Kinetics and Mechanism of Decomposition of Cefazolin Ester in Phosphate Buffer Solution

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We evaluated the rate and mechanism of decomposition of cefazolin methyl ester (Δ^3 ester) in phosphate buffer (pH 8.4).

The decomposition of Δ^3 ester proceeded simultaneously *via* 3 pathways. The first pathway is production of Δ^2 ester (rate k_{12}) by isomerization and production of Δ^2 acid (k_{23}) by hydrolysis of the Δ^2 ester. The second pathway is the cleavage reaction of the β-lactam ring and simultaneous elimination of the substituent at position 3 (k_{14}). The third pathway is production of Δ^3 acid (k_{15}) by hydrolysis of the carboxylic ester at position 4.

Kinetic analysis of each pathway was performed. The reaction rate constant from Δ^2 ester to Δ^2 acid (k_{23}) was the highest, and the reaction rate constant for the production of Δ^2 ester by isomerization (k_{12}) was similar to that for the elimination of the position 3 substituent by cleavage of the β-lactam ring (k_{14}). The rate of production of Δ^3 acid by hydrolysis of the position 4 carboxylic ester was the lowest.

These results show that Δ^3 ester decomposition under a basic condition predominantly occurs through cleavage reaction of the β-lactam ring and Δ^2 ester production by isomerization.

Since the production of Δ^2 acid markedly depended on the production of Δ^2 ester by isomerization, Δ^2 ester production is the rate-limiting step of this pathway.

Key words cephalosporin; isomerization; cefazolin methyl ester; kinetics; decomposition

In general, cephalosporin compounds for injection are not appropriate for oral administration. This is due to the relatively low pK_a value of the carboxylic acid at position 4. Since the carboxylic acid is dissociated in the intestinal tract, the drug is only slightly fat-soluble and is poorly absorbed. To improve this, many attempts have been made to increase fat solubility by esterification of the carboxylic acid at position 4,[1] but so far without success.

One reason for this may be the low stability of esterified cephalosporin compounds in solution. Several investigators[2] have evaluated the decomposition of esterified cephalosporin compounds under basic conditions. Cephalosporin compounds after esterification of the position 4 carboxylic acid tend to isomerize (Δ^3 ester→Δ^2 ester; refer to Chart 1) under a basic condition, and this has been considered to be the cause of inactivation of these compounds. However, we previously observed that the β-lactam ring generally tends to be cleaved under basic conditions. Speculating that there is another pathway of decomposition, we evaluated the mechanism of decomposition and performed kinetic analysis in the case of cefazolin methyl ester (Δ^3 ester).

Experimental

General Cefazolin esters and acids were prepared by conventional methods.[3] Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Bruker AC200P spectrometer (Germany) using tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a JASCO IR-810 spectrometer (Tokyo). The melting points were determined with a Büchi 535 apparatus (Germany).

High-Performance Liquid Chromatography (HPLC) Instrument and Analytical Conditions HPLC was performed using a Waters chromatography system (510 pump, variable-wave-length detector set at 254 nm) and a Shimadzu C-R3A Chromatopac.

HPLC was carried out on a Cosmosil C-18 column (4.6 × 150 mm) Nacalai Tesque, Inc. using the following solvent systems: A, 2.27% disodium hydrogenphosphate 12·H_2O solution; B, 0.47% citric acid in 30% methanol. The column temperature was 40 °C, and the flow rate was 1.0 ml/min.

Materials and Reagents Cefazolin, sodium salt (cefazolin sodium) and 5-methyl-1,3,4-thiadiazol-2-thiol (MTT) were supplied by the pharmaceutical section of our laboratories. Δ^2 Acid was kindly supplied by Mr. Kitaguchi of our section.

Preparation of Δ^3 Ester Methyl iodide (4.1 g) was added to a solution of cefazolin (10.9 g) in dimethylformamide (DMF) (57 ml)/*p*-dioxane (43 ml) and the mixture was stirred overnight at room temperature. After addition of water (120 ml), ethyl acetate (180 ml) and a sufficient quantity of NaCl to induce separation, the organic layer was washed successively with water and saturated NaCl, and then dried over anhydrous sodium sulfate. Removal of the solvent and treatment of the residue with ether yielded 8.7 g of crystalline Δ^3 ester. mp: 180—182 °C, IR (Nujol): 1768 (β lactam), 1710 (ester), 1680 cm^{-1} (amide). ^{1}H-NMR (DMSO-d_6) δ: 2.69 (3H, s, CH_3), 3.78 (3H, s, OCH_3), 3.73 (2H, dd, J=18 Hz, CH_2), 4.39 (2H, dd, J=13.5 Hz, CH_2), 5.14 (1H, d, J=5 Hz, CH), 5.37 (2H, dd, J=17 Hz, NCH_2CO), 5.75 (1H, ABC pattern, J=8, 5 Hz, CH), 9.37 (1H, s, NCH), 9.59 ppm (1H, d, J=8 Hz, CONH).

