and the resulting colourless crystals are collected by filtration, washed with acetone and dried over P₂O₅ at room temperature and under reduced pressure (about 1 mmHg) for about 3 hours. By the above procedure there is obtained 0.23 g of the said carboxylic acid-1/2 HCl salt [water content 1.8% (K.F.)].

Elemental analysis (C₁₆H₁₇N₉O₅S₃ · 1/2 HCl · 1/2 H₂O · 1/6 (CH₃)₂CO

Calcd. : C, 36.13; H, 3.58; N, 22.98; S, 17.53; Cl, 3.23

Found : C, 36.51; H, 3.41; N, 23.35; S, 17.51; Cl, 2.81

The NMR value of this product shows that it is solvated with 1/6th mol of acetone. The powder X-ray diffraction pattern and the IR values of the above compound are in agreement with the pattern and spectrum of the compound obtained in Example 1.

Example 4

0.50 g of the sodium salt of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid is dissolved in 10 ml of water. While the solution is stirred at 23°C, 0.3 ml of a 48% aqueous solution of HBr is added. The resinous substance which has been

obtained in a small amount is separated off and the remaining solution is stirred at 23°C for 2 hours, whereby colourless crystals separate out. The crystals are collected by filtration, washed with water and dried over P₂O₅ at 23°C under reduced pressure (about 1 mmHg) for about 10 hours. 0.27 g of the said carboxylic acid-1/2 HBr [water content 0.3% (K.F.)] is obtained by the above procedure.

- 5 Elemental analysis (C₁₆H₁₇N₅O₅S₃·1/2HBr) 5
 Calcd: C, 34.81; H, 3.20; N, 22.84; Br, 7.24
 Found: C, 34.71; H, 3.28; N, 22.82; Br, 6.90
 IR (KBr)cm⁻¹: 1780 (β-lactam)

The powder X-ray diffraction pattern of this product attests to its crystallinity.

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Example 5

In 20 ml of water there is suspended 0.50 g of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, and, while the suspension is stirred at 23°C, 0.4 ml of a 48% aqueous solution of HBr is added. A small amount of insoluble material is filtered off and the filtrate is stirred at 23°C for 16 hours. The resulting colourless crystals are collected by filtration, washed with water and dried over P₂O₅ at 23°C under reduced pressure (about 1 mmHg) for about 7 hours. 0.40 g of the said carboxylic acid-1/2 HBr [water content 0.5% (K.F.)] is obtained by the above procedure.

Elemental analysis (C₁₆H₁₇N₅O₅S₃·1/2HBr)

Calcd: C, 34.81; H, 3.20; N, 22.84; Br, 7.24

- 20 Found: C, 34.46; H, 3.31; N, 22.60; Br, 7.39 20

In powder X-ray diffraction pattern and IR, this product is identical with the compound obtained in Example 4.

Example 6

- 25 (1) In 720 ml of acetone there are suspended 78.5 g of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, and, under cooling at 5°C and stirring, 72 ml of water are added. The solution thus obtained is filtered through Celite (Trade Mark) and washed with a mixture of acetone (65 ml) and water (3 ml). The filtrate and washings are combined and, under cooling at 5°C and stirring, 23 ml of 12N HCl are added. The mixture is stirred for 1.5 hours and after 30 removal of the cooling equipment, stirred further for 1.5 hours. The resulting colourless crystals are collected 30 with a glass filter and washed with 500 ml of acetone. A humid nitrogen gas, which is prepared by passing gaseous nitrogen through a bottle containing water, is passed over the above crystals at the rate of 1.9 l/min. to 3.3 l/min. for 10 hours. The crystals (water content 8.9% as measured for a sample by K.F.) were dried over silica gel (blue) in a desiccator under reduced pressure. By the above procedure there are 35 obtained 65.0 g of the said carboxylic acid-monohydro-chloride. 35

Elemental analysis (C₁₆H₁₇N₅O₅S₃·HCl·1.5H₂O)

Calcd.: C, 33.42; H, 3.68; N, 21.92; S, 16.73; Cl, 6.17

Found: C, 33.59; H, 3.56; N, 22.16; S, 16.79; Cl, 5.94

This product contains 4.6% of water (K.F., calcd. 4.7%) and 110 ppm of acetone.

- 40 NMR (90 MHz, DMSO-d₆)δ: 3.57, 3.81 (each 1H, doublet, J=18Hz), 3.93(6H, singlet), 4.19, 4.38 (each 1H, doublet, J=13Hz), 5.12(1H, doublet, J=5Hz), 5.73(1H, Quartet, J=5, 9Hz), 6.90(1H, singlet), 9.78(1H, doublet, J=5Hz). IR(KBr)cm⁻¹: 1770 (β-lactam) 40

The powder X-ray diffraction pattern of this product attests to its crystallinity.

- 45 (2) In 2 ml of water there is suspended 0.20 g of the carboxylic acid-monohydrochloride obtained at (1) 45 above and the suspension is stirred at 26°C for 10 minutes (in the course of stirring, a change is observed in the crystal form). The resulting crystals are collected by filtration, washed with water and dried over P₂O₅ under reduced pressure (above 1 mmHg) for about 5 hours. 0.17 g of the said carboxylic acid-1/2 HCl [water content 0.9% (K.F.)] is obtained by the above procedure.

Elemental analysis (C₁₆H₁₇N₅O₅S₃·1/2HCl·1/4H₂O)

- 50 Calcd: C, 35.97; H, 3.40; N, 23.59; Cl, 3.32 50

Found: C, 35.95; H, 3.11; N, 23.49; Cl, 3.08

In powder X-ray diffraction and IR values, this product is identical with the compound obtained in Example 1.

55 CLAIMS 55

1. A crystalline hemi-acid salt comprising one molecule of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid and a half-molecule of HCl or HBr.
- 60 2. A process for producing a crystalline hemi-acid salt comprising one molecule of 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid and a half-molecule of HCl or HBr, which comprises reacting 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, or a salt or an ester thereof, with HCl or HBr.
- 65 3. A process according to Claim 2, wherein HCl or HBr is used in a proportion of from 0.5 to 10 moles per 65



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mole of 7 β -[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid.

4. A process according to Claim 2, wherein the crystalline hemi-acid salt is collected from an aqueous solvent solution thereof.
- 5 5. A process according to Claim 4, wherein the aqueous solvent is water. 5
6. An antibiotic composition which comprises a crystalline hemi-acid salt as defined in Claim 1 and a non-toxic alkali metal hydroxide or alkali metal salt.
7. An antibiotic composition according to Claim 6, wherein the non-toxic alkali metal salt is sodium te, carbonate, sodium hydrogen carbonate or trisodium phosphate.
- 10 8. An antibiotic composition according to Claim 6, wherein the composition is stored in a sealed container. 10
9. An antibiotic composition according to Claim 8, wherein the sealed container is a vacuum-sealed vial.
10. A process according to any of Claims 2 to 5 substantially as herein described with reference to any of the specific examples.