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Studies on the Stability of Δ^2 and Δ^3 Cephem Esters. II. Comparative Stability Studies of Δ^2 and Δ^3 Cephems at Various pHs and the Degradation Process of Δ^2 Cephem Esters

Shigeto NEGI,* Motosuke YAMANAKA, Yuki KOMATSU, Akihiko TSURUOKA, Itaru TSUKADA, and Norio MINAMI

Eisai Co., Ltd., Tsukuba Research Laboratories, 1–3 Tokodai 5-chome, Tsukuba, Ibaraki, Japan.

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The instability of Δ^2 cephem prodrug-type ester (5a) under acidic conditions prompted us to investigate the comparative stability of Δ^3 and Δ^2 cephems at various pHs. The Δ^2 cephem ester 5a was found to show marked instability under neutral (pH 7) and acidic conditions (1 M HCl) compared with the Δ^3 cephem ester (4a). A comparative study between Δ^3 (4c) and Δ^2 cephem acid (5c) at pH 7 and in 1 M HCl solution showed that the Δ^2 acid 5c was as stable as the Δ^3 acid 4c at pH 7, while 5c was less stable than 4c in 1 M HCl. Isolation of the degraded compounds demonstrated that the C-4 ester moiety was hydrolyzed in the initial stage to afford 5c, which was further degraded to more polar substances. This instability was observed in other types of Δ^2 cephem esters (5d—f). Here we report comparative stability studies and elucidation of the degradation products.

Key words oral cephalosporin; cephem ester; cephem stability

E1101 (Fig. 1), a broad spectrum antibacterial agent,[1] has been developed as a third generation oral cephalosporin. As reported in our previous paper,[2] we found in the course of process research into E1101 that a Δ^2 cephem prodrug type ester (**5a**) was less stable under formamido acidic cleavage conditions (methanesulfonic acid in methanol) than its Δ^3 counterpart (**4a**). Little has been published about the reactivity of Δ^2 cephems. Cooker *et al.*[3] reported that Δ^2 cephem acids were not reactive in nucleophilic substitution reactions at the C-3 position under alkaline conditions, while Δ^3 cephem acids reacted with various types of nucleophiles at the C-3 position. Saikawa *et al.*[4] reported that Δ^2 cephem esters were more reactive at the C-3 position with nucleophiles such as tetrazole under nonaqueous acidic conditions than were Δ^3 cephems. However, they provided no information on the instability of Δ^2 cephem esters under acidic and basic conditions.

Thus, we were prompted to carry out research on comparative stability studies in aqueous solutions at various pHs between Δ^2 and Δ^3 cephems and to elucidate the degradation mechanisms. We report here on the marked difference in stability between Δ^2 and Δ^3 cephem esters (**5a**, **4a**) and the stability of Δ^2 and Δ^3 cephem acids (**5c**, **4c**) as well as several types of Δ^2 cephem esters (**5d**—**f**), and suggest why Δ^2 cephem esters show such instability. We also deal with the degradation processes.

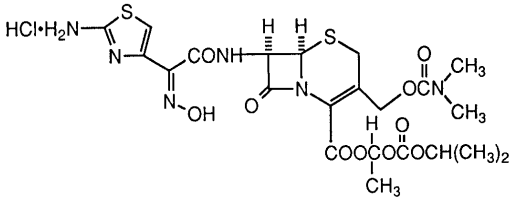


Fig. 1. The Structure of E1101

* To whom correspondence should be addressed.

Results and Discussion

As shown in Chart 1, several Δ^2 cephem esters (**5d**—**f**) were synthesized in a manner similar to that used for **5a**.[2] Firstly, the Δ^3 cephem acid sodium salts (**1a**,[5] **1f**) were esterified with the appropriate esterifying reagent, such as methyl iodide or pivaloyloxymethyl iodide in *N*,*N*-dimethylacetamide (DMA), to give Δ^3 cephem esters (**2d**—**f**). The Δ^3 esters (**2d**—**f**) were then treated with 1 molar eq of triethylamine at room temperature to afford *ca.* 3:2 mixtures of Δ^2 (**3d**—**f**) and Δ^3 cephems esters (**2d**—**f**). The Δ^2 cephem esters (**3d**—**f**) were separated by chromatography and the C-7 formamido substituent was removed in the presence of methanesulfonic acid in methanol at 3 °C to yield the desired 7-amino-Δ^2-cephem esters (**5d**—**f**). The Δ^3 cephem acid **4c** and the Δ^2 cephem acid **5c** were prepared according to the scheme as shown in Chart 2. 7-Amino-Δ^3-cephem acid diphenylmethyl ester (**4b**)[1] was treated with trifluoroacetic acid in the presence of anisole to afford **4c** as the trifluoroacetic acid salt, which was neutralized with aqueous $NaHCO_3$, purified by octadecyl silica (ODS) column chromatography and precipitated with aqueous HCl solution to give **4c** as a zwitterion form. On the other hand, **4b** was treated with 1 molar eq of triethylamine to afford *ca.* a 2:3 mixture of **4b**/**5b**. Because this mixture was inseparable, the diphenylmethyl ester moiety was removed with trifluoroacetic acid in the presence of anisole to give a mixture of the trifluoroacetates of **4c** and **5c**, which were separated on ODS column chromatography to afford the desired Δ^2 cephem acid **5c** in the form of the trifluoroacetate.

