

Stability and Degradation Pattern of Cefpirome (HR 810) in Aqueous Solution

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The stability and degradation pathways of a new semi-synthetic cephalosporin, 1-[[[(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*Z*)-(O-methyloxime) sulfate (cefpirome sulfate, HR 810), were studied.

Cefpirome in various buffer solutions was allowed to stand at 40 °C and its degradation patterns were investigated by high performance liquid chromatography. Cefpirome was stable in the region of pH 4—7 and slightly unstable beyond this range.

In aqueous solution from the neutral to alkaline regions, the produced degradation products were: 1-[[[(6*R*,7*S*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*Z*)-(O-methyloxime) (epi-cefpirome); 1-[[[(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*Z*)-(O-methyloxime) (*A*₂-cefpirome); 2-[[[(2-amino-4-thiazolyl)((*Z*)-methoxyimino)acetyl]amino]acetaldehyde; and 6,7-dihydro-5*H*-1-pyridine. On the other hand, 1-[[[(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*E*)-(O-methyloxime) (*anti*-cefpirome), 2-[[[(2-amino-4-thiazolyl)-((*Z*)-methoxyimino)acetyl]aminomethyl]-1,2,5,7-tetrahydro-7-oxo-4*H*-furo[3,4-*d*]-[1,3]thiazine, and 6,7-dihydro-5*H*-1-pyridine were produced in strongly acidic solution or under irradiation by artificial sunlight.

Keywords cephalosporin; cefpirome; HR 810; stability; degradation pattern; aqueous solution

1-[[[(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*Z*)-(O-methyloxime), sulfate (cefpirome sulfate, HR 810, hereinafter referred to as cefpirome, Chart 1)¹⁾ is a novel, semisynthetic cephalosporin antibiotic codeveloped as parenteral use by Hoechst AG (FRG) and Roussel Uclaf (France).

Cefpirome possesses potent antibacterial activities against gram-positive microorganisms including *Staphylococcus aureus* and gram-negative microorganisms such as *Pseudomonas aeruginosa* and has a very broad antibacterial spectrum.²⁾

The present paper reports the stability and degradation pattern of cefpirome in aqueous solution and the chemical structure of its degradation products.

Stability of Cefpirome in Aqueous Solution Aqueous cefpirome solutions (1%, pH 1—9; ionic strength, 0.1) were stored at 40 °C and the residual cefpirome amount in each solution was determined in course of time by high performance liquid chromatography (HPLC, method A) to calculate the degradation rate. In the pH region of 1—9, a linear relationship was obtained between the logarithms of the residual percent and time, indicating that cefpirome, as well as penicillins and the other cephalosporins,³⁾ appeared to be degraded in aqueous solution according to

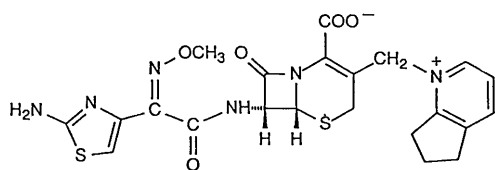


Chart 1. Structure of Cefpirome (HR 810)

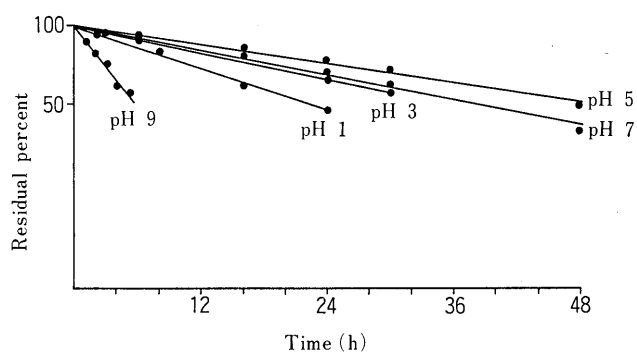


Fig. 1. Plots of Observed Pseudo-First-Order Kinetic Degradation of Cefpirome at Various pH Values, 40 °C, and Ionic Strength of 0.1

pH 1 (0.1 N HCl), pH 3 (formic acid–sodium formate), pH 5 (acetic acid–sodium acetate), pH 7 (monobasic potassium phosphate–dibasic sodium phosphate), pH 9 (sodium bicarbonate–sodium carbonate).

