Studies on Orally Active Cephalosporin Esters. III.[1] Effect of the 3-Substituent on the Chemical Stability of Pivaloyloxymethyl Esters in Phosphate Buffer Solution

Masao MIYAUCHI,* Hideshi KURIHARA, Koichi FUJIMOTO, Isao KAWAMOTO, Junya IDE and Hideo NAKAO

Sankyo Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140, Japan. *Received December 9, 1988*

The effect of substituents at the C-3 position on the degradation kinetics of the pivaloyloxymethyl (POM) ester of Δ^3 cephalosporin in phosphate buffer solution (pH 6—8) was investigated. In the degradation, the isomerization process to the Δ^2 ester was the rate-determining step. In this study, the logarithm of the isomerization rate to the Δ^2 ester ($\log k_{12}$) correlated with the carbon-13 nuclear magnetic resonance chemical shift difference value at C-3 and C-4 of the Δ^3 ester ($\Delta\delta$ (4–3)). The energy level of the lowest unoccupied molecular orbital (LUMO) of the Δ^3 esters also correlated with $\log k_{12}$. The electronic properties at the C-2 position had no effect on the isomerization reaction. On the other hand, the logarithm of the isomerization rate back to the Δ^3 ester ($\log k_{21}$) correlated with the van der Waals volume (MV) of the 3-substituent. These results show that the substituent at the C-3 position influences mainly the electronic structure of the conjugated π-bond system ($C_3=C_4-C_{4'}=O$) and consequently affects the feasibility of isomerization to the Δ^2 ester, *i.e.*, the stability to degradation.

Keywords cephalosporin; substituent effect; isomerization; deprotonation; ^{13}C-NMR; LUMO; MNDO; Δ^2 cephalosporin; prodrug

In the previous paper[1] we reported on the degradation kinetics of pivaloyloxymethyl (POM) esters of Δ^3 cephalosporins with various substituents at the C-3 position in phosphate buffer, in which the isomerization process from Δ^3 to Δ^2 ester is the rate-determining step. Rate constants of the isomerization varied with the 3-substituents in the range of 0.059—0.600 h^{-1}.

The effect of the 3-substituents on the reactivity of the β-lactam ring of cephalosporins has been investigated in detail by many groups. A number of parameters have been proposed to correlate with the reactivity.[2] However, the effect of the 3-substituents on the isomerization of the double bond at the C-3 position of cephalosporin esters is not known yet.

In this paper the effect of the 3-substituents on the degradation kinetics of the cephalosporin esters is discussed quantitatively based on the results of carbon-13 nuclear magnetic resonance (^{13}C-NMR) and semi-empirical molecular orbital calculations.

Results and Discussion

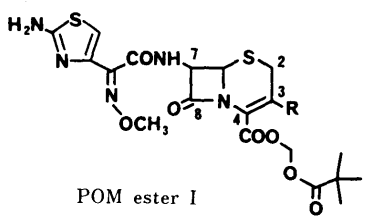
Cephalosporin POM esters used in this study are depicted in Chart 1. As discussed in the previous paper, they degraded in phosphate buffer solution according to the kinetic model shown in Chart 2.[1] First-order rate constants k_{mn} calculated for the degradation at pH 6.86 were employed in the following discussions.

3-Substituent Effect on Isomerization Rate k_{12} The mechanism of isomerization between the Δ^3 ester and Δ^2 ester is postulated to be as shown in Chart 3. The isomerization rate from the Δ^3 to the Δ^2 ester, k_{12}, should depend on the deprotonation rate at the C-2 position, *i.e.*, the feasibility of enolization of the α,β-unsaturated carbonyl moiety including the positions C-2, C-3, C-4, and C-4'. ^{13}C-NMR spectra were measured to estimate the effect of 3-substituents on the electronic status at these positions.[3] The chem-

TABLE I. ^{13}C-NMR Chemical Shifts at the C-2, C-3, C-4 and C-4' Positions and $\Delta\delta$ (4–3)[a]

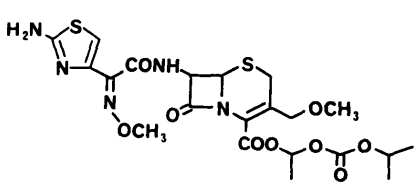
No.	3-Substituent	C-2	C-3	C-4	C-4'	$\Delta\delta$ (4–3)[b]
Ia	CH_2OCH_3	26.3	133.0	123.3	160.3	− 9.7
Ib	$CH_2OCH_2CH_3$	26.5	133.5	123.0	160.3	−10.5
Ic	CH_2OAc	26.8	128.5	124.8	160.1	− 3.7
Id	CH_2OIox	26.6	128.7	124.7	160.1	− 4.0
Ie	CH_2SCH_3	28.3	133.4	123.5	160.4	− 9.9
If	CH_2SCH_2CN	27.9	129.4	124.8	160.4	− 4.6
Ig	CH_2STz	28.7	130.5	124.8	160.4	− 5.7
Ih	CH_2STh	26.9	129.7	125.5	159.9	− 4.2
Ii	CH_2Tet	26.9	124.9	126.0	160.0	+ 1.1
Ij	CH_3	30.8	135.2	122.0	160.7	−13.2
Ik	H	24.4	121.9	127.3	160.0	+ 5.4