We reported[2] that the Δ^2 cephem ester **5a** was less stable under the formamido deprotection condition (methanesulfonic acid in methanol) than its corresponding Δ^3 cephem ester **4a**. It was difficult to explore the degradation processes because a number of peaks were observed. In order to study this phenomenon further, comparative stability studies on Δ^2 and Δ^3 cephems in aqueous solution were carried out.

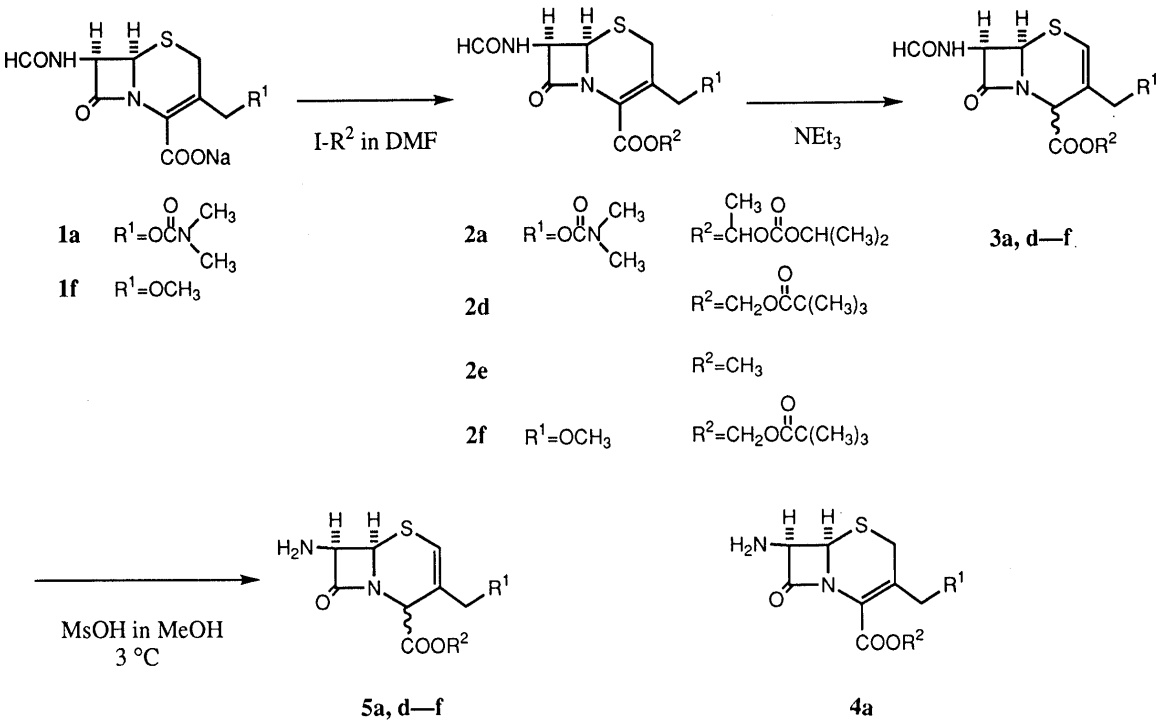


Chart 1

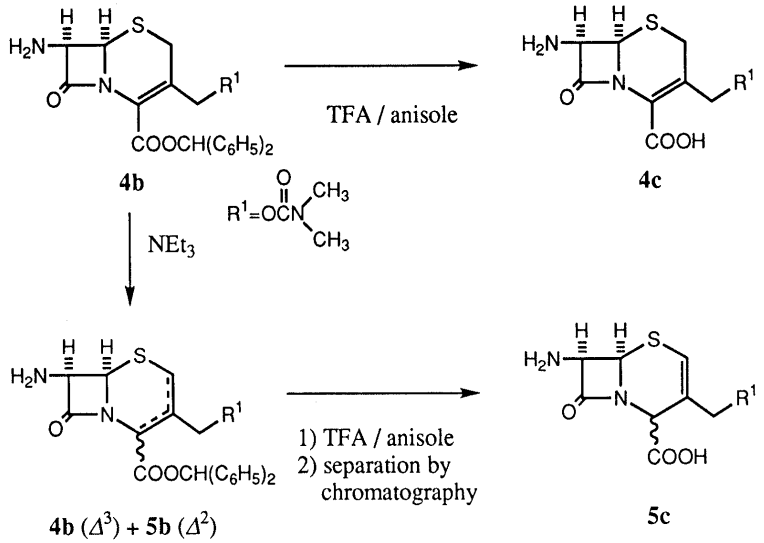


Chart 2

Figures 2 and 3 show the results of the stability studies of both **4a** and **5a** in solutions of various pHs, *i.e.*, pH 7, 5, 3 and 1, and 1 M HCl at 30 °C, respectively. Due to the poor solubility of **4a** and **5a** in water, the experiments were conducted in a 1 : 9 mixture of CH_3CN/H_2O, and the residue concentration was determined by HPLC using ethyl *p*-hydroxybenzoate as an internal standard. The Δ^3 cephem ester **4a** was quite stable at various pHs, and this finding was consistent with the result in the previous report.[6] In particular, **4a** was stable in the range of pH 1—3, but was rather unstable at neutral pH (pH 7), being transformed to the Δ^2 cephem ester **5a**. The Δ^2 cephem ester **5a** exhibited marked instability at pH 7 and 1 M HCl but showed rather good stability within the range of pH 5 to pH 1. Thus, **5a** was found to be more easily degraded than the corresponding Δ^3 cephem ester **4a**.