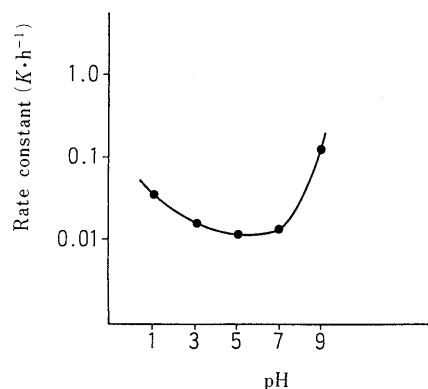


Fig. 2. log *K*-pH Profile of Degradation of Cefpirome in Aqueous Solution at 40 °C and Ionic Strength of 0.1

pH 1 (0.1 N HCl), pH 3 (formic acid–sodium formate), pH 5 (acetic acid–sodium acetate), pH 7 (monobasic potassium phosphate–dibasic sodium phosphate), pH 9 (sodium bicarbonate–sodium carbonate).

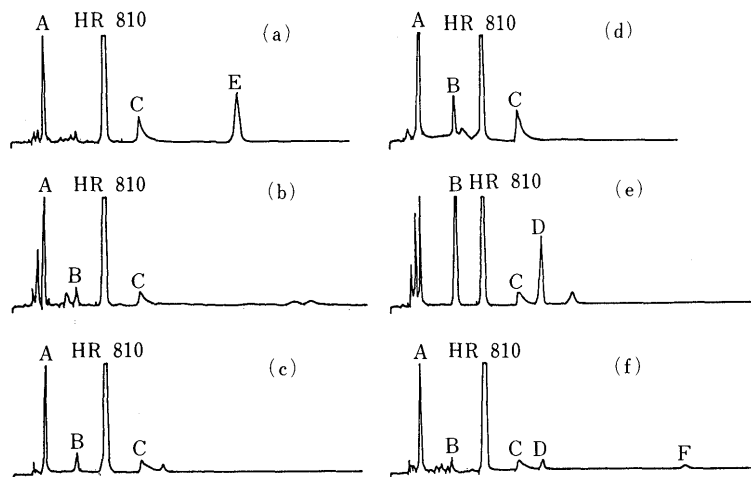


Fig. 3. High Performance Liquid Chromatograms of Degraded Cefpirome in Aqueous Solution of Different pH Values

pH 1 (0.5N HCl), pH 3–8 (McIlvaine buffer solution), pH 13 (0.5N NaOH). HPLC (method B): column, Nucleosil 10C₁₈ 4.0 × 300 mm; mobile phase, 1% KH₂PO₄:CH₃CN=8:1; flow rate, 1 ml/min; injection volume, 20 μl. (a) pH 1, 40°C, 24 h. (b) pH 3, 40°C, 24 h. (c) pH 7, 40°C, 24 h. (d) pH 8, 40°C, 24 h. (e) pH 13, room temp., 5 min. (f) pH 7, 30°C, artificial sunlight, 24 h.

pseudo-first-order kinetics (Fig. 1). The pH–rate profile of cefpirome degradation is shown in Fig. 2. In the buffer solutions used in the present test, cefpirome as well as the other cephalosporins was stable at pH 4–7, slightly unstable at pH 3 or lower and promptly degraded at pH 9 or higher.

Degradation Pattern of Cefpirome in Aqueous Solution
Aqueous cefpirome solutions (1%) of various pH values were allowed to stand at room temperature (pH 13 solution), at 30°C under artificial sunlight (pH 7) and at 40°C (pH's 1, 3, 7 and 8), and the individual aged solutions were subjected to HPLC (method B) in course of time. The results of HPLC are shown in Fig. 3 and the main degradation products under the individual conditions are listed in Table I. Degradation product A was formed under all conditions except a strongly alkaline region and found to be a main degradation product from its integrated peak area. Degradation product B was formed in weakly acidic to strongly alkaline regions and increased in amount as the alkalinity was raised. Degradation product D was formed at pH 7 (40°C, 24 h) and pH 13 (room temperature, 5 min) but hardly detected at pH 8 (40°C, 24 h). However, degradation product D was considered to be formed mainly under alkaline conditions and less stable than other degradation products such as degradation product B because it was detected as a large peak as compared with that of degradation product B at pH 8 (40°C, 8 h). Degradation product C was detected under individual conditions and increased in amount in course of time. Degradation product E was found only under strongly acidic conditions. Degradation product F was detected in very trace specifically under artificial sunlight.

