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Acidic Degradation of Cefodizime (THR-221) and Structural Elucidation of the Products. I

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Cefodizime sodium (CDZM, THR-221) is a new semi-synthetic cephalosporin. The acidic degradation of cefodizime and structural elucidation of the degradation products were investigated. In acidic solution, cefodizime was converted to the *anti*-form of cefodizime [THR-221-A] and the Δ_3-cephem form [THR-221-B]. In another degradation pathway, cefodizime was degraded to desacetyl cefotaxime [THR-221-I] and mercaptothiazole [THR-221-IV]. THR-221-I was further changed to desacetyl cefotaxime lactone [THR-221-IIa] and its *anti*-form [THR-221-IIb], two isomers of deslactam desacetyl cefotaxime lactone [THR-221-III and -III′], deslactam descarboxy desacetyl cefotaxime lactone [THR-221-IVa] and its *anti*-form [THR-221-IVb] and methoxyimino-aminothiazolyl aldehyde [THR-221-V].

Based on the structural elucidation, the acidic degradation pathways of cefodizime were determined.

Keywords cefodizime; antibiotic; acidic degradation; structural elucidation

Cefodizime sodium (CDZM, THR-221; disodium (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-[[[5-(carboxylatomethyl)-4-methyl-2-thiazolyl]thio]methyl]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate) is a semi-synthetic cephalosporin derivative containing a *syn*-methoxyimino-aminothiazolyl group at the C-7 position and a mercaptothiazolyl group at the C-3 position, developed by Hoechst AG (Frankfurt, F.R.G.) and Roussel Uclaf (Paris, France).[2] This antibiotic has been demonstrated to have a broad spectrum of activity against gram-positive and gram-negative organisms, to be stable against β-lactamases, and to have a longer half-life in blood than other cephem antibiotics.[3]

The acidic degradation of cefodizime was studied, the degradation products were isolated, and their chemical structures were elucidated. This paper reports the results obtained.

Experimental

Materials and Reagents Cefodizime sodium was supplied by Hoechst AG (Frankfurt, F.R.G.) and authentic samples of degradation products of cefodizime were synthesized by Hoechst AG and in our laboratories. Cefoteram pivoxil was also synthesized in our laboratory. Acetonitrile was of liquid chromatographic reagent grade and other chemicals used were all purchased from Wako Pure Chemicals (Osaka, Japan).

The McIlvaine buffer solution (pH 3.0) was prepared by mixing 4.11 ml of 0.2 M Na_2HPO_4 and 15.89 ml of 0.1 M citric acid. The mobile phases used were as follows: the acetate buffer solution (pH 5.0) was prepared by dissolving 3.00 g of acetic acid in *ca.* 950 ml of deionized water, adjusted to pH 5.0 with 5 N NaOH, and made up to a 1.0-l volume with deionized water; and the phosphate buffer solution (pH 6.8) was prepared by mixing equal volumes of 5 mM KH_2PO_4 and 5 mM Na_2HPO_4.

Instruments Infrared (IR) spectra were measured on a Hitachi 260-50 IR spectrophotometer (Tokyo, Japan) by the KBr tablet method. Measurement of ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were carried out using a JEOL FX-100 Fourier-transform nuclear magnetic resonance spectrometer coupled with an FAFT 70 data system (Tokyo, Japan). ^{1}H-NMR spectra were measured in dimethylsulfoxide-d_6 (DMSO-d_6) using tetramethylsilane as an internal standard. A JEOL DX-303 mass spectrometer linked to a JMA DA5100 data system was used for the measurement of electron impact mass spectra (EIMS) and fast atom bombardment mass spectra (FABMS). FABMS were measured using water or DMSO as a solvent and a mixture of glycerol and thioglycerol (1:1, v/v) as a matrix on the target. EIMS were obtained under the following conditions: ionization energy, 70 eV; ionization current, 300 μA; and acceleration voltage, 3.0 kV. Most of the degradation products of cefodizime, as well as cefodizime and other cephem antibiotics, gave no molecular ion peaks in the EIMS. The FAB ionization method was thus used in most cases. A Shimadzu LC-6 high-performance liquid chromatographic system consisted of two LC-6A pumps, an SPD-6A variable wavelength ultraviolet (UV) detector, a CTO-6A column oven, a SIL-6A automatic injector and a C-R3A Chromatopac data system (Kyoto, Japan).

High-Performance Liquid Chromatography (HPLC) The time-dependent degradation of cefodizime in aqueous acidic solution was monitored by HPLC using reversed-phase columns, Radial NOVA-PAK C_{18} and Radial pak μC_{18} (Waters Assoc., MA, U.S.A.), and gradient systems.

i) HPLC Condition 1: Column, Radial NOVA-PAK C_{18} (5 μm), 100 × 8 mm i.d.; column temperature, room temperature; mobile phase, acetonitrile/50 mM acetate buffer (pH 5.0), a linear gradient of 0→50% acetonitrile (2%/min); flow rate, 1.5 ml/min; and detector, UV 254 nm, 0.08 a.u.f.s.

ii) HPLC Condition 2: Column, Radial pak μC_{18} (10 μm), 100 × 8 mm i.d.; column temperature, room temperature; mobile phase, acetonitrile/5 mM phosphate buffer (pH 6.8), a linear gradient of 0→50% acetonitrile (2%/min); flow rate, 1.5 ml/min; and detector, UV 254 nm, 0.32 a.u.f.s.