Figure 4 shows the stability of Δ^3 and Δ^2 cephem acids (**4c**, **5c**) at pH 7 and in 1 M HCl aqueous solution. In general, **4c** was found to be more stable in both pH 7 and 1 M HCl than **5c**. Interestingly, **5c** was unstable in 1 M HCl, but its degradation was slower than that of **5a**. The chemical behaviors of **5c** and **5a** at pH 7 showed a noteworthy difference, that is, **5c** was rather stable at pH 7, while **5a** was rapidly degraded, as shown in Figs. 3 and 4. Therefore, we anticipated that the degradation mechanism of the Δ^2 cephem acid **5c** would be different from that of the Δ^2 cephem ester **5a**.

In order to elucidate this degradation reaction, we carried out isolation and structural determination studies on the decomposition products of **5a** in pH 7 and 1 M HCl solutions. The experiments were carried out under the same conditions as used for the stability studies described

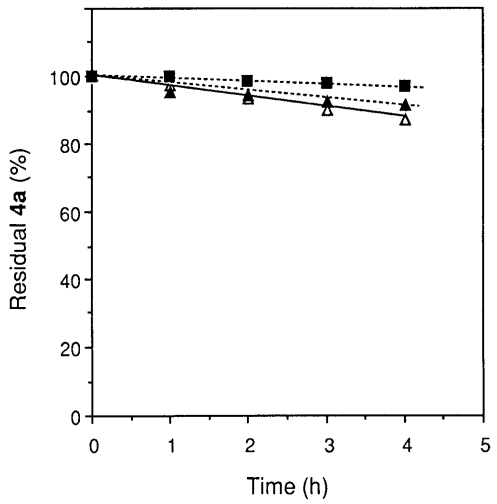


Fig. 2. Time Course of Reaction of **4a** at Various pHs
△, pH 7; ■, pH 5, pH 1; ▲, 1 M HCl.

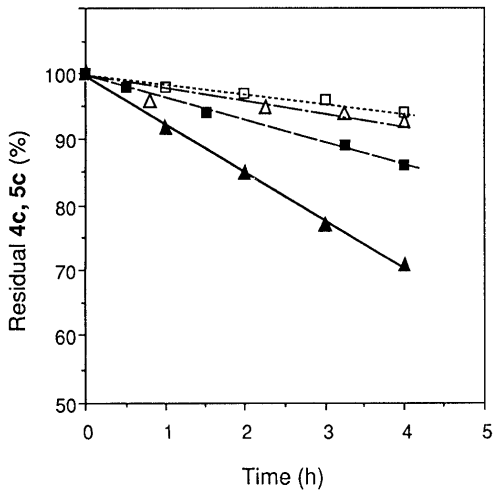


Fig. 4. Time Course of the Reactions of **4c** and **5c**
□, **4c**, pH 7; ■, **4c**, 1 M HCl; △, **5c**, pH 7; ▲, **5c**, 1 M HCl.

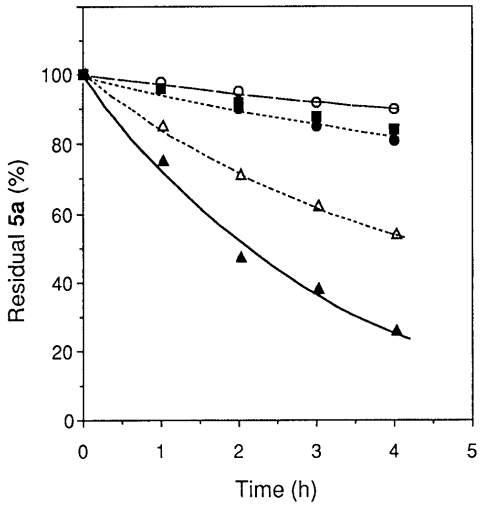


Fig. 3. Time Course of Reaction of **5a** at Various pHs
△, pH 7; ○, pH 5; ●, pH 3; ■, pH 1; ▲, 1 M HCl.

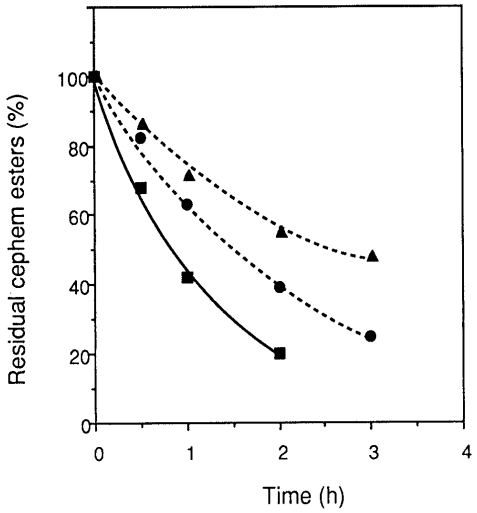


Fig. 5. Reactions of Other Cephem Esters in 1 M HCl
■, **5d**; ▲, **5e**; ●, **5f**.