Preparation of Δ^2 Ester For preparation, 40% KOH solution (30 ml) and ether (90 ml) were cooled in an ice bath. Methylnitrosourea (8.1 g) was added to the mixture with stirring and cooling.

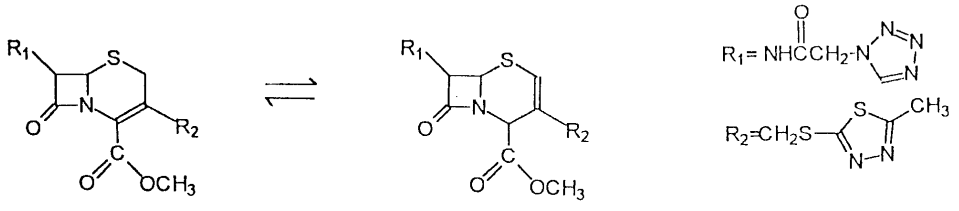

Chart 1

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The ether layer become yellow, and the organic layer was added to Δ^2 acid in ethylacetate with stirring. Stirring and cooling were continued until the yellow color disappeared. The organic layer was washed with 2% $NaHCO_3$ and dried over anhydrous sodium sulfate. The crystal of Δ^3 ester (0.6 g) was obtained by the concentration of the organic layer. mp: 97—99 °C, IR (Nujol): 1770 (β lactam), 1732 (ester), 1672 cm^{-1} (amide). ^{1}H-NMR (DMSO-d_6) δ: 2.68 (3H, s, CH_3), 3.32 (3H, s, OCH_3), 4.18 (2H, dd, J=14 Hz, CH_2), 5.17 (1H, d, J=4 Hz, CH), 5.2 (1H, d, J=1.5 Hz, CH), 5.37 (1H, dd, J=17 Hz, NCH_2CO), 5.50 (1H, ABC pattern, J=8, 4 Hz, CH), 6.75 (1H, d, J=1.5 Hz, CH), 9.36 (1H, s, NCH), 9.58 ppm (1H, d, J=8 Hz, CONH).

Procedure for Kinetic Study[4] of Cefazolin Ester in Phosphate Buffer Solution About 10 mg of cefazolin methyl ester (Δ^3 ester) was dispersed and dissolved in 500 ml of 1/10 M phosphate buffer with a pH of 8.4 (194 ml of 1/10 M hydrogenphosphate $12\cdot H_2O$ and 306 ml of 1/10 M sodium tetraborate $10\cdot H_2O$) at 40 °C for about 5 min, passed through a membrane filter (pore size, 0.2 μm), and used as test solution.

This solution was placed in a cylindrical glass vessel with a jacket (1 l, 100 mm × 150 mm), kept at 40±1 °C, and stirred using a Rushton-type stirrer (6 blade; blade length, 50 mm) and a Heidon 600 G stirring motor.

The decomposition solution of Δ^3 ester (10 μl) was serially sampled and directly injected into the HPLC column. To determine the sample concentration, standard solutions of Δ^3 ester, Δ^2 ester, and Δ^3 acid, Δ^2 acid, and MTT were prepared, and calibration curves were obtained.

1) Calibration Curve of Δ^3 Ester: A calibration curve was obtained under the above measurement conditions using Δ^3 ester standard solution in the concentration range of 2.33—23.3 μg/ml. A good linear relationship was observed. The regression equation was $Y=1795X+15$: $\gamma=1.0000$; Y, peak area (μV·s); X, concentration (μg/ml).

2) Calibration Curve of Δ^2 Ester: A calibration curve was obtained under the above measurement conditions using Δ^2 standard solution in the concentration range of 0.47—4.7 μg/ml. A good linear relationship was observed. The regression equation was $Y=1882X+31$: $\gamma=1.0000$; Y, peak area; X, concentration (μg/ml).

3) Calibration Curve of Δ^3 Acid: A calibration curve was obtained under the above measurement conditions using Δ^3 acid standard solution in the concentration range of 0.46—4.6 μg/ml. A good linear relationship was observed. The regression equation was $Y=1971X+13$: $\gamma=0.9999$; Y, peak area; X, concentration (μg/ml).

4) Calibration Curve of Δ^2 Acid: A calibration curve was obtained under the above measurement conditions using Δ^2 acid standard solution in the concentration range of 0.46—9.2 μg/ml. A good linear relationship was observed. The regression equation was $Y=2554X-33$: $\gamma=1.0000$; Y, peak area; X, concentration (μg/ml).