Degradation of Cefodizime in Aqueous Acidic Solutions Cefodizime was dissolved in McIlvaine buffer (pH 3.0) to a concentration of 100 μg/ml. Each solution, after standing at 35 °C for 1,2,4,6 and 8 d, was directly injected into the HPLC apparatus. The degradation products were analyzed under HPLC conditions 1 and 2. A solution of 100 μg/ml was also prepared by dissolving cefodizime in 0.5 N HCl solution and then allowed to stand at 100 °C for 15, 30, 45 and 60 min. The chromatograms under HPLC condition 2 were recorded for each preparation.

Isolation of THR-221-A and -IIa Each aqueous cefodizime solution (0.2 g/0.5 ml) was diluted with 5 ml of 0.5 N HCl. After standing at 100 °C for 30 min (for THR-221-IIa) or 60 min (for THR-221-A), the reaction solution was neutralized with 1 N NaOH. For isolation of THR-221-A, the resultant solution was extracted twice with 20 ml of chloroform and then twice with 20 ml of ethyl acetate. The resulting aqueous layer containing THR-221-A was subjected to fractionation by HPLC [column, Radial pak μC_{18}; mobile phase, CH_3CN: 5 mM phosphate buffer (pH 6.8) = 10:90 (v/v); flow rate, 2.0 ml/min; and detector, UV 310 nm]. The fractionated solution was lyophilized to obtain a crude sample. This crude sample was dissolved in 2.0 ml of deionized water, and applied to a CHP-20P column (particle size 150—300 μm, 200 × 14 mm i.d., Mitsubishi Chemical Industry, Tokyo, Japan), which was washed with deionized water, and then eluted with 20% acetonitrile aqueous solution. The eluate was evaporated and lyophilized to yield 10 mg of THR-221-A. For isolation of THR-221-IIa, on the other hand, the neutralized solution was extracted twice with 20 ml of ethyl acetate. The extract was reduced to dryness to obtain 20 mg of THR-221-IIa.

Isolation of THR-221-B Cefodizime (0.2 g) was dissolved in 1 ml of deionized water and heated at 100 °C for 120 min. The reaction solution was washed by extraction three times with 4 ml of ethyl acetate followed by fractionation of the aqueous layer by HPLC [column, Radial pak μC_{18}; mobile phase, CH_3CN: 5 mM phosphate buffer (pH 6.8) = 8 : 92 (v/v); flow rate, 2.0 ml/min; and detector, UV 340 nm]. The fraction of THR-221-B was concentrated, lyophilized and then treated with a Sep-Pak C_{18} cartridge (Waters Assoc.). The sample dissolved in deionized water was applied to the activated cartridge, which was washed with 1.0 ml of deionized water and then eluted with 4 ml of methanol. The eluate was concentrated to dryness to obtain *ca.* 1.0 mg of THR-221-B.

Isolation of THR-221-I Cefodizime (1.0 g) was dissolved in 100 ml of 0.1 M KH_2PO_4 solution (pH 5.0) and the solution was reacted at 60 °C for 20 h. Following the addition of 1 ml of acetic acid, the solution was applied to a CHP-20P column (200 × 20 mm i.d.), which was washed with 100 ml of 0.1% acetic acid and then with 0.1% acetic acid containing 10% methanol followed by elution with 0.1% acetic acid containing 30% methanol. The eluate was concentrated to remove methanol and lyophilized to obtain 60 mg of crude THR-221-I. This crude sample (30 mg) was dissolved in 2 ml of 50 mM phosphate buffer solution (pH 7.0), then the solution was fractionated by HPLC [column, Radial NOVA-PAK C_{18}; mobile phase, CH_3CN : 0.1% acetic acid = 5 : 95 (v/v); flow rate, 2.0 ml/min; and detector, UV 254 nm]. The fraction was concentrated and lyophilized to yield 3 mg of THR-221-I.

Isolation of THR-221-IIb, -IVa and -IVb To 2.5 ml of aqueous solution containing 0.2 g of cefodizime, 2.5 ml of of 1 N HCl was added. The solution was left at 100 °C for 60 min and then neutralized with 1 N NaOH. The solution was extracted twice with 20 ml of chloroform and the chloroform layer was concentrated to dryness. The residue was fractionated by HPLC [column, Radial pak μC_{18}; mobile phase, CH_3CN : 5 mM phosphate buffer (pH 6.8) = 20 : 80 (v/v); flow rate, 2.0 ml/min; and detector, UV 310 nm]. Each fraction was concentrated to remove acetonitrile and extracted with ethyl acetate. From each extract, 8 mg of THR-221-IIb, 6 mg of THR-IVa and 9 mg of THR-IVb were obtained, respectively. THR-221-IVa was further purified by preparative thin-layer chromatography (TLC) [Merck, Darmstadt, F.R.G., Kieselgel type 60 F_{254}; developing solvent, $CHCl_3$: MeOH = 5 : 1 (v/v); and extracting solvent, $CHCl_3$: MeOH = 1 : 1 (v/v)].