above, except for the concentration of substrate **5a**, which was 6 or 28 times greater than that in the kinetic study. Firstly, in the pH 7 experiment, the reaction proceeded at a rate similar to that in the kinetic run; a peak at a retention time of 3.8 min was observed at the beginning, and several new peaks appeared in the more polar regions on the HPLC chart as time passed. After 12 h, the reaction mixture was chromatographed on an ODS column, and the column was eluted with 9% aqueous CH_3CN solution. The fractions containing the desired substances were collected, but the product fractions (the retention times = 2.3—3.5 min) were found to contain several substances. One compound, the retention time of which was 3.8 min, was identified as the same compound as the Δ^2 cephem acid **5c** by ^{1}H-NMR. Next, an attempt was made to identify the degradation compounds in 1 M HCl. At the initial stage, the peak at the retention time of 3.8 min appeared, as in the pH 7 reaction, and the Δ^2 cephem acid **5c** was also isolated. However, compared with the pH 7 reaction, many polar compounds were observed by HPLC in the 1 M HCl solution as time passed. These degraded compounds were quite polar and were eluted on an ODS preparative chromatogram as a single peak. The ^{1}H-NMR analysis of this multi-component isolated material showed no peaks at around δ 3.0, which would have corresponded to the dimethylcarbamoyl substituent. Therefore, it was clear that the C-3 (*N*,*N*-dimethylcarbamoyloxy)methyl substituent had been transformed. These isolated products showed marked instability and were easily and rapidly converted to several substances even at −20 °C. We thought that the β-lactam ring was cleaved and then concomitant reactions occurred. We concluded that new isolation methods were required to clarify the degradation processes of **5a** or **5c** under acidic conditions or the study should be carried out using other Δ^2 cephem acids with a more lipophilic substituent at the C-7 position.

Chart 3 shows the putative degradation pathway of **5a** in pH 7 and 1 M HCl solution. In this study, we were only able to show that the initial stage of the degradation processes of Δ^2 cephem esters was hydrolysis of the C-4 ester substituent. At pH 7, the Δ^2 cephem ester **5a** was hydrolyzed to the Δ^2 cephem acid **5c** which was stable at pH 7, and so hydrolysis was likely to be the main reaction. In contrast, in 1 M HCl, **5a** was also changed to **5c**, but

the resultant **5c** was further converted to many polar substances.

We were interested to see if this finding was general or not, so several Δ^2 cephem esters (**5d**—**f**) bearing different substituents at the C-4 position or the C-3 position were prepared and subjected to the reaction in 1 M HCl. The results are shown in Fig. 5. The pivaloyloxymethyl ester (POM) (**5d**), the substituent of which is often used in cephem prodrug esters such as CFTM-PI[7a] or ME1207[7b] (Fig. 6) was also degraded at a rate similar to that of **5a**, and the cephem acid **5c** was also observed by HPLC analysis. This result indicated that hydrolysis reaction at the C-4 ester might have taken place. The methyl ester (**5e**) was also found to be decomposed, although the hydrolysis reaction proceeded at a lower rate compared to that of **5a** and **5d**. Therefore, a double prodrug-type ester such as an alkoxycarbonyloxyalkyl or acyloxyalkyl ester is likely to be cleaved more easily than ordinary alkyl esters such as the methyl ester. Compound **5f** modified with a methoxymethyl substituent at the C-3 position and the POM ester at the C-4 position was also found to be degraded at a somewhat lower rate than 3-(*N*,*N*-dimethylcarbamoyloxymethyl) cephem esters (**5a**, **d**). The effect of the C-3 substituent on this hydrolysis reaction is still unclear, although the reaction was influenced by the nature of the C-3 substituent. In summary, all Δ^2 cephem esters were found to be hydrolyzed easily in 1 M HCl solution to yield Δ^2 cephem carboxylic acids, regardless of the substituent at the C-3, C-4, or C-7 position. 7-Acyl cephems, such as 7-acetamido cephem 1-(isopropoxycarbonyloxy)ethyl (PR) ester, were found to be degraded in a similar way (data not shown).