5) Calibration Curve of MTT: A calibration curve was obtained under the above measurement conditions using MTT standard solution in the concentration range of 0.66—2.64 μg/ml. A good linear relationship was observed. The regression equation was $Y=1993X+3$: $\gamma=0.9999$; Y, peak area; X, concentration (μg/ml).

Results and Discussion

The decomposition rate of cephalosporin ester compounds has been studied over a wide pH range from weakly acid to weakly basic. In each study, the decomposition rate constant at a weakly acid to neutral condition was lower than that under a weakly basic condition, though comparison was difficult. Cephalosporin compounds with a β-lactam ring have been reported to show elimination of the substituent at position 3 at the time of cleavage of the β-lactam ring.[5] Based on these findings, we used cefazolin methyl ester (Δ^3 ester) with a MTT group at position 3 and a tetrazolylacetoamide group at position 7 as a sample and evaluated its decomposition process at a pH of 8.4.

Decomposition Kinetics and Mechanism of Cefazolin Methyl Ester in Phosphate Buffer Solution The decomposition reaction of Δ^3 ester (I) in phosphate buffer solution (1/10 M; pH, 8.4) at 40±1 °C was evaluated by HPLC under the above conditions. As shown in Fig. 1, 4 peaks

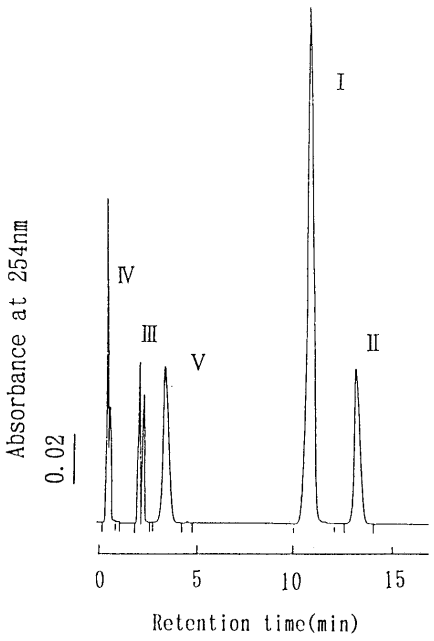

Fig. 1. HPLC Pattern of the Decomposed Δ^3 Ester

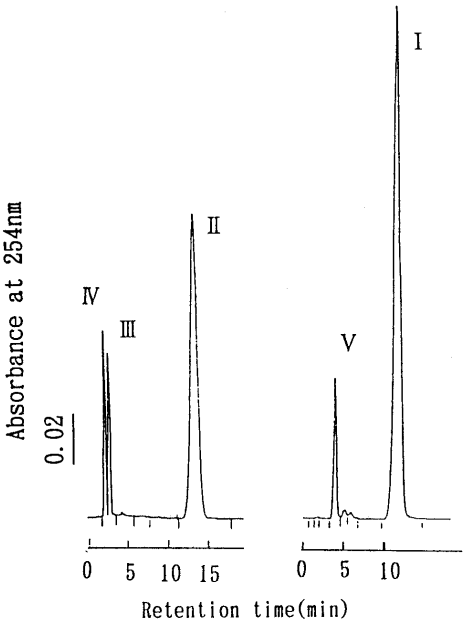

Fig. 2. HPLC Pattern of Standard Cefazolin Derivatives

Peak assignments: peak I, Δ^3 ester; peak II, Δ^2 ester; peak III, Δ^2 acid; peak IV, MTT; peak V, Δ^3 acid.

of compounds derived from Δ^3 ester decomposition products were observed. Each peak showed a retention time equal to that of the corresponding authentic sample (Fig. 2). The retention times of Δ^3 ester, Δ^3 acid, Δ^2 ester, Δ^2 acid, and MTT were 10.9, 3.9, 13.1, 2.5, and 2.1 min, respectively. With the decrease in the peak area of Δ^3 ester, the starting material, Δ^2 ester (II) gradually increased, reaching a maximum after about 1 h, and decreasing thereafter. On the other hand, Δ^2 acid (III) gradually increased after a short lag time. The peak areas of MTT (IV) and Δ^3 acid (V) increased, but the increase in Δ^3 acid was very slight compared with that of MTT.