Isolation of THR-211-III and -III′ Cefodizime (2 g) was suspended in 2 l of McIlvaine buffer (pH 3.0) and reacted at 40 °C for 66 h. This solution was applied to a CHP-20P column (80 mm × 20 mm i.d.), which was washed with 40 ml of 10% MeOH solution and eluted with 100 ml of 50% methanol. The methanol eluate was evaporated to remove methanol and lyophilized. The resultant residue was dissolved in 20 ml of deionized water and extracted thrice with 60 ml of ethyl acetate at pH 3.0. The ethyl acetate layer was concentrated to dryness and dissolved in methanol. Fractionation on a preparative silica gel TLC plate [developing solvent, $CHCl_3$: MeOH : acetic acid = 10 : 2 : 1 (v/v); and extracting solvent, methanol] was then carried out. The fractions were further purified by HPLC [column, Radial pak μC_{18}; mobile phase, methanol: 20 mM KH_2PO_4 = 15 : 85 (v/v); flow rate, 1.5 ml/min; and detector, UV 254 nm]. Each fraction was concentrated and adjusted to pH 3—4 with 1 N HCl followed by extraction thrice with 40 ml of ethyl acetate. Each ethyl acetate extract was concentrated to dryness to obtain 5 mg of THR-221-III and 3 mg of THR-221-III″.

Isolation of THR-221-V and Its Hydrazone Derivatization Cefodizime (3 g) was dissolved in 300 ml of 0.1 M KH_2PO_4 solution (pH 5.0) and the solution was allowed to stand at 60 °C for 48 h. The solution thus obtained was concentrated to a volume of *ca.* 40 ml, adjusted to pH 3—4 with acetic acid, and filtered to remove the precipitate. The filtrate was applied to a CHP-20P column (60 × 20 mm i.d.), which was washed with *ca.* 100 ml of distilled water and eluted with 20% methanol solution. The eluate was reduced and lyophilized to yield 430 mg of crude sample. This crude sample dissolved in 50 ml of ethanol was reacted with 0.9 g of 2,4-dinitrophenylhydrazine and 1 ml of concentrated HCl. The mixture was refluxed for 2 min and ethanol was evaporated off. Next, 5 ml of a mixture of acetone and tetrahydrofuran (1 : 1, v/v) was added. The solution was neutralized with a saturated $NaHCO_3$ solution and extracted five times with 50 ml of ethyl acetate. The ethyl acetate layer was concentrated and the residue obtained was purified by silica gel column chromatography [Wakogel C-200; 250 × 20 mm i.d.; developing solvent, ethyl acetate : benzene : methanol = 10 : 10 : 1 (v/v)]. Remaining impurities were removed by dissolving them with a small amount of ethyl acetate : benzene : methanol (10 : 10 : 1, v/v), leaving 10 mg of the hydrazone derivative of THR-221-V.

Isolation of THR-221-VI Cefodizime (1.0 g) was dissolved in 20 ml of 0.5 N HCl. The solution was left at 37 °C for 48 h, filtered, neutralized with Na_2CO_3 and extracted with chloroform (40 ml × 2) and then with ethyl acetate (40 ml × 2). The aqueous layer was concentrated, applied to a CHP-20P column (200 × 20 mm i.d.) and eluted with deionized water. The eluate was lyophilized to obtain 45 mg of THR-221-VI.

Results and Discussion

Degradation of Cefodizime in Aqueous Acidic Solutions

Figure 1 shows chromatograms (HPLC condition 1) of the degradation products in McIlvaine buffer solution at pH 3.0, and Fig. 2 shows the time-dependent changes of the chromatograms of cefodizime and its degradation products, prepared by plotting the relative peak areas of the compounds.

The degradation of cefodizime in McIlvaine buffer solution at pH 3.0 proceeded very rapidly. In the initial step, THR-221-I, -IIa and -VI as major products and THR-221-III and -B as minor products were formed, and in the final step, THR-221-III′, -IVa, -IVb and -V were additionally observed. The amounts of THR-221-B were very low during the degradation. The amounts of THR-221-I, -IIa and -III present decreased gradually, while THR-221-IVa, -IVb and -V increased in amount over time. From the results obtained, it was concluded that THR-221-I, -IIa and -VI

