Alkaline and Neutral Degradation of Cefodizime (THR-221) and Structural Elucidation of the Products. II[1)]

Eiji Matsushima, Yoshinori Minami, Ryotaro Azuma, Ken-ichiro Yoshida, Yukihiko Umeno and Teruyoshi Marunaka*,[2)]

Biological Research Laboratory, Taiho Pharmaceutical Co., Ltd., Hiraishi, Kawauchi-cho, Tokushima 771-01, Japan. *Received June 15, 1988*

Cefodizime sodium (CDZM, THR-221) is a new semi-synthetic cephalosporin. The alkaline and neutral degradation of cefodizime and structural elucidation of its degradation products were investigated. In aqueous alkaline and neutral solutions, cefodizime was degraded to the C-7 positional isomer of the *β*-lactam ring [THR-221-C], Δ_3-cephemform [THR-221-B], methoxyimino-aminothiazolyl aldehyde [THR-221-V] and mercaptothiazole [THR-221-VI].

Based on the structural elucidation, the alkaline and neutral degradation pathways of cefodizime were determined.

Keywords cefodizime; antibiotic; alkaline degradation; structural elucidation; neutral degradation

Cefodizime sodium (CDZM, THR-221, disodium (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyimino-acetamido]-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 in derivative, developed by Hoechst AG (Frankfurt, F.R.G.) and Roussel Uclaf (Paris, France).[3)]

We previously investigated the acidic degradation of cefodizime and structural elucidation of the degradation products.[1)] In acidic solution, cefodizime was degraded to the *anti*-form of the methoxyimino group of cefodizime [THR-221-A], Δ_3-cephem form [THR-221-B], desacetyl cefotaxime [THR-221-I] and mercaptothiazole [THR-221-VI], while THR-221-I further changed to desacetyl cefotaxime lactone [THR-221-IIa] and its *anti*-form [THR-221-IIb], two isomers (2*R* and 2*S*) 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]. The present paper describes the alkaline and neutral degradation of cefodizime. The structure of a new degradation product [THR-221-C] was elucidated.

Experimental

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

The buffer solutions used were prepared as follows: the McIlvaine buffer solution (pH 7.0) was prepared by mixing 16.47 ml of 0.2 M Na_2HPO_4 and 3.53 ml of 0.1 M citric acid; and the Clark–Lubs buffer solution (pH 9.0) was prepared by adding 50 ml of a mixture of 0.2 M boric acid and 0.2 M KCl to 21.3 ml of 0.2 N NaOH and 128.7 ml of deionized water. The preparations of acetate buffer (pH 5.0) and phosphate buffer (pH 6.8) solutions used as mobile phase were described in the previous report.[1)]

Instruments The infrared (IR) spectra were recorded on a Hitachi 260-50 IR spectrophotometer (Tokyo, Japan) by the KBr tablet method. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were measured with a JEOL FX-100 Fourier-transform nuclear magnetic resonance spectrometer, coupled with an FAFT 70 data system (Tokyo, Japan). For ^{1}H-NMR spectra measurement in dimethylsulfoxide-d_6 (DMSO-d_6), tetramethylsilane was used as an internal standard. A JEOL DX-303 mass spectrometer linked to a JMA-DA5100 data system was used to measure fast atom bombardment mass spectra (FABMS); the conditions were described in the previous report.[1)] A Shimadzu (Kyoto, Japan) LC-6 high-performance liquid chromatographic system[1)] was used for separation of the degradation products.

High-Performance Liquid Chromatography (HPLC) The degradation products of cefodizime in aqueous alkaline and neutral solutions were detected under the HPLC condition 1 described in the previous report,[1)] using a Radial NOVA-PAK C_{18} column (5 μm, 100 × 8 mm i.d., Waters Assoc., MA., U.S.A.).

Degradation of Cefodizime in Buffer and Aqueous Solutions Cefodizime was dissolved in McIlvaine buffer (pH 7.0), Clark–Lubs buffer (pH 9.0), 1 N NaOH solution or distilled water to a concentration of 100 μg/ml. Buffer solutions after standing at 35 °C for 1, 2, 4, 6 and 8 d were directly injected into the chromatograph. The aqueous NaOH solution was stored at room temperature (18 °C) and the aqueous neutral solution was heated at 100 °C and the samples of each solution were periodically directly analyzed under HPLC condition 1 to identify the products.