a) Spectra were taken in $CDCl_3$ at 67.7 MHz with tetramethylsilane as an internal standard. Chemical shifts are given in δ (ppm). *b*) $\Delta\delta$ (4–3) indicates the chemical shift difference between C-4 and C-3.



-R
Ia -CH_2OCH_3
Ib -$CH_2OCH_2CH_3$
Ic -CH_2OCCH_3 (O)
Id -CH_2OIox
Ie -CH_2SCH_3
If -CH_2SCH_2CN

-R
Ig -CH_2STz
Ih -CH_2STz
Ii -CH_2Tet
Ij -CH_3
Ik -H



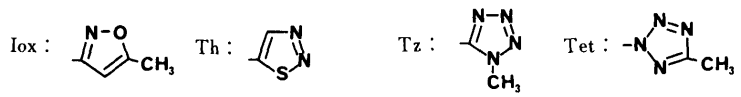


Chart 1

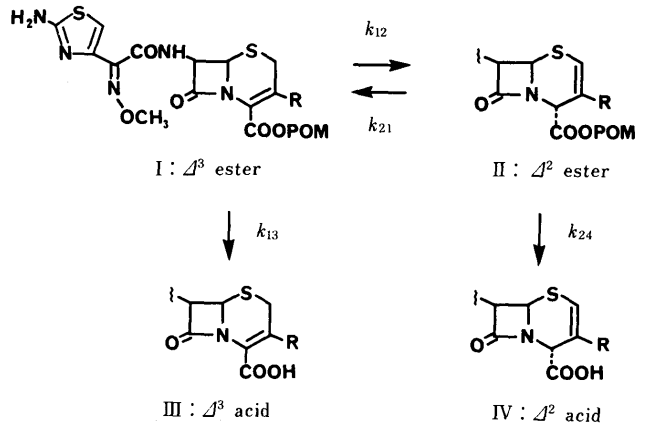


Chart 2

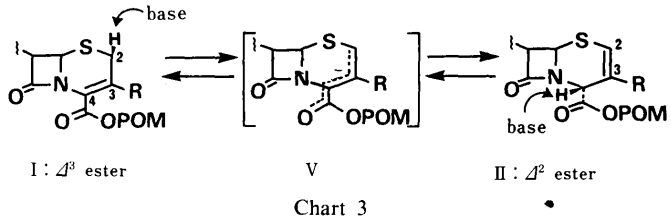


Chart 3

ical shifts at the C-2, C-3 and C-4 positions were markedly affected by the 3-substituents (see Table I).

The difference of chemical shifts at the C-3 and C-4 positions, $\Delta\delta$(4–3), can be used as an indicator of the inductive effect of 3-substituents because the polarity of the $C_3=C_4$ double bond depends on the inductive property of 3-substituents.[2b,c,g-i] As can be seen in Fig. 1, the difference of chemical shifts, $\Delta\delta$(4–3), shows a fairly good linear correlation with the logarithm[4] of the isomerization rate constant $\log k_{12}$. Equation 1 indicates the regression between $\log k_{12}$ and the parameter; 95% confidence intervals are given in parentheses along with the correlation coefficient (r), standard deviation (s) and sample number (n). Compound Ik (R=H) which widely deviates to the region of large parameter value was omitted from the regression; the deviation could be attributed to the structural factor of Ik because it has no methylenic carbon at the C-3 position.

$$\log k_{12} = -0.2225 + 0.0667\Delta\delta\ (4\text{–}3) \quad (1)$$
$$(\pm 0.1487)(\pm 0.0196)$$
$$n=10,\ s=0.108,\ r=0.941$$

The chemical shift at the C-2 position δ(C2), which was expected to indicate the feasibility of deprotonation at this position, did not correlate with the isomerization rate $\log k_{12}$.