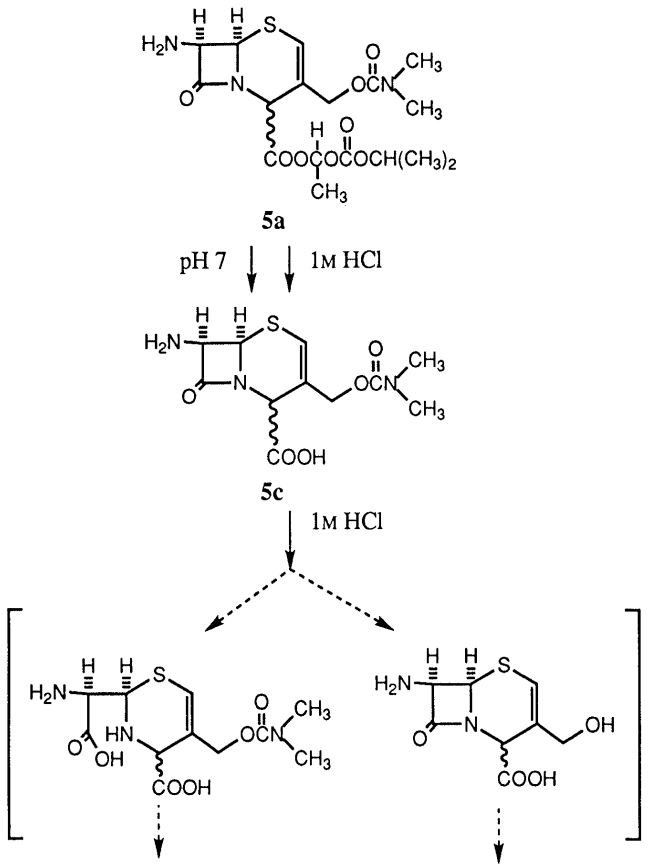


Chart 3. Putative Degradation Pathway of **5a**

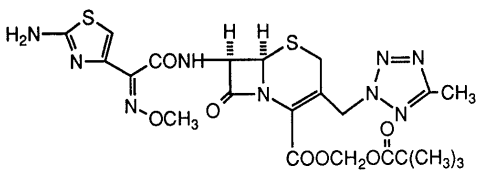
The reason why Δ^2 cephem esters were more sensitive to hydrolysis than Δ^3 cephem esters is thought to be because Δ^3 cephems are a type of conjugated unsaturated carboxylic acid ester, whereas Δ^2 cephems are a type of unconjugated saturated carboxylic acid ester. However, the reason for the instability of Δ^2 cephem acids in acidic solution remains unclear, because we could not clarify the degradation processes of **5c**.

We reported in our previous paper[2] that the Δ^2 cephem ester **5a** was unstable under the formamido cleavage conditions, *i.e.*, methanesulfonic acid in methanol, and this reaction yielded numerous inseparable polar substances. In the course of studying this degradation reaction, the reaction of **5a** under this acidic cleavage condition has become clearer. This reaction was conducted in methanol, and at the initial stage, the transesterification of the PR ester **5a** to the methyl ester **5e** mainly occurred, along with hydrolysis, and then the methyl ester was assumed to be degraded in more complex ways at the C-3 position or at the β-lactam ring to afford polar substances.

In summary, Δ^2 cephem esters were susceptible to hydrolysis under acidic and basic conditions compared to Δ^3 cephem esters, because Δ^3 cephem is a conjugated carboxylic acid, whereas Δ^2 cephem is an unconjugated carboxylic acid. Δ^2 Cephem acid was found to be stable under neutral conditions, but less stable under strongly acidic conditions such as in 1 M HCl solution. These findings will be useful for large-scale synthesis of cephem prodrug esters, and helpful in considering the metabolism of Δ^3 cephem prodrug esters such as E1101, because these esters are well known to be isomerized to Δ^2 cephem esters during gastrointestinal absorption.[8]

Experimental

Melting points were determined using a Yamato MP21 melting point apparatus. IR spectra were recorded on either a Hitachi 260-30 or a Nicolet 205 FT-IR spectrometer. Mass spectra were recorded on a JEOL JMS HX100, and ^{1}H-NMR spectra were recorded on a Varian UNITY 400 using tetramethylsilane (TMS) as an internal standard. Commercially available organic solvents were used, and evaporation and concentration were carried out under reduced pressure below 30 °C.



CFTM-PI

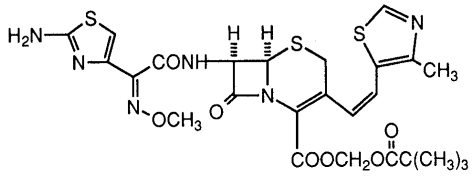


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Fig. 6

Pivaloyloxymethyl (6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-formamido-Δ^3-cephem-4-carboxylate (2d) Pivaloyloxymethyl iodide (0.77 g, 3.3 mmol) was added to a solution of **1a**[5] (1.0 g, 2.8 mmol) in *N*,*N*-dimethylformamide (DMF) (8 ml) and the mixture was stirred for 1 h. The reaction mixture was diluted with EtOAc (100 ml), and washed with water (100 ml) and then with brine. The combined extracts and washing were dried over $MgSO_4$ and concentrated *in vacuo* to give 1.2 g of crude **2d** (100%). The residue was used for the next step without further purification. IR (Nujol): 1780, 1760, 1690, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (9H, s, $C(CH_3)_3$), 2.91 (3H, s, CH_3), 2.92 (3H, s, CH_3), 3.48 and 3.59 (2H, ABq, J=17 Hz, CH_2O), 4.84 and 5.16 (2H, ABq, J=14 Hz, H-2), 5.00 (1H, d, J=5 Hz, H-6), 5.84 and 5.94 (2H, ABq, J=6 Hz, CO_2CH_2O), 5.92 (1H, dd, J=5, 8 Hz, H-7), 6.28 (1H, d, J=8 Hz, CONH), 8.28 (1H, s, HCO).

Pivaloyloxymethyl (6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-formamido-Δ^2-cephem-4-carboxylate (3d) A solution of **2d** (1.0 g, 2.2 mmol) in a mixture of triethylamine (0.25 g, 2.5 mmol) and tetrahydrofuran (THF, 10 ml) was stirred at room temperature for 18 h to give a 2:3 mixture of **2d**/**3d**. EtOAc (100 ml) and H_2O (100 ml) were added to this mixture, and the organic layer was washed with brine, dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was purified by chromatography (Wako-gel C-200, dichloromethane–EtOAc (4:1)) to give 0.25 g of **3d** as a yellow oil (25% yield). IR (Nujol): 1770, 1740, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (9H, s, $C(CH_3)_3$), 2.92 (6H, s, $N(CH_3)_2$), 4.61 and 4.69 (2H, ABq, J=13 Hz, CH_2O), 5.09 (1H, s, H-4), 5.30 (1H, d, J=4 Hz, H-6), 5.78 (1H, dd, J=4, 8 Hz, H-7), 5.83 (2H, s, CO_2CH_2O), 6.36 (1H, d, J=8 Hz, CONH), 6.48 (1H, s, H-2), 8.28 (1H, s, HCO).