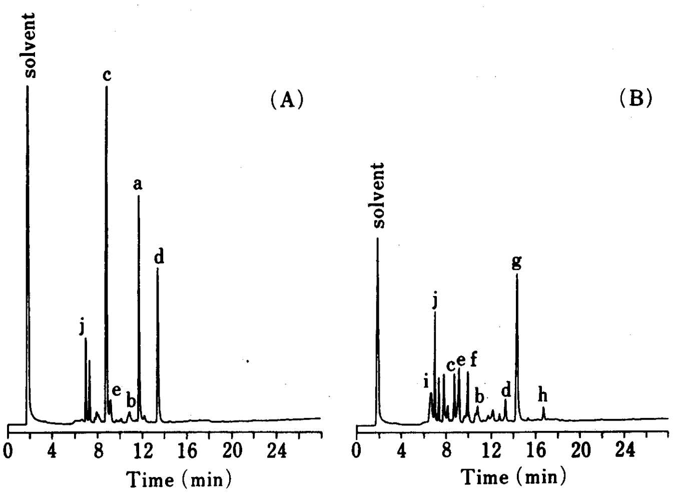

Fig. 1. Chromatograms of Cefodizime (THR-221) and Its Degradation Products in McIlvaine Buffer (pH 3.0) after (A) 1 d and (B) 6 d at 35 °C

a, THR-221; b, THR-221-B; c, THR-221-I; d, THR-221-IIa; e, THR-221-III; f, THR-221-III′; g, THR-221-IVa; h, THR-221-IVb; i, THR-221-V; j, THR-221-VI.

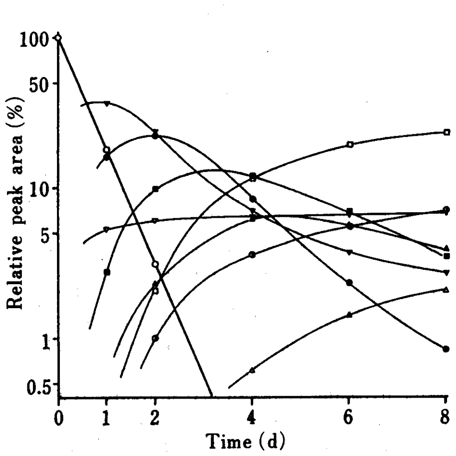

Fig. 2. Changes in Cefodizime (THR-221) and Its Degradation Products in McIlvaine Buffer (pH 3.0) at 35 °C

○, THR-221; ▼, THR-221-I; ●, THR-221-IIa; ■, THR-221-III; ▲, THR-221-III′; □, THR-221-IVa; △, THR-221-IVb; ○, THR-221-V; ▽, THR-221-VI.

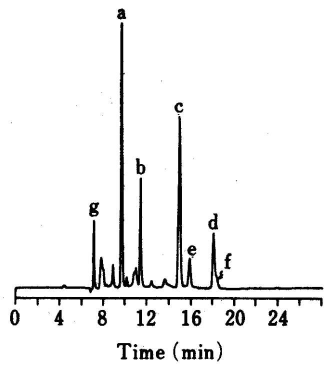

Fig. 3. Chromatogram of Cefodizime (THR-221) and Its Degradation Products in 0.5 N HCl after 60 min at 100 °C

a, THR-221; b, THR-221-A; c, THR-221-IIa; d, THR-221-IIb; e, THR-221-IVa; f, THR-221-IVb; g, THR-221-VI.

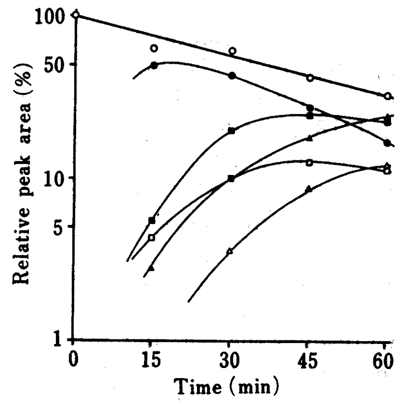

Fig. 4. Changes in Cefodizime (THR-221) and Its Degradation Products in 0.5 N HCl at 100 °C

○, THR-221; ▲, THR-221-A; ●, THR-221-IIa; ■, THR-221-IIb; □, HTR-221-IVa; △, THR-221-IVb.

are initial degradation products and THR-221-IVa, -IVb and -V are final products.

Next, the time-dependent degradation of cefodizime in 0.5 N HCl was investigated. The chromatogram (HPLC condition 2) of products formed and their time-dependent changes are shown in Figs. 3 and 4.

THR-221-A and -IIb in addition to THR-221-IIa, -IVa, -IVb and -VI were detected, but no peaks of THR-221-I, -III, -III′ and -B could be detected. The amounts of THR-221-IIa and -IVa decreased gradually, while THR-221-IIb and -IVb increased in amount over time. The changes of THR-221-IIa and -IIb, and THR-221-IVa and -IVb were quite similar, suggesting the same degradation pathway. From these results, the products and extent of acidic degradation appear to be influenced to some degree by the conditions employed.

Chemical Structure of Degradation Products of Cefodizime The FABMS, EIMS, IR and ^{1}H-NMR data for cefodizime and its degradation products, isolated as described under Experimental, are summarized in Tables I and II. On the basis of these results, the chemical structures of the degradation products were elucidated.