Then, identification of the chemical structures of these 6 main degradation products was attempted. Small peaks and those near the tip of the elution were not subjected to structural determination because of low formation amounts, difficulty in purification by HPLC due to the polarity, etc.

Structure of Degradation Products **1) Degradation Product A** It has been reported that aldehyde derivatives are produced as degradation products of cephalosporin antibiotics.⁴⁾ Especially from ceftaram pivoxil which as well

TABLE I. List of Main Degradation Products

Condition	Degradation product (Retention time, min)					
	A (ca. 4)	B (ca. 8)	C (ca. 16)	D (ca. 19)	E (ca. 29)	F (ca. 37)
pH 1 40°C	○	—	○	—	○	—
pH 3 40°C	○	○	○	—	—	—
pH 7 40°C	○	○	○	○	—	—
pH 8 40°C	○	○	○	○	—	—
pH 13 Room temperature	—	○	○	○	—	—
pH 7 Artificial sunlight	○	○	○	○	—	○

○: formed. —: not formed.

as cefpirome has the same side chain at the 7 position, an aldehyde compound is isolated and identified as a dinitrophenylhydrazine derivative.⁵⁾ Then, with the expectation that cefpirome also yields a similar aldehyde compound, degradation product A was subjected to isolation and derivatization according to the method in the literature. The proton nuclear magnetic resonance (¹H-NMR), infrared (IR) absorption and mass spectra of degradation product A-dinitrophenylhydrazine derivative were identical with those in the literature, so degradation product A was determined to be 2-[[[(2-amino-4-thiazolyl) ((Z)-methoxyimino)acetyl]amino]acetoaldehyde. Furthermore, degradation product A was confirmed to be produced also by the hydrolysis of (Z)-N-(2-diethoxyethyl)-2-(2-formaminothiazol-4-yl)-2-methoxyimino acetamide (I) synthesized separately (Chart 2).

2) Degradation Product B Degradation product B was estimated to be an isomer of cefpirome since its fast atom bombardment mass spectrum (FAB-MS) showed the same molecular ion peak as that of cefpirome. When compared with ¹H-NMR spectrum of cefpirome, 6- and 7-positioned protons at δ 5.28 and δ 5.87 were shifted to δ 5.41 and δ

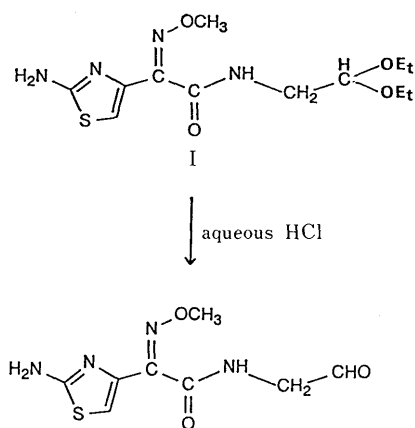


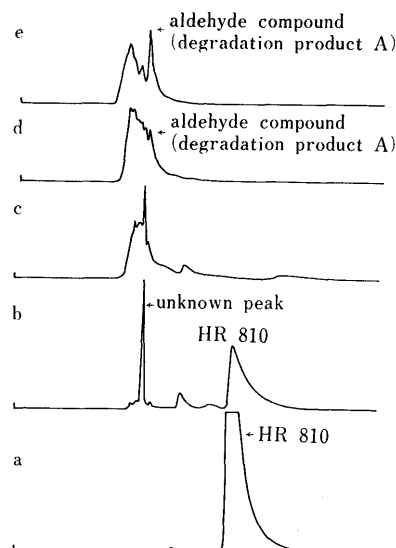
Chart 2. Synthesis of Degradation Product A

5.50 in the spectrum of degradation product B. Signals due to the 2-positioned methylene proton at δ 3.31 and δ 3.57 disappeared and new signals were seen at δ 4.61 and δ 6.54. Therefore, degradation product B was identified as 1-[[[(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*Z*)-(O-methyloxime) (Δ_2 -cefpime) in which the double bond at C-3 of cefpirome had been isomerized at C-2. Furthermore the ¹H-NMR and HPLC data of degradation product B were identical with those of the authentic compound synthesized separately.