Semi-empirical molecular orbital calculations were made for model structures VIa—k in Chart 4 to search for correlations with physicochemical properties. Initial coordinates of the model VIa were constructed on the basis of the X-ray crystallographic result for (1*S*)-1-(isopropyloxycarbonyloxy)ethyl 7β-amino-3-methoxymethyl-3-cephem-4-carboxylate hydrochloride.[5] Initial coordinates of other models were constructed by replacing the methoxymethyl group of VIa with the desired 3-substituents, which were made up by using standard or X-ray crystallographic data. Computations were carried out with geometry optimizations of the

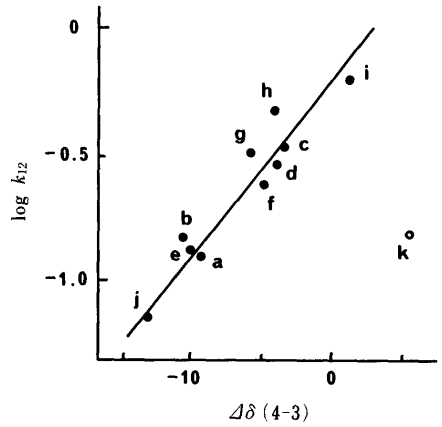


Fig. 1. Correlation between $\log k_{12}$ and $\Delta\delta$ (4–3)

k_{12} (h^{-1}) is the rate constant of isomerization from the Δ^3 ester I to the Δ^2 ester II. $\Delta\delta$ (4–3) (ppm) is the difference of ^{13}C-NMR chemical shifts for C-3 and C-4 in the Δ^3 ester I. Compound Ik is omitted from the regression.
$\log k_{12} = -0.2225 + 0.0667\Delta\delta$ (4–3) $n=10$, $s=0.108$, $r=0.941$.

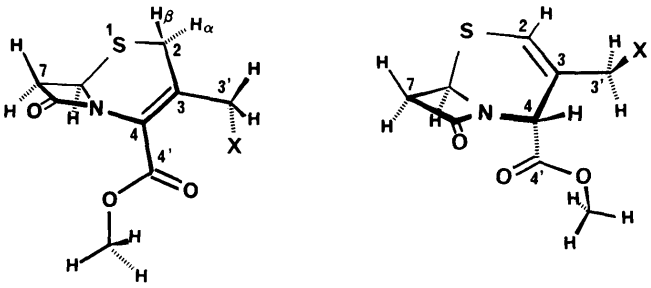


Chart 4

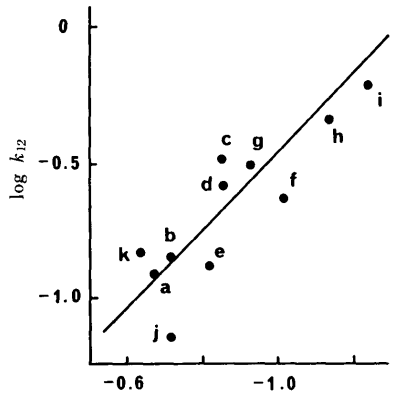


Fig. 2. Correlation between $\log k_{12}$ and LUMO Energy Levels

k_{12} (h^{-1}) is the rate constant of isomerization from the Δ^3 ester I to the Δ^2 ester II. LUMO energy levels were computed by the MNDO method for model structures VIa—k.
$\log k_{12} = -1.718 - 1.209$ LUMO $n=11$, $s=0.182$, $r=0.801$.

$C_3=C_4$ double bond and the 3-substituent, including their peripheral regions, using an MNDO method.[6]

Among the molecular orbital information computed for the model structures, the energy levels of the lowest unoccupied molecular orbital (LUMO) were found to correlate with $\log k_{12}$ as shown in Fig. 2. Equation 2 indicates the regression. The LUMO energy level of each model compound and the frontier electron population of each

Table II. LUMO Energy Levels and Frontier Electron Populations Computed for Model Structures VIa—k[a]

No. 3-Substituent	VIa CH_2OCH_3	VIb $CH_2OCH_2CH_3$	VIc CH_2OAc	VId CH_2OIox	VIe CH_2SCH_3	VIf CH_2SCH_2CN	VIg CH_2STz	VIh CH_2STh	VIi CH_2Tet	VIj CH_3	VIk H
LUMO energy level											
(eV)	−0.689	−0.714	−0.849	−0.851	−0.811	−1.018	−0.919	−1.154	−1.224	−0.708	−0.636
Frontier electron population											
H_α	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
H_β	0.025	0.024	0.024	0.024	0.024	0.023	0.022	0.022	0.021	0.026	0.029
C2	0.010	0.009	0.010	0.009	0.008	0.007	0.007	0.007	0.006	0.011	0.014
C3	0.787	0.797	0.773	0.771	0.761	0.735	0.703	0.727	0.697	0.803	0.818
C4	0.619	0.600	0.630	0.646	0.616	0.623	0.666	0.616	0.692	0.594	0.575
C4′	0.192	0.201	0.188	0.178	0.181	0.175	0.150	0.170	0.149	0.196	0.217
O[b]	0.158	0.161	0.158	0.153	0.151	0.147	0.135	0.147	0.137	0.161	0.172
C3′	0.007	0.006	0.009	0.012	0.013	0.020	0.041	0.016	0.049	0.001	—

a) MO calculation was performed using an MNDO method. b) Ester carbonyl oxygen.