The IR spectra of THR-221-A, -B and -I showed the absorptions of the carboxy group in the β-lactam ring, amido bond and carboxyl group. In the IR spectra of THR-221-IIa and -IIb, besides the carboxy group in the β-lactam ring, amido bond and carboxyl group, new characteristic absorptions of a γ-lactone carbonyl group were observed. The IR spectrum of THR-221-III showed the absorptions of the amido band, carboxyl group and γ-lactone carbonyl group, but not that of the carboxy group in the β-lactam ring. In the IR spectra of THR-221-IVa and -IVb, the absorptions of the amido bond and γ-lactone carbonyl group, but no absorptions of the carboxy group in the β-lactam ring and carboxyl group were noted.

The FABMS of THR-221-A and -B gave quasi-molecular ion peaks corresponding to the molecular weight of the free form of cefodizime. The quasi-molecular ions of THR-221-IIa and -IIb have 18 m.u. less than THR-221-I, those of THR-221-III and -III′ have 18 m.u. more than THR-221-IIa and -IIb and those of THR-221-IVa and -IVb have 44 m.u. less than THR-221-III and -III′.

A comparison of the ^{1}H-NMR spectra of THR-221-A with that of cefodizime indicated close coincidence in most of the signals, though those of the proton at C-5′ of the aminothiazolyl group and the protons of the methoxy group were shifted downfield from δ 6.72 to 7.45 and from δ 3.83 to 3.95, respectively. These chemical shifts may possibly have been due to *syn*- and *anti*-isomers of the methoxyimino group.[4]

From these results and a spectral comparison with an authentic synthetic sample, THR-221-A was identified as

TABLE I. MS and IR Data for Cefodizime (THR-221) and Its Degradation Products

Degradation product	FABMS m/z	EIMS m/z	IR (cm^{-1}, $\nu_{C=O}$)			
			β-Lactam carbonyl group	Amide bond	Carboxyl group	γ-Lactone carbonyl group
Cefodizime (THR-221)	629 $(M+1)^+$	—	1760	1650 (sh)	1590	—
THR-221-A	585 $(M+1)^+$	—	1763	1670	1622	—
THR-221-B	585 $(M+1)^+$	—	1753	1655 (sh)	1600	—
THR-221-I	414 $(M+1)^+$	—	1760	1662	1620	—
THR-221-IIa	396 $(M+1)^+$	—	1781	1668	1615	1750 (sh)
THR-221-IIb	396 $(M+1)^+$	—	1791	1668	1612	1750 (sh)
THR-221-III	414 $(M+1)^+$	—	—	1660	1630 (sh)	1750
THR-221-III′[a]	414 $(M+1)^+$	—				
THR-221-IVa	370 $(M+1)^+$	—	—	1652	—	1740
THR-221-IVb	370 $(M+1)^+$	—	—	1668	—	1750
THR-221-V[b]	423 $(M+1)^+$	—	—	1670	—	—
THR-221-VI	190 $(M+1)^+$	189 $(M)^+$	—	1692	—	—

a) The IR spectrum was not measured because only a trace amount was available. *b*) As the hydrazone derivative.