3) Degradation Product C Degradation product C was formed under all the severe conditions, especially basic conditions. Cefpirome was degraded as time went on and the degraded cefpirome solution evolved an odor specific to pyridine, suggesting release of 2,3-cyclopentenopyridine at the C-3 side chain of cefpirome. The ¹H-NMR and IR spectra of the separated oily material were identical with those of the authentic compound, 6,7-dihydro-5*H*-1-pyridine (2,3-cyclopentenopyridine).

4) Degradation Product D Since degradation product D exhibited the same molecular ion peak as that of cefpirome in FAB-mass spectrometry, it was estimated to be an isomer of cefpirome. When compared with the ¹H-NMR spectrum of cefpirome, protons at the 6- and 7-positions shifted upward from δ 5.28 and δ 5.87 to δ 5.06 and δ 5.12, respectively, in that of degradation product D, and the coupling constant also changed from 4.9 to 1.8 Hz. These changes were considered to be caused by differences in the chemical shift and coupling constant between α - and β -isomers of the substituent at the 7-position.⁶⁾ As this was also supported by a carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum, degradation product D was determined to be 1-[[[(6*R*,7*S*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*Z*)-(O-methyloxime) (*epi*-cefpime) having an epimer at the 7-position of cefpirome. Degradation product D was also confirmed to be an isomer of cefpirome since retreatment of isolated degradation products B and D with 0.1*N* NaOH caused mutual conversion to cefpirome, degradation product B and degradation product D.

5) Degradation Product E Degradation product E was

Fig. 4. High Performance Liquid Chromatograms of Degraded Cefpirome in β -Lactamase Solution

HPLC (method C): column, TSKgel 2000 SW; mobile phase, 0.5% NaCl; flow rate, 1 ml/min; injection volume, 20 μ l. a, initial; b, 1 h; c, 4 h; d, 8 h; e, 24 h.

formed under strongly acidic conditions. In the IR spectrum of degradation product E, an absorption band from the β -lactam ring disappeared and at 1738 cm^{-1} an absorption band probably from lactone was observed. The electron impact mass spectrum (EI-MS) revealed that degradation product E has a molecular weight smaller than that of cefpirome by 145. In the ¹H-NMR spectrum, a signal due to 2,3-cyclopentenopyridine at the 3-position side chain of cefpirome was not seen and a proton supposed to be from the amino group at δ 5.97 and methylene protons at δ 3.50, δ 3.57 and δ 4.83 were observed. These spectral data indicated that degradation product E was 2-[[[(2-amino-4-thiazolyl) ((*Z*)-methoxyimino)acetyl]aminomethyl]-1,2,5,7-tetrahydro-7-oxo-4*H*-furo[3,4-*d*] [1,3]thiazine in which the 3-position side chain had been eliminated and lactonized and the β -lactam ring had been decarbonized by hydrolysis.

6) Degradation Product F Showing the same ion peak as cefpirome in the FAB-MS, degradation product F was supposed to be an isomer of cefpirome. When the ¹H-NMR spectrum of degradation product F was compared with that of cefpirome, there was no difference except that a proton at the 5-position of thiazol was largely shifted to the lower field from δ 7.13 to δ 7.53. This spectral change was considered to have originated from the *syn-anti* isomerization of the methoxyimino group.⁷⁾ Degradation product F was identified as an *anti*-isomer of cefpirome, 1-[[[(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-6,7-dihydro-5*H*-1-pyridinium hydroxide, inner salt, 7²-(*E*)-(O-methyloxime) (*anti*-cefpime). The spectral data of degradation product F corresponded to those of the authentic compound synthesized separately.