Pivaloyloxymethyl (6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)-methyl-Δ^2-cephem-4-carboxylate (5d) Methanesulfonic acid (120 mg, 1.2 mmol) was added to a solution of **3d** (230 mg, 0.52 mmol) in MeOH (2.3 ml) at 3 °C, and the mixture was stirred at the same temperature for 14 h. EtOAc (100 ml) and H_2O (100 ml) were added to this mixture, and the whole was neutralized with aqueous $NaHCO_3$ solution. The organic layer was then separated, and the aqueous layer was washed with EtOAc (50 ml). The combined organic layers were washed with H_2O, then brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by chromatography (Wako gel C-200, dichloromethane : EtOAc = 4 : 1) to give 40 mg of **5d** as a pale yellow oil (19% yield). IR (Nujol): 1780, 1740, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (9H, s, $C(CH_3)_3$), 2.90 (6H, s, $N(CH_3)_2$), 4.59 and 4.65 (2H, ABq, J=14 Hz, CH_2O), 4.60 (1H, d, J=4 Hz, H-6), 5.04 (1H, s, H-4), 5.18 (1H, d, J=4 Hz, H-7), 5.80 (2H, s, CO_2CH_2O), 6.49 (1H, s, H-2). HR-MS *m*/*z*: Calcd for $C_{17}H_{25}N_3O_7S$: 416.1491. Found: 416.1501.

2f—**5f** were prepared by methods similar to those described for **2d**—**5d**.

Pivaloyloxymethyl (6*R*,7*R*)-7-Formamido-3-methoxymethyl-Δ^3-cephem-4-carboxylate (2f) ^{1}H-NMR ($CDCl_3$) δ: 1.23 (9H, s, $C(CH_3)_3$), 3.34 (3H, s, OCH_3), 3.58 (2H, s, CH_2O), 4.32 (2H, s, H-2), 5.01 (1H, d, J=5 Hz, H-6), 5.86 and 5.93 (2H, ABq, J=6 Hz, CO_2CH_2O), 5.90—5.95 (1H, m, H-7), 6.36 (1H, d, J=9 Hz, CONH), 8.29 (1H, s, HCO).

Pivaloyloxymethyl (6*R*,7*R*)-7-Formamido-3-methoxymethyl-Δ^2-cephem-4-carboxylate (3f) IR (Nujol): 1780, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (9H, s, $C(CH_3)_3$), 3.30 (3H, s, OCH_3), 3.87 and 4.06 (2H, ABq, J=12 Hz, CH_2O), 5.09 (1H, s, H-4), 5.30 (1H, d, J=4 Hz, H-6), 5.77 (1H, dd, J=4, 9 Hz, H-7), 5.82 (2H, ABq, J=6 Hz, CO_2CH_2O), 6.35 (1H, s, H-2), 6.38 (1H, d, J=9 Hz, CONH), 8.28 (1H, s, HCO).

Pivaloyloxymethyl (6*R*,7*R*)-7-Amino-3-methoxymethyl-Δ^2-cephem-4-carboxylate (5f) IR (Nujol): 1780, 1740, 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (9H, s, $C(CH_3)_3$), 3.30 (1H, s, OCH_3), 3.85 and 4.07 (2H, ABq, J=13 Hz, CH_2O), 4.61 (1H, d, J=4 Hz, H-6), 5.05 (1H, s, H-4), 5.21 (1H, d, J=4 Hz, H-7), 5.78 and 5.81 (2H, d, J=6 Hz, CO_2CH_2O), 6.37 (1H, s, H-2). HR-MS *m*/*z*: Calcd for $C_{15}H_{22}N_2O_6S$: 358.1199. Found: 358.1183.

(6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^3-cephem-4-carboxylic Acid (4c) Diphenylmethyl (6*R*,7*R*)-7-amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^3-cephem-4-carboxylate[1] (**4b**, 2 g, 4.3 mmol) was added to a solution of anisole (2 g), and trifluoroacetic acid (4 g) in an ice bath, and the mixture was stirred at the same temperature for 30 min. Diisopropyl ether (100 ml) was added to the mixture, and the resultant precipitate was collected by filtration to give a crude product. This precipitate was dissolved in aqueous $NaHCO_3$ solution and purified by chromatography (YMC-343-7, 9% CH_3CN) to afford 400 mg of the sodium salt. The solid was dissolved in water and the solution was adjusted with 1 N HCl to pH 3. The resultant precipitate was collected by filtration to give 450 mg of **4c** (35% yield). An analytical sample was obtained after further purification, mp 169—171 °C (dec.). IR (Nujol): 1790, 1710, 1680 cm^{-1}. ^{1}H-NMR (Me_2SO-d_6) δ: 2.81 (3H, s, CH_3), 2.84 (3H, s, CH_3), 3.43 and 3.58 (2H, ABq, J=17 Hz, H-2), 4.01 and 4.98 (2H, ABq, J=12 Hz, CH_2O), 4.75 (1H, d, J=5 Hz, H-6), 5.99 (1H, d, J=5 Hz, H-7). *Anal.* Calcd for $C_{11}H_{15}N_3O_5$: C, 43.85; H, 5.02; N, 13.95. Found: C, 43.60; H, 5.04; N, 13.95.