TABLE II. ^{1}H-NMR Data for Cefodizime (THR-221) and Its Degradation Products

Degradation product	Chemical shift (δ ppm) in DMSO-d_6
Cefodizime (THR-221)	3.28, 3.58 (d, J=16 Hz, 4-H$_2$), 4.08, 4.56 (d, J=13 Hz, 3-CH$_2$), 4.99 (d, J=5 Hz, 6-H), 5.59 (dd, J=5, 8 Hz, 7-H), 9.56 (d, J=8 Hz, 7-NH), 3.83 (s, OCH$_3$), 6.72 (s, 5′-H), 7.27 (br s, NH$_2$), 2.16 (s, CH$_3$), 3.30 (s, CH$_2$COO$^-$)
THR-221-A	3.30, 3.58 (d, J=17 Hz, 4-H$_2$), 4.09, 4.54 (d, J=13 Hz, 3-CH$_2$), 4.96 (d, J=4 Hz, 6-H), 5.59 (dd, J=4, 9 Hz, 7-H), 9.33 (d, J=9 Hz, 7-NH), 3.95 (s, OCH$_3$), 7.45 (s, 5′-H), 7.12 (br s, NH$_2$), 2.16 (s, CH$_3$), 3.25 (s, CH$_2$COO$^-$)
THR-221-B	6.18 (s, 4-H), 4.02, 4.31 (d, J=13 Hz, 3-CH$_2$), 5.33 (br s, 6-H), 5.39 (m, 7-H), 9.77 (d, J=7 Hz, 7-NH), 4.59 (s, 2-H), 3.82 (s, OCH$_3$), 6.75 (s, 5′-H), 7.26 (br s, NH$_2$), 2.16 (s, CH$_3$), 3.26 (s, CH$_2$COO$^-$)
THR-221-I	3.56 (br s, 4-H$_2$), 4.26 (br s, 3-CH$_2$), 5.11 (d, J=5 Hz, 6-H), 5.73 (dd, J=5, 8 Hz, 7-H), 9.58 (d, J=8 Hz, 7-NH), 3.84 (s, OCH$_3$), 6.75 (s, 5′-H), 7.22 (br s, NH$_2$), 12.95 (br s, 2-COOH)
THR-221-IIa	3.79 (br s, 4-H$_2$), 5.05 (br s, 3-CH$_2$), 5.16 (d, J=5 Hz, 6-H), 5.94 (dd, J=5, 8.5 Hz, 7-H), 9.69 (d, J=8.5 Hz, 7-NH), 3.85 (s, OCH$_3$), 6.75 (s, 5′-H), 7.23 (br s, NH$_2$)
THR-221-IIb	3.79 (br s, 4-H$_2$), 5.05 (br s, 3-CH$_2$), 5.14 (d, J=5 Hz, 6-H), 5.93 (dd, J=5, 9 Hz, 7-H), 9.54 (d, J=9 Hz, 7-NH), 3.96 (s, OCH$_3$), 7.48 (s, 5′-H), 7.15 (br s, NH$_2$)
THR-221-III	3.66 (br s, 6-H$_2$), 4.86 (br s, 5-CH$_2$), *ca.* 4.8 (m, 2-H), *ca.* 4.85 (m, 2-CH), 9.05 (d, J=9 Hz, CONH), 5.74 (d, J=6 Hz, 3-NH), 3.81 (s, OCH$_3$), 6.85 (s, 5′-H), 7.19 (br s, NH$_2$)
THR-221-III′	3.54 (br s, 6-H$_2$), 4.84 (br s, 5-CH$_2$), 4.74 (dd, J=6, 8.5 Hz, 2-H), 4.51 (t, J=8.5 Hz, 2-CH), 9.15 (d, J=8.5 Hz, CONH), 6.21 (d, J=6 Hz, 3-NH), 3.80 (s, OCH$_3$), 6.84 (s, 5′-H), 7.20 (br s, NH$_2$)
THR-221-IVa	3.57 (br s, 6-H$_2$), 4.84 (br s, 5-CH$_2$), 4.56 (m, 2-H), *ca.* 3.45 (m, 2-CH$_2$), 8.75 (br t, J=5 Hz, CONH), 6.00 (d, J=5 Hz, 3-NH), 3.82 (s, OCH$_3$), 6.89 (s, 5′-H), 7.19 (br s, NH$_2$)
THR-221-IVb	3.57 (br s, 6-H$_2$), 4.83 (br s, 5-CH$_2$), 4.52 (m, 2-H), 3.45 (m, 2-CH$_2$), 8.62 (br t, J=5 Hz, CONH), 6.05 (d, J=5 Hz, 3-NH), 3.95 (s, OCH$_3$), 7.42 (s, 5′-H), 7.09 (br s, NH$_2$)
THR-221-V as its hydrazone derivative	3.86 (s, OCH$_3$), 4.11 (br t, CH$_2$), 6.81 (s, 5-H), 7.22 (br s, 2-NH$_2$), 8.03 (t, J=4 Hz, CH=N), 8.96 (t, J=6 Hz, CONH) Dinitrophenyl hydrazone moiety: 7.91 (d, J=9 Hz), 8.33 (dd, J=9, 2.5 Hz), 8.86 (d, J=2.5 Hz), 11.49 (br s, NH)
THR-221-VI	2.05 (s, CH$_3$), 3.57 (s, CH$_2$), *ca.* 3.5 (SH), *ca.* 13.0 (COOH)

an *anti*-isomer of cefodizime, (−)-(6*R*,7*R*)-7-[(*E*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-[[[5-(carboxymethyl)-4-methyl-2-thiazolyl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

On comparing the ^{1}H-NMR spectrum of THR-221-B with that of cefodizime, the signal of the proton at C-6 of the β-lactam ring was found to have shifted downfield from δ4.99 to 5.33 and that of the proton at C-7, upfield from δ5.59 to 5.39. The signals of two protons at C-4 of cefodizime were not observed, and broad singlet peaks at δ6.18 due to the vinyl proton and at δ4.59 were newly observed. Δ_2-Cephem compounds having a double bond between the C-2 and C-3 position can be converted into Δ_3-cephem compounds having a double bond between the C-3 and C-4 position in alkaline solution.[5] The configuration of COOH at the C-2 position could not be clearly determined from these spectral data, but THR-221-B was confirmed to have an α-configuration, as also reported for other related compounds.[6]

From these results, THR-221-B was identified as (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-[[[5-(carboxymethyl)-4-methyl-2-thiazolyl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid, having a Δ_3-cephem structure.

In the ^{1}H-NMR spectrum of THR-221-I, having a molecular weight of 413 and a β-lactam ring, no signal due to a mercaptothiazole moiety was observed and broad singlet signals at δ4.26 and 3.55, attributed to the methylene group at C-3 and the protons at C-4, were noted. Other signals showed chemical shifts similar to those of cefodizime.

THR-221-I was thus identified as desacetyl cefotaxime, (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-hydroxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, having a hydroxymethyl group at the C-3 position, formed through cleavage of the mercaptothiazolyl moiety of cefodizime.