Estimated Degradation Pathway of Cefpirome Cefpirome was allowed to react with β -lactamase and the degradation of cefpirome was followed by HPLC (method C) using a gel column. The results are given in Fig. 4. With the gel column, 2,3-cyclopentenopyridine was not eluted. However, the analysis of the same solution with an ODS

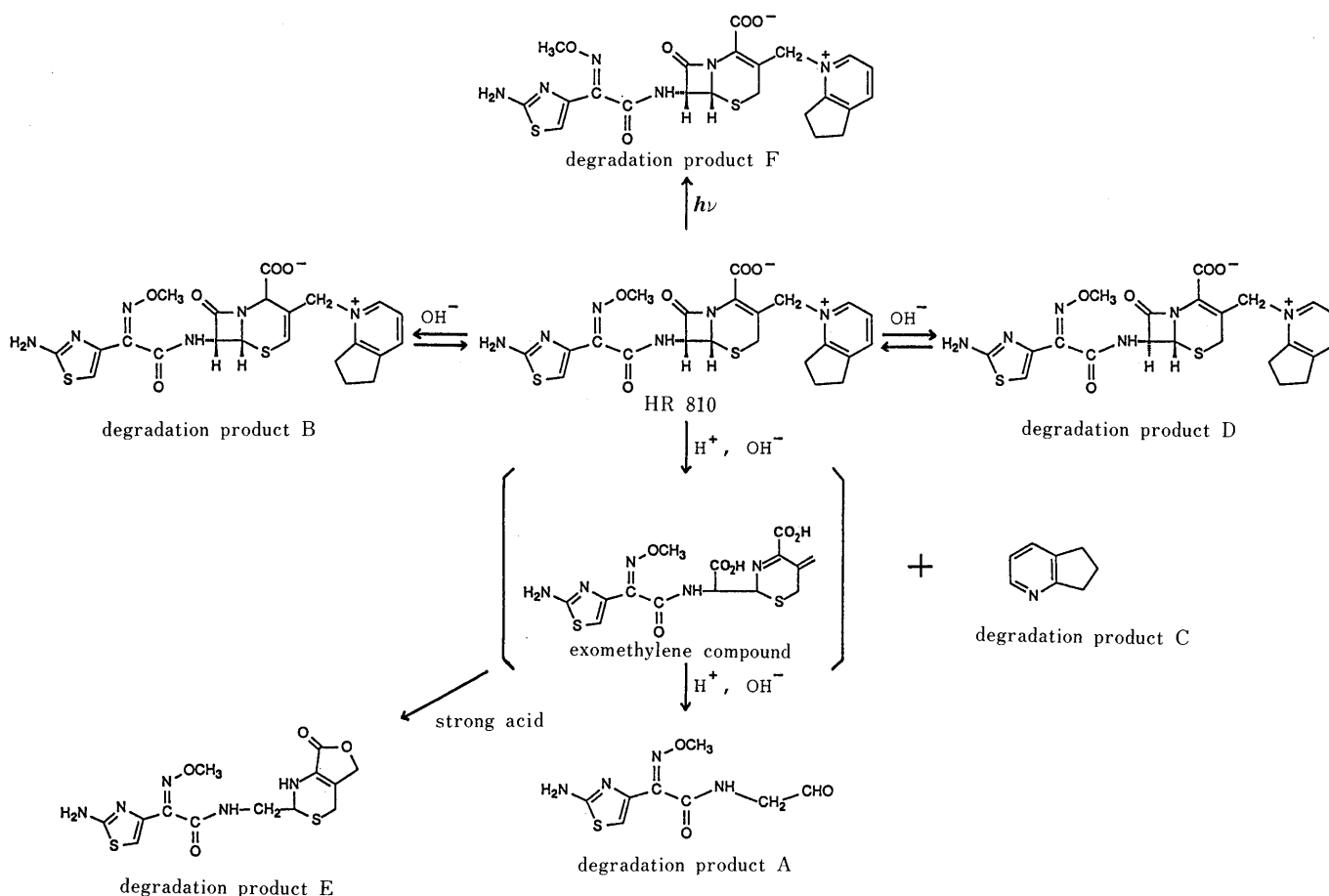


Chart 3. Estimated Degradation Pathway of Cefpirome

column indicated that cefpirome had been decomposed by β -lactamase to give an unknown peak and a peak from 2,3-cyclopentenopyridine on the chromatogram. The unknown peak in Fig. 4 degraded in a complicated manner over the course of time to form an aldehyde compound, a main degradation product of cefpirome. These time-course changes were similar to the degradation behavior of cefpirome in aqueous solution. The structure of this unknown peak could not be identified due to its instability (very labile in the acidic region) but was estimated to be a compound having an exomethylene structure. Reportedly cephalosporin antibiotics having a substituent with higher elimination ability at the 3-position form a degradation product, so-called exomethylene compound, by cleavage of the β -lactam ring.⁸⁾ Therefore, cefpirome having the 3-positioned 2,3-cyclopentenopyridine group of high elimination ability was assumed to undergo skeletal decomposition initiated by the cleavage of a β -lactam ring. The degradation pathway of cefpirome estimated from the above-mentioned considerations is shown in Chart 3. Mainly under alkaline conditions, cefpirome is degraded to form degradation product B in which the double bond of the cephem structure has been isomerized from Δ_3 to Δ_2 and degradation product D having an epimer at the 7-position. Under artificial sunlight isomerization of the methoxyimino group occurs to give degradation product F. In weakly alkaline to weakly acidic regions cefpirome is supposed to be degraded mainly by β -lactam ring cleavage to give degradation product C and an exomethylene

compound, the latter of which then forms degradation product A during the process of degradation. Under strongly acidic conditions, the exomethylene compound is supposed to produce degradation product E.

Polymers produced in aged cephalosporin antibiotics are estimated to be formed in the process of complicated degradation of exomethylene compounds.

Experimental