(6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^2-cephem-4-carboxylic Acid Trifluoroacetate (5c) A solution of **4b** (10 g, 21.4 mmol) and triethylamine (2.5 g, 24.8 mmol) in dichloromethane (100 ml) was stirred at room temperature for 24 h. Dichloromethane (300 ml) and H_2O (200 ml) were added to the mixture, and the organic layer was separated. The extract was washed with water, then brine, and dried over $MgSO_4$. It was then concentrated *in vacuo* to yield a 2:3 mixture of **4b**/**5b**. Trifluoroacetic acid (20 ml) and anisole (5 ml) were added to the residue and the mixture was stirred for 1 h. Diisopropyl ether (500 ml) was added to the reaction mixture, and the resultant precipitate was collected by filtration. This precipitate was dissolved in 0.5% aqueous trifluoroacetic acid solution and purified by ODS chromatography (YMC-343-7, 0.5% trifluoroacetic acid) to give 1.8 g of **5c** as a pale yellow glass-like solid (28% yield). IR (Nujol): 1790, 1725, 1650 cm^{-1}. ^{1}H-NMR (CD^3OD) δ: 2.90 (3H, s, CH_3), 2.93 (3H, s, CH_3), 4.70 and 4.81 (2H, ABq, J=12 Hz, CH_2O), 4.97 (1H, d, J=4 Hz, H-6), 5.10 (1H, s, H-4), 5.44 (1H, d, J=4 Hz, H-7), 5.59 (1H, s, H-2). HR-MS *m*/*z*: Calcd for $C_{11}H_{15}N_3O_5S$: 302.0811 (M+H). Found: 302.0791.

Methyl (6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-formamido-Δ^3-cephem-4-carboxylate (2e) Methyl iodide (3 g, 21.1 mmol) was added to a solution of **1a** (5 g, 14.2 mmol) in DMF (40 ml). The reaction mixture was stirred for 1 h, diluted with EtOAc (400 ml), and washed with H_2O (200 ml) and then brine. The organic solution was dried over $MgSO_4$ and concentrated *in vacuo*. Diisopropyl ether (100 ml) was added to the residue, and the crystals were collected by filtration to afford 3.4 g of **2e** as colorless crystals (70% yield), mp 166—168 °C (dec.). IR (Nujol): 1790, 1720, 1690, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.91 (3H, s, NCH_3), 2.93 (3H, s, NCH_3), 3.46 and 3.60 (2H, ABq, J=18 Hz, CH_2O), 3.88 (3H, s, CO_2CH_3), 4.86 and 5.17 (2H, ABq, J=14 Hz, H-2), 5.01 (1H, d, J=5 Hz, H-6), 5.91 (1H, dd, J=5, 10 Hz, H-7), 6.40 (1H, d, J=10 Hz, CONH), 8.29 (1H, s, HCO). *Anal.* Calcd for $C_{13}H_{17}N_3O_6S$: C, 45.47; H, 4.99; N, 12.24. Found: C, 45.33; H, 4.90; N, 12.15. MS *m*/*z*: 344 (M+H).

Methyl (6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-formamido-Δ^2-cephem-4-carboxylate (3e) A solution of **2e** (3.4 g, 9.9 mmol) in a mixture of triethylamine (1.01 g, 10 mmol) and THF (40 ml) was stirred at room temperature for 18 h to give a 2:3 mixture of **2e**/**3e**. EtOAc (300 ml) and H_2O (200 ml) were added to this mixture, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by chromatography using silica gel with dichloromethane–EtOAc (19:1) to give 1.2 g of **3e** as a yellow oil (35% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.93 (6H, s, $N(CH_3)_2$), 3.82 (3H, s, CO_2CH_3), 4.68 (2H, s, CH_2O), 5.06 (1H, s, H-4), 5.32 (1H, d, J=4 Hz, H-6), 5.79 (1H, dd, J=4, 8 Hz, H-7), 6.47 (1H, s, H-2), 8.28 (1H, s, HCO).

Methyl (6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^2-cephem-4-carboxylate (5e) Methanesulfonic acid (56 mg, 0.58 mmol) was added to a solution of **3e** (100 mg, 0.29 mmol) in MeOH (1 ml) at 3 °C, and the mixture was stirred at the same temperature for 22 h. EtOAc (100 ml) and H_2O (10 ml) were added to this mixture; the whole was neutralized with aqueous $NaHCO_3$ solution, and the organic layer was separated, washed with H_2O, then brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by chromatography (YMC-343-7, 12% MeOH) to give 42 mg of **5e** as a yellow oil (46% yield). IR (Nujol): 1780, 1760, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.92 (6H, s, $N(CH_3)_2$), 3.79 (3H, s, CO_2CH_3), 4.60—4.70 (3H, m, CH_2O, H-6), 5.02 (1H, s, H-4), 5.22 (1H, d, J=4 Hz, H-7), 6.49 (1H, s, H-2). HR-MS *m*/*z*: Calcd for $C_{12}H_{17}N_3O_5S$: 315.0889. Found: 315.0855.