Isolation of THR-221-C Cefodizime (0.2 g) was dissolved in 1 ml of 1 N NaOH. The solution was left at room temperature (18 °C) for 60 min. The neutralized solution with 1 N HCl was then fractionated by HPLC [column, Radial pak μC_{18} (10 μm), 100 × 8 mm i.d.; mobile phase, acetonitrile: 5 mM phosphate buffer (pH 6.8) = 10 : 90 (v/v); flow rate, 2.0 ml/min; and detector, UV 340 nm]. The fraction of THR-221-C was concentrated and lyophilized. The crude samples obtained were dissolved in 2.0 ml of distilled water and applied to a CHP-20P column (particle size 150—300 μm, 200 × 20 mm i.d., Mitsubishi Chemical Industry, Tokyo, Japan), washed with distilled water and eluted with 20% acetonitrile aqueous solution. The sample thus obtained was again lyophilized to yield *ca.* 2 mg of THR-221-C. FABMS *m/z*: 585 $(M^{+1})^+$. ^{1}H-NMR (DMSO-d_6) δ: 3.30, 3.64 (d, $J=17$ Hz, 4-H_2), 3.99, 4.39 (d, $J=13$ Hz, 3-CH_2), 4.64 (d, $J=2$ Hz, 6-H), 4.78 (dd, $J=2$, 8 Hz, 7-H), 9.76 (d, $J=8$ Hz, 7-NH), 3.84 (s, OCH_3), 6.78 (s, 5′-H), 7.24 (br s, NH_2), 2.15 (s, CH_3), 3.24 (s, CH_2). IR (KBr): 1752 ($\nu_{C=O}$ of the *β*-lactam ring), 1660 ($\nu_{C=O}$ of the amido bond), 1595 ($\nu_{C=O}$ of COO^-). Retention time (t_R) under HPLC condition 1: 12.6 min.

Results and Discussion

Degradation of Cefodizime in Aqueous Alkaline and Neutral Solutions The changes in the chromatograms of cefodizime and its degradation products in Clark–Lubs buffer solution at pH 9.0 and McIlvaine buffer solution at pH 7.0 are shown in Fig. 1.

Cefodizime was relatively stable in buffer solution at pH 9.0. In the initial step of degradation, THR-221-C was predominantly formed and THR-221-D, -V, -VI and -B were also detected. The amount of THR-221-V, the final product,[1)] increased gradually on prolonged reaction. In the neutral buffer, cefodizime was fairly stable, but after considerable degradation, trace amounts of THR-221-B, -C, -D, -V and -VI were noted. In 1 N NaOH solution, it rapidly degraded even at room temperature to form THR-221-B as the major product in addition to THR-221-C, -VI

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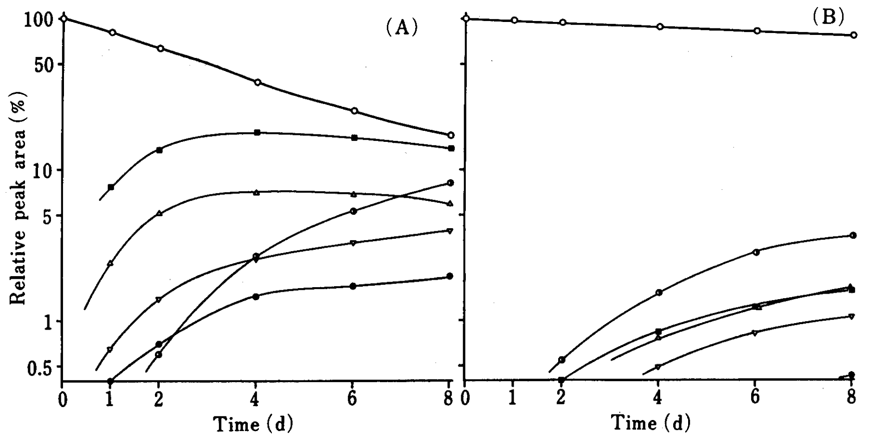

Fig. 1. Changes in Cefodizime (THR-221) and Its Degradation Products in (A) Clark–Lubs Buffer Solution (pH 9.0) and (B) McIlvaine Buffer Solution (pH 7.0) at 35 °C

○, THR-221; ●, THR-221-B; ■, THR-221-C; △, THR-221-D; ○, THR-221-V and ▽, THR-221-VI.