Cefpirome used had been synthesized by Hoechst AG (FRF). All the reagents used were of analytical grade. NMR spectra were recorded on a JEOL FX-90Q spectrometer or a JEOL JNM-GX400 spectrometer. The other apparatuses used were a Hitachi 285 IR spectrophotometer, a Hitachi 340 ultraviolet (UV) spectrophotometer, a JEOL DX-300 mass spectrometer and a Toa Electronics HM-18E pH meter.

HPLC HPLC was done on a JASCO SR-II instrument equipped with a UVIDEK 100-V UV detector and a Hewlett Packard HP 3380A integrator. Method A: Column, Nucleosil 5C₁₈ 4.6 i.d. \times 150 mm; eluent, mixture of 0.2% ammonium acetate (adjusted to pH 4.65 with acetic acid) and acetonitrile (7:1); flow rate, 1.5 ml/min; injection volume, 20 μ l. Method B: Column, Nucleosil 10C₁₈ 4.6 i.d. \times 300 mm; eluent, mixture of 1% monobasic potassium phosphate and acetonitrile (8:1); flow rate, 1.0 ml/min; injection volume, 20 μ l. Method C: Column, TSK gel 2000SW 7.5 i.d. \times 600 mm; eluent, 0.5% sodium chloride solution; flow rate, 1 ml/min; injection volume, 20 μ l.

Stability of Cefpirome in Aqueous Solution Cefpirome was dissolved in the respective buffer solutions to a concentration of 1%, allowed to stand at 40 $^\circ$ C, and subjected to HPLC (method A). The buffer solutions were 0.1 in ionic strength and included 0.1 N HCl (pH 1), formic acid-sodium formate (pH 3), acetic acid-sodium acetate (pH 5), monobasic potassium phosphate-dibasic sodium phosphate (pH 7) and sodium bicarbonate-sodium carbonate (pH 9).

Degradation Pattern of Cefpirome in Aqueous Solution Cefpirome was

dissolved in the respective buffer solutions to a concentration of 1%, and solutions of pH's 1, 3, 7 and 8 were stored at 40 °C in a dark room and pH 13 solution was left at room temperature. Separately, pH 7 solution was allowed to stand at 30 °C under artificial sunlight. These solutions were sampled in the course of time and subjected to HPLC (method B). The buffer solutions used were McIlvaine buffer solution (pH 3—8), 0.5 N HCl (pH 1) and 0.5 N NaOH (pH 13). The ionic strength was adjusted to about 0.5 with KCl.

Semi-preparative HPLC HPLC was done on a Waters Associates M-600 multi-solvent system equipped with a Waters Associates 484 UV detector and a Nucleosil 7C₁₈ column (22.2 × 300mm). The operating conditions were: eluent, mixture of water and acetonitrile (8 : 1); flow rate, 10 ml/min; and injection volume, 1 ml.

Decomposition of Cefpirome by β -Lactamase To 2 ml of cefpirome solution in 1% phosphate buffer solution (pH 7.0), 4 ml of crude β -lactamase solution prepared from a clinical isolate (*Bacteroides fragilis*)⁹⁾ was added at room temperature, and the mixture was subjected to HPLC (method C) in the course of time.