Kinetic Runs Substrates **4a**, **5a**, **d**, **e**, **f** were each dissolved in a solution of CH_3CN–phosphate buffer, or Britton–Robinson buffer or HCl aqueous solution (1:9, v/v) of pH 7, 5, 3, or 1, or in 1 M HCl at a concentration of 3×10^{-2} mM. The reaction mixture was stirred in a water bath equipped with a thermocontroller set at 30 °C. Substrates **4c** and **5c** were each dissolved in a solution of MeOH–pH 7 phosphate buffer (1:9, v/v) or 1 M HCl solution at a concentration of 3×10^{-2} mM. Experiments were carried out in a way similar to that described above.

The concentrations of **4a**, **c**, **5a**, **c**—**f** were determined by HPLC.

HPLC Conditions The HPLC conditions were as follows: apparatus, LC-10A system (Shimadzu Co., Kyoto, Japan) equipped with a UV detector (SPD-10A, Shimadzu), Rheodyne type 7125 injection valve and integrated data analyzer (C-R4A, Shimadzu). Stationary phase: AM312 5C18 packed column (YMC AM-312, Kyoto, Japan). Column temperature: room temperature. Detection: UV 254 nm. For **4a** and **5a** the mobile phase was CH_3CN : 0.1% aqueous ammonium acetate solution = 30 : 70 (v/v). Flow rate: 2.0 ml/min. Retention times (min): 14.2, 15.9 (**4a**), 12.4 (**5a**), and 8.0 (ethyl *p*-hydroxybenzoate: internal standard). For **4c** and **5c** the mobile phase was CH_3CN : 0.35% aqueous perchloric acid solution = 50 : 950 (v/v). Flow rate: 2.0 ml/min. Retention times (min): 9.6 (**4c**), 9.0 (**5c**) and 6.3 (anthranic acid: internal standard). For **5d** the mobile phase was CH_3CN : 0.1% aqueous ammonium acetate solution = 30 : 70 (v/v). Flow rate: 2.0 ml/min. Retention times (min): 16.0 (**5d**) and 8.0 (ethyl *p*-hydroxybenzoate: internal standard). For **5e** the mobile phase was CH_3CN : 0.1% aqueous ammonium acetate solution = 20 : 80 (v/v). Flow rate: 2.0 ml/min. Retention times (min): 8.3 (**5e**) and 17.2 (benzoic acid: internal standard). For **5f** the mobile phase was CH_3CN : 0.1% aqueous ammonium acetate solution = 32 : 68 (v/v). Flow rate: 2.0 ml/min. Retention times (min): 7.5 (**5f**) and 6.7 (ethyl *p*-hydroxybenzoate: internal standard)

Isolation of Degradation Products of 5a In 1 M HCl: A solution of **5a** (500 mg, 1.16 mmol) in 1 M HCl aqueous solution (15 ml) was stirred at 30 °C for 2 h. The reaction mixture was charged on an ODS column (YMC-343-7), and the column was eluted with 9% CH_3CN. The desired fractions were collected separately and lyophilized to give three samples as follows: the first fraction, 30 mg; the second fraction, 30 mg; and the third fraction, 110 mg. HPLC analysis and ^{1}H-NMR spectra showed that the compound of the third fraction was identical with **5c**. The first and second fractions were found to contain many substances by HPLC analysis.

In pH 7 Solution: A solution of **5a** (110 mg, 0.26 mmol) in a mixture of pH 7 phosphate buffer and CH_3CN (15 ml) was stirred at 30 °C for 12 h. The reaction mixture was charged on an ODS column (YMC-343-7) and the column was eluted with H_2O. Fractions containing the desired substances were collected and treated in a manner similar to that described above to afford 10 mg of **5c**.

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References

1) Negi S., Yamanaka M., Sugiyama I., Komatsu Y., Sasho M., Tsuruoka A., Kamada A., Tsukada I., Hiruma R., Katsu K., Machida Y., *J. Antibiot.*, **47**, 1057—1525 (1994).
2) Negi S., Yamanaka M., Komatsu Y., Tsuruoka., Tsukada I., Minami N., *Chem. Pharm. Bull.*, **43**, 1998—2000 (1995).
3) Cooker J. D., Eardley S., Gregory I., Hall M. E., Long A. G., *J. Chem. Soc.*, (*C*), **1966**, 1142—1151.
4) Saikawa I., Takano S., Momonoi K., Takakura I., Tanaka K., Kutani C., *Chem. Pharm. Bull.*, **33**, 5534—5538 (1985).
5) S. Negi., Yamanaka M., Sugiyama I., Komatsu Y., Tsuruoka A., Kamada A., Tsukada I., Machida Y., *Chem. Pharm. Bull.*, **43**, 1031 (1995).
6) Saikawa I., Tai M., Sakai H., Yamamoto Y., Sugimoto Y., Demachi K., Kanai K., Nakano J., Sadaki H., *Yakugaku Zasshi*, **106**, 452—465 (1986).
7) *a*) Sadaki H., Imaizumi H., Inaba T., Hirakawa T., Murotani Y., Watanabe Y., Minami S., Saikawa I., *Yakugaku Zasshi*, **106**, 129—146 (1986); *b*) Sakagami S., Atsumi K., Tamura A., Yoshida T., Nishikawa K., Fukatsu S., *J. Antibiot.*, **43**, 1047—1050 (1990).
8) Saikawa I., Tai M., Sugimoto Y., Imaizumi H., Takeda K., Sadaki H., *Yakugaku Zasshi*, **106**, 466—477 (1986).