We consider that the presence of MTT is noteworthy. Many researchers have ignored elimination of the

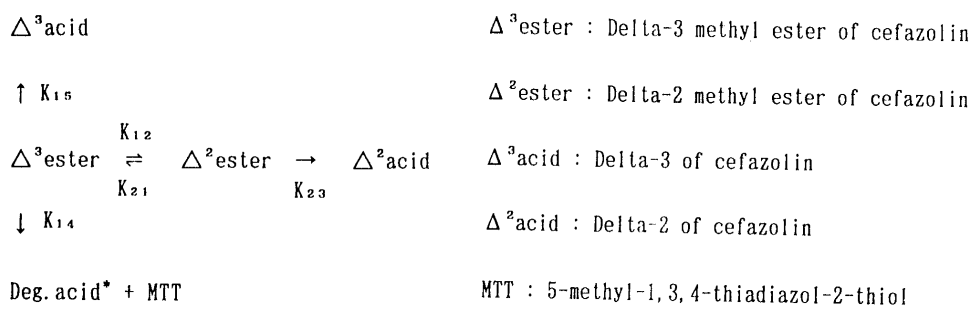

Chart 2

substituent at position 3 at the time of cleavage of the β-lactam ring under a basic condition. Therefore, we propose the decomposition model shown in Chart 2 based on the findings in the previous and present studies.

Δ^3 ester, the starting material, is decomposed *via* 3 pathways under a basic condition. One is Δ^2 ester formation accompanied with isomerization and Δ^2 acid formation by hydrolysis. In this process, interconversion was reported to be possible between Δ^3 ester and Δ^2 ester but not between Δ^3 ester and Δ^2 acid produced by hydrolysis.[2)] The second pathway is cleavage of the β-lactam ring and simultaneous elimination of MTT at position 3. The third pathway is formation of Δ^3 acid by hydrolysis of the carboxylic ester at position 4.

Miyauchi *et al.*[2b)] found that the decomposition of the pivaloyloxymethyl ester of cephalosporin is a pseudo-first-order reaction. In our study, the decomposition of Δ^3 ester was also found to be pseudo first order.

Based on the above proposed model (Chart 2), the following reaction rate equations were obtained.

$$d[\mathrm{I}]/dt = -(k_{12}+k_{14}+k_{15})[\mathrm{I}]+k_{21}[\mathrm{II}]$$
$$d[\mathrm{II}]/dt = k_{12}[\mathrm{I}]-(k_{21}+k_{23})[\mathrm{II}]$$
$$d[\mathrm{III}]/dt = k_{23}[\mathrm{II}]$$
$$d[\mathrm{IV}]/dt = k_{14}[\mathrm{I}]$$
$$d[\mathrm{V}]/dt = k_{15}[\mathrm{I}]$$

The reaction rate constants k_{12}, k_{21}, k_{23}, k_{14}, and k_{15} calculated using MULTI[6)] were 0.426 (S.D.: 0.0403), 0.199 (S.D.: 0.0241), 1.90 (S.D.: 0.134), 0.288 (S.D.: 0.0299), and 0.0994 (S.D.: 0.00119), respectively. As shown in Fig. 3, the values obtained in the experiment were consistent with calculated values, supporting the validity of the model proposed in Chart 2.

The relationship of the reaction rate constants can be summarized as follows:

$$k_{23} \gg k_{12} > k_{14} > k_{21} > k_{15}$$

This relationship shows that the major decomposition products are Δ^2 acid produced by isomerization and MTT produced by cleavage of the β-lactam ring. The production of Δ^3 acid is slight.

The decomposition of cephalosporin ester compounds under a basic condition has been considered to consist predominantly of Δ^2 acid production by isomerization. However, our results suggest the importance of cleavage of the β-lactam ring in decomposition. Since Δ^2 acid formation markedly depends on Δ^2 ester formation by isomerization of Δ^3 ester, Δ^2 ester formation is a rate-limiting step of this reaction.

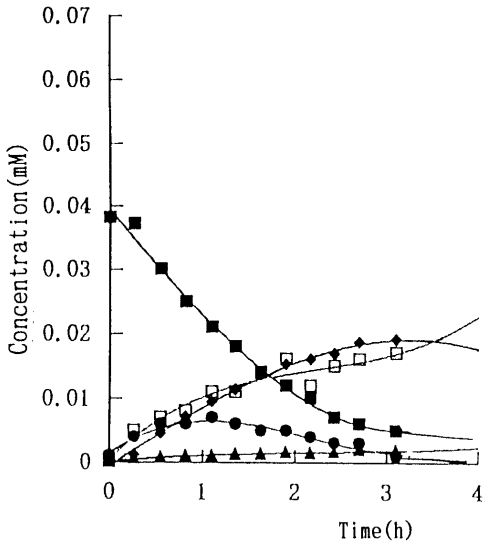

Fig. 3. The Time Course of Δ^3 Ester (■), Δ^2 Ester (●), Δ^3 Acid (▲), Δ^3 Acid (◆), MTT (□) during the Decomposition of Δ^3 Ester
The lines were simulated based on the scheme shown in Chart 2.

Further studies are planned using other cephalo compounds on the isomerization into Δ^2 under basic conditions and the elimination behavior of the substituent at position 3 due to cleavage of the β-lactam ring.

References and Notes

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