Isolation and Purification of Degradation Products **1) Degradation Product A** Cefpirome, 5 g, was dissolved in 0.1 N hydrochloric acid and allowed to stand at 40 °C for 4 d. After the addition of 300 ml of acetone, insoluble material was removed by filtration. The filtrate was injected into a Diaion HP21 column (Mitsubishi Chemical Industries Co., Ltd.) and eluted with 20% aqueous acetone solution. After evaporation of acetone under reduced pressure, the eluate was lyophilized. The residue was dispersed in methanol and insoluble material was removed by filtration. To this methanol solution, dinitrophenylhydrazine was added and the mixture was allowed to react at room temperature for 2 h. From the reaction mixture methanol was evaporated and the residue was washed with aqueous solution of monohydrogen sodium carbonate. Then, the residue was dissolved in methanol and the solution was repeatedly treated with active carbon to give 15 mg of the dinitrophenylhydrazine derivative of degradation product A. IR (KBr): 3470, 3350, 3300, 1680, 1615, 1330 cm⁻¹. ¹H-NMR (90 MHz, DMSO-*d*₆) δ : 3.9 (3H, s, OCH₃), 4.1 (2H, t-like, NHCH₂), 6.8 (1H, s, thiazolyl-C₅-H), 7.2 (2H, br s, NH₂), 7.9 (1H, d, *J* = 6.6 Hz), 8.0 (1H, t-like, CH = N), 8.3 (1H, dd, *J* = 6.6, 1.4 Hz), 8.8 (1H, d, *J* = 1.4 Hz), 8.9 (1H, t-like, NH), 11.4 (1H, br s, NH). FAB-MS *m/z*: 423 (M⁺ + 1).

2) Degradation Product B Cefpirome, 2 g, was dissolved in 50 ml of 0.1 N sodium hydroxide, and the solution was allowed to stand at room temperature for 30 min, adjusted to pH 3—4 with hydrochloric acid, and filtered to remove insoluble material. The filtrate was fractionated by semi-prep. HPLC and, after evaporation of acetonitrile under reduced pressure, lyophilized to give 4 mg of degradation product B. ¹H-NMR (400 MHz, D₂O) δ : 2.30 (2H, m, C₆''-H₂), 3.18 (2H, m, C₅''-H₂), 3.35 (2H, m, C₇''-H₂), 3.98 (3H, s, OCH₃), 4.61 (1H, s, C₄-H), 5.28, 5.39 (2H, ABq, *J* = 15.3 Hz, C₃-CH₂), 5.41 (1H, d, *J* = 2.5 Hz, C₆-H), 5.50 (1H, d, *J* = 2.5 Hz, C₇-H), 6.54 (1H, s, C₂-H), 7.06 (1H, s, C₅'-H), 7.76 (1H, t-like, *J* = 6.3 Hz, C₃'-H), 8.28 (1H, d, *J* = 7.6 Hz, C₄''-H), 8.49 (1H, d, *J* = 5.8 Hz, C₂''-H). ¹³C-NMR (400 MHz, D₂O) δ : 24.85 (t), 33.20 (t), 34.32 (t), 55.68 (d), 55.75 (d), 62.57 (d), 64.60 (t), 65.30 (q), 116.17 (d), 121.27 (s), 126.08 (d), 128.14 (d), 143.12 (s), 143.38 (d), 143.93 (d), 148.95 (s), 150.62 (s), 164.80 (s), 167.24 (s), 167.37 (s), 173.53 (s), 174.71 (s). FAB-MS *m/z*: 515 (M⁺ + 1).

3) Degradation Product C Cefpirome, 0.5 g, was dissolved in 50 ml of 0.1 N sodium hydroxide, and after standing at room temperature for 2 h, the solution was extracted with ethyl acetate. The ethyl acetate layer was treated with active carbon and concentrated under reduced pressure to give 52 mg of oily material, degradation product C. IR (CHCl₃): 2960, 1420 cm⁻¹. ¹H-NMR (90 MHz, CDCl₃) δ : 2.1 (2H, m, C₆-H₂), 2.9 (4H, q, C_{5,7}-H₂ × 2), 7.1 (1H, dd, *J* = 6.7, 4.3 Hz, C₃-H), 7.5 (1H, d, *J* = 6.7 Hz,

C₄-H), 8.3 (1H, d, *J* = 4.3 Hz, C₂-H), EI-MS *m/z*: 119 (M⁺).