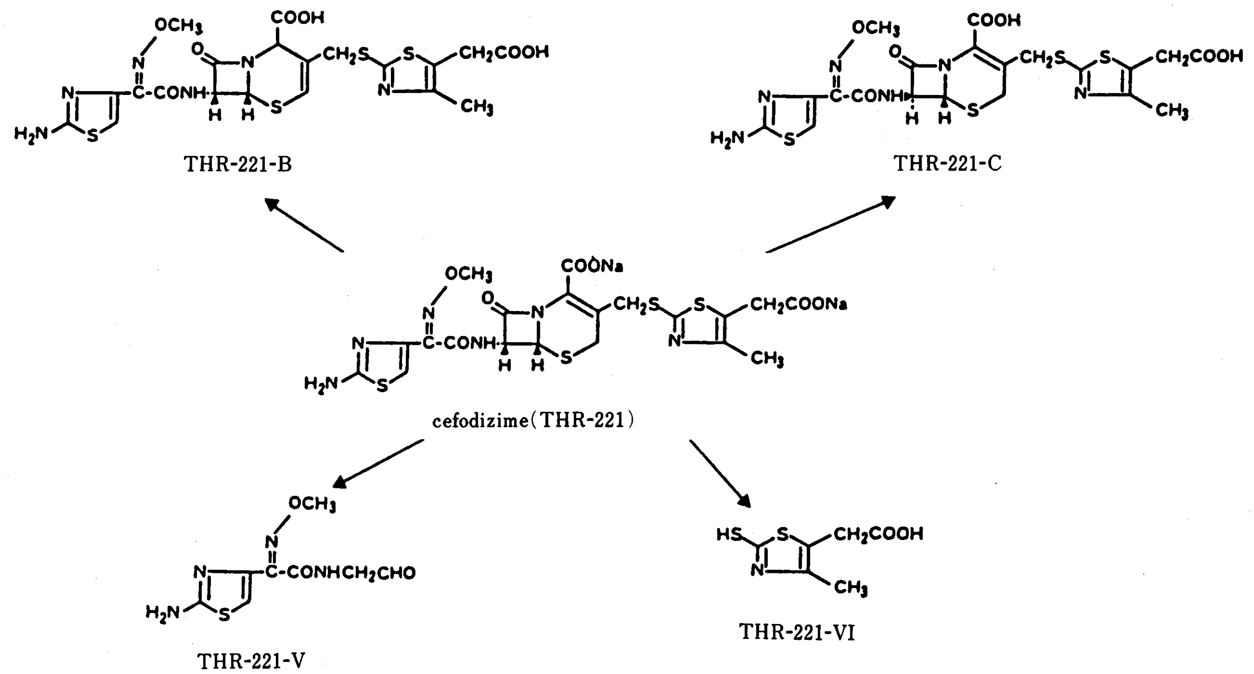

Chart 1. Degradation Pathways of Cefodizime (THR-221) in Aqueous Alkaline and Neutral Solutions

and various trace products. The degradation of cefodizime in deionized water gave similar patterns, and thus the degradation of cefodizime in neutral solution was considered to be essentially the same as that in aqueous alkaline solution.

Chemical Structures of Degradation Products of Cefodizime On comparing the ^{1}H-NMR spectrum of THR-221-C (molecular weight of 584, and containing a cephem ring) with that of cefodizime,[1] differences in the chemical shifts and coupling constants (J) of the protons at C-6 and C-7 of the β-lactam ring were observed. That is, the signals of the protons at C-6 and C-7 shifted upfield from δ 4.99 to 4.64 and from δ 5.59 to 4.78, respectively, and the J value of the C-6 and C-7 protons showed a small value of 2 Hz in contrast to 5 Hz in cefodizime. This J value agrees quite well with the coupling constants (1.8 Hz) of (6*R*,7*S*)-cephalosporins reported by Gutowski.[4]

From these results, THR-221-C was identified as a C-7 positional isomer of cefodizime, (−)-(6*R*,7*S*)-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-2-ene-2-carboxylic acid.

The chemical structures of THR-221-D (t_R, 7.4 min) and other minor degradation products could not be elucidated because of their instability or because the amounts available were too small to permit isolation.

Degradation Pathways of Cefodizime From the chemical structural elucidations, the degradation pathways of cefodizime in the aqueous alkaline and neutral solutions are considered to be as given in Chart 1.

From cefodizime, a 7-positional isomer [THR-221-C] was mainly formed. The formation of the Δ_3-cephem compound [THR-221-B], methoxyimino-aminothiazolyl aldehyde [THR-221-V] and mercaptothiazole [THR-221-VI] was also observed, whereas that of desacetyl cefotaxime [THR-221-I] and a series of degradation products confirmed in acidic degradation was not recognized.

The degradation pattern of cefodizime under alkaline and neutral conditions differed considerably from that under acidic conditions. Under alkaline and neutral conditions, the degradation of the 3-positional side chain may be slower, while the isomerization at C-7 is faster.

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References and Notes

1) T. Marunaka, E. Matsushima, Y. Minami, R. Azuma, K. Yoshida and Y. Umeno, *Chem. Pharm. Bull.*, **37**, 367 (1989).
2) Present address: *Biwako Research Institute, Otsuka Pharmaceutical Co., Ltd., 1-11-1, Karasaki, Ohtsu, Shiga 520-01, Japan.*
3) J. Blumbach, W. Durckheimer, E. Ehlers, K. Fleischmann, N. Klesel, M. Limbert, B. Mencke, J. Reden, K. H. Scheunemann, E. Schrinner, G. Seibert and M. Worm, *J. Antibiot.*, **40**, 29 (1987).
4) G. E. Gutowski, *Tetrahedron Lett.*, **21**, 1779 (1980).

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