The ^{1}H-NMR spectrum of THR-221-IIa was coincident with that of THR-221-IIb, except that the signal of the proton at C-5′ of the aminothiazole moiety of THR-221-IIb shifted downfield from δ6.75 of THR-221-IIa to δ7.48, suggesting the *anti*- and *syn*-configurations of the two compounds.

From these spectral data and comparison with authentic samples synthesized from cefotaxime,[7] THR-221-IIa and -IIb were identified as the *syn*- and *anti*-isomers of desacetyl cefotaxime lactone, (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-hydroxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid lactone and (−)-(6*R*,7*R*)-7-[(*E*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-hydroxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid lactone.

A comparison of the ^{1}H-NMR spectrum of THR-221-III with that of THR-221-III′ indicated them to be quite similar. The signals due to the protons at C-2 and the 2-positional side chain of the thiazine ring were observed and a new doublet signal was attributed to the proton of 3-NH. These degradation products appear to be compounds produced by cleavage of the β-lactam ring of THR-221-IIa. Furthermore, the signals of two protons at C-6 and the methylene group at C-5 in the thiazine ring (δ3.66 and 4.86 for THR-221-III and δ3.54 and 4.84 for THR-221-III′) suggest that these compounds may each have a lactone moiety, as in the case of THR-221-IIa, and on the basis of the chemical shifts of the methoxyimino group, it could be elucidated to be in *syn*-form cofiguration. Additionally, in the degradations of THR-221-III and -III′ in 0.5 N HCl at 40 °C, THR-221-III′ from the former compound and THR-221-III from the latter compound were formed.

These compounds thus appear to be isomers. THR-221-III and -III′ are thus considered to be the C-2 positional isomers of the thiazine ring (2*R*- or 2*S*-desacetyl deslactam cefotaxime lactone), 2-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-2-[(2*R*)-5-hydroxymethyl-4-carboxylic acid lactone-2,3-dihydro-6*H*-2-(1,3-thiazinyl)]acetic acid and 2-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-2-[(2*S*)-5-hydroxymethyl-4-carboxylic acid lactone-2,3-dihydro-6*H*-2-(1,3-thiazinyl)]acetic acid.

In the ^{1}H-NMR spectrum of THR-221-IVa, remarkable changes of the signals of the proton at C-2 and methylene protons of the side chain at C-2 of the thiazine ring were observed. The ^{1}H-NMR spectum of THR-221-IVb coincided precisely with that of THR-221-IVa, except that the signals of the proton at C-5′ of the aminothiazole moiety was shifted downfield, suggesting the *anti*-form of the methoxyimino group. THR-221-IVa and -IVb are possibly formed from THR-221-III and -III′ by decarboxylation.

From the above mentioned results, THR-221-IVa and -IVb were concluded to be *syn*- and *anti*-isomers of the methoxyimino group and were identified as 2-[(*Z*)-2-(2-amino-4-thizolyl)-2-methoxyiminoacetamido]-2-[5-hydroxymethyl-4-carboxylic acid lactone-2,3-dihydro-6*H*-2-(1,3-thiazinyl)] methane and 2-[(*E*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-2-[5-hydroxymethyl-4-carboxylic acid lactone-2,3-dihydro-6*H*-2-(1,3-thiazinyl)] methane, respectively (*syn*- and *anti*-deslactam descarboxy desacetyl cefotaxime lactone). These products showed spectra completely coincident with those of authentic synthetic samples.

THR-221-V could not be isolated due to its instability. However, since this product showed the same retention time of HPLC as the aldehyde-form reported to be a degradation product of cefteram pivoxil with the same aminothiazolyl-methoxyimino group at the 7-position as cefodizime,[8] cefodizime may be degraded to produce a similar aldehyde-form. Thus, the hydrazone derivative of THR-221-V was obtained according to the method re-