4) Degradation Product D Cefpirome was treated in a similar manner to that for degradation product B, and the resultant filtrate was fractionated by semi-prep. HPLC and lyophilized to give 1 mg of degradation product D. ¹H-NMR (400 MHz, D₂O) δ : 2.32 (2H, m, C₆''-H₂), 3.19 (2H, m, C₅''-H₂), 3.37 (2H, m, C₇''-H₂), 3.17, 3.54 (2H, ABq, *J* = 16 Hz, C₂-H₂), 3.97 (3H, s, OCH₃), 5.06 (1H, d, *J* = 1.8 Hz, C₆-H), 5.12 (1H, d, *J* = 1.8 Hz, C₇-H), 5.25, 5.35 (2H, ABq, *J* = 15 Hz, C₃-CH₂), 6.97 (1H, s, C₅'-H), 7.77 (1H, t-like, *J* = 7 Hz, C₃''-H), 8.28 (1H, d, *J* = 7.6 Hz, C₄''-H), 8.50 (1H, d, *J* = 6.1 Hz, C₂''-H). ¹³C-NMR (400 MHz, D₂O) δ : 24.82 (t), 28.98 (t), 33.24 (t), 34.41 (t), 58.99 (d), 61.54 (t), 65.16 (d), 65.53 (q), 110.72 (s), 115.86 (d), 128.28 (d), 138.13 (s), 143.00 (s), 143.23 (d), 148.83 (s), 150.23 (s), 164.63 (s, two peaks), 167.20 (s), 170.04 (s), 173.63 (s). FAB-MS *m/z*: 515 (M⁺ + 1).

5) Degradation Product E Cefpirome, 15 g, was dissolved in 400 ml of 0.5 N HCl, allowed to stand at 40 °C for 48 h, and adjusted to pH 3—4 with aqueous sodium hydroxide solution. This solution was extracted with ethyl acetate, and after evaporation of ethyl acetate under reduced pressure, the residue was purified by column chromatography (column, silica gel; eluent, ethyl acetate:methanol=9:1). The resulting crystal was recrystallized from a small amount of methanol to give 465 mg of degradation product E. IR (KBr): 1738, 1650, 1572, 1035 cm⁻¹. ¹H-NMR (90 MHz, DMSO-*d*₆) δ : 3.50 (2H, m, C₂-CH₂), 3.75 (2H, br s, C₆-H₂), 3.83 (3H, s, OCH₃), 4.5 (1H, m, C₂-H), 4.83 (2H, br s, C₅-CH₂), 5.97 (1H, d, *J* = 5.7 Hz, NH), 6.87 (1H, s, C₅'-H), 7.17 (2H, br s, NH₂), 8.71 (1H, t, *J* = 5.7 Hz, CONH). EI-MS *m/z*: 369 (M⁺), 338, 214, 183 (100).

6) Degradation Product F 2% aqueous cefpirome solution (pH 7), 100 ml, was irradiated with artificial sunlight (cumulative light intensity: about 1000000 lux) at 35—40 °C. This solution was fractionated by semi-prep. HPLC in a similar manner to that for degradation product B and 1 mg of a lyophilized degradation product F was obtained. ¹H-NMR (90 MHz, D₂O) δ : 2.30 (2H, m, C₆''-H₂), 3.0—3.5 (6H, m, C₂-H₂, C_{5,7}''-H₂ × 2), 4.07 (3H, s, OCH₃), 5.25 (1H, d, *J* = 4 Hz, C₆-H), 5.30, 5.50 (2H, ABq, *J* = 16 Hz, C₃-H₂), 5.86 (1H, d, *J* = 4 Hz, C₇-H), 7.53 (1H, s, C₅'-H), 7.76 (1H, t-like, *J* = 7 Hz, C₃''-H), 8.25 (1H, d, *J* = 7 Hz, C₁₀''-H), 8.49 (1H, d, *J* = 7 Hz, C₂''-H). FAB-MS *m/z*: 515 (M⁺ + 1).

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