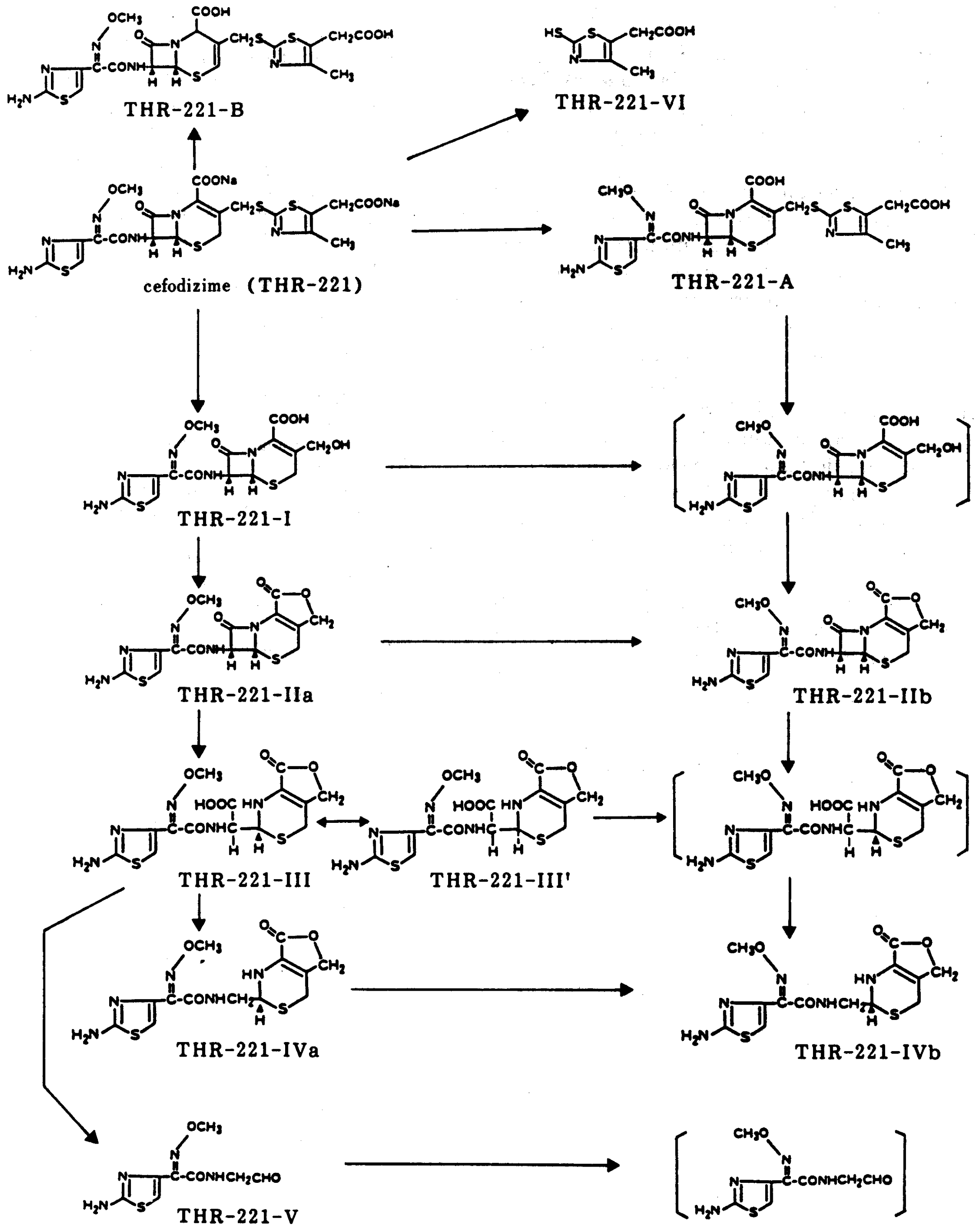

Chart 1. Degradation Pathway of Cefodizime (THR-221) in Aqueous Acidic Solution

ported for the aldehyde-form product of cefteram pivoxil.[8] The spectral data for the hydrazone derivative of THR-221-V agreed with those reported for the derivative of the aldehyde degraded from cefteram pivoxil.[8]

On the basis of these results, THR-221-V was elucidated as 2-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]acetaldehyde. The formation of similar aldehyde compounds has also been reported for other cephalosporin antibiotics, such as cephalothin,[9] cephaloridine,[10] cephacetrile[11] and cefoperazone.[12]

THR-221-VI was also identified as [5-(carboxymethyl)-4-methyl-2-thiazolyl]thiol or (2-mercapto-4-methyl-5-thiazolyl)acetic acid from the various spectra and a comparison with a authentic sample.

Other degradation products were too unstable under neutral or acidic conditions, and isolation by column chromatography or other methods was difficult. Thus, their chemical structures could not be elucidated.

Degradation Pathways of Cefodizime in Aqueous Acidic Solution On the basis of chemical structural elucidation, the degradation pathways of cefodizime are considered to be as illustrated in Chart 1.

In the initial step of degradation in buffer solution at pH 3.0, cefodizime is degraded to THR-221-I and THR-221-VI by hydrolysis of the mercaptothiazole moiety at C-3, and then THR-221-I is converted to the lactone compound, THR-221-IIa. The subsequent degradation pathway is considered to be as follows: formation of two isomers, THR-221-III and -III′, by cleavage of the β-lactam ring of THR-221-IIa, formation of THR-221-IVa by decarboxylation of THR-221-III and -III′ and finally formation of THR-221-V. In the final step of degradation in buffer solution at pH 3.0 or in HCl solution, degradation products of the *anti*-form of the methoxyimino group, such as THR-221-A, -IIb and -IVb, are produced. Thus, severe acidic degradation appears to be necessary for isomerization of the methoxyimino moiety.

Powdered and lyophilized samples of cefodizime were exposed to direct solar rediation for 100 d. THR-221-A as a major product and THR-221-VI were formed and no other acidic degradation products could be detected.

It was found that no metabolites of cefodizime were present in plasma, urine and other biological materials *in vivo* experiments. However, it was also found that two metabolites, having the methoxyimino-aminothiazolyl moiety, were present in human feces after incubation at 37 °C for 24 h.[13] THR-221-I, -IIa, -III and -III′ were identified as metabolites of cefotaxime.[14]

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References and Notes

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