atom in the LUMO are listed in Table II.[7] LUMO of each model structure was shown to consist mainly of the conjugated π-bond system ($C_3=C_4-C_{4'}=O$) and to be perturbed from the $C_{3'}$-X σ-bond which is down for the "α-face." These results indicate that inductive 3-substituents, which should lower the LUMO energy levels through the perturbation, increase the isomerization rate from Δ^3 to Δ^2 ester since the LUMO energy levels should influence the feasibility of the initial stage of the isomerization process in which the proton at the C-2 position is taken off and the intermediate enolate anion V is constructed from the C_2-H_β σ-bond and π-bond system of the LUMO.[7b] It is also indicated that the axial proton (H_β) having the larger coefficient may be removed in the isomerization process.

$$\log k_{12} = -1.718 - 1.209\,\mathrm{LUMO} \quad (2)$$
$$(\pm 0.605)(\pm 0.681)$$
$$n=11,\ s=0.182,\ r=0.801$$

The calculated net atomic charges at the C-2 carbon and at the H-2$_\beta$ hydrogen did not correlate with the isomerization rate $\log k_{12}$ or the ^{13}C-NMR chemical shift δ(C2).

These results indicate that the isomerization rate k_{12} is markedly influenced by the electron-inductive property of the substituent at the C-3 position. The electronic structure of the C_2–$C_3=C_4$–$C_{4'}=O$ moiety, especially the energy level of the reactive frontier orbital (LUMO), was found to be an important factor which controls the feasibility of isomerization from the Δ^3 to the Δ^2 ester. The electronic property at the C-2 position estimated from the MNDO method and the ^{13}C-NMR chemical shift seemed to be unrelated to the feasibility of deprotonation at the C-2 position.

Since this isomerization process is the rate-determining step of degradation, the chemical stability of cephalosporin esters may be estimated from these experimental or computed parameters without measurements of the degradation rate constants.

3-Substituent Effect on Isomerization Rate k_{21} The isomerization rate from the Δ^2 ester back to the Δ^3 ester, k_{21}, did not correlate with $\Delta\delta$(4–3). The electronic effect of the 3-substituent, therefore, is no more important in this process than in that from the Δ^3 to Δ^2 ester described above. On the other hand, the steric parameter, van der Waals volume, of the 3-substituent (MV) linearly correlated with the logarithm of the rate constant k_{21}, as shown in Fig.

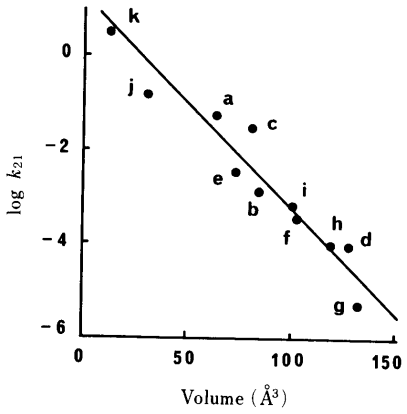


Fig. 3. Correlation between $\log k_{12}$ and van der Waals Volume of the 3-Substituent

k_{21} (h^{-1}) is the rate constant of isomerization from the Δ^2 ester II to the Δ^3 ester I. The van der Waals volumes were calculated using TRIBBLE.
$\log k_{21} = 0.809 - 4.068$ MV/100 $n=11$, $s=0.535$, $r=0.951$.

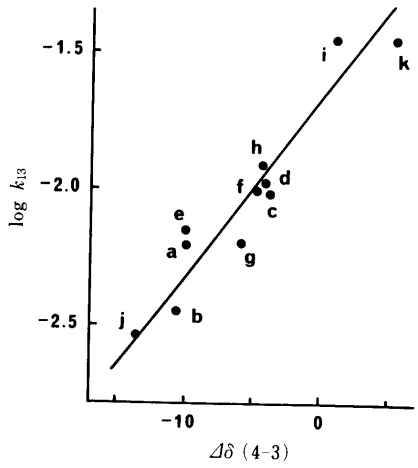


Fig. 4. Correlation between $\log k_{13}$ and $\Delta\delta$ (4–3)

k_{13} (h^{-1}) is the rate constant of hydrolysis from the Δ^3 ester I to the Δ^3 acid III.
$\log k_{13} = -1.7064 + 0.0605\Delta\delta$ (4–3) $n=11$, $s=0.116$, $r=0.948$.

3.[8] Equation 3 indicates the regression between $\log k_{21}$ and MV value. This observation can be interpreted as follows. The bulky 3-substituent sterically interrupts deprotonation at the C-4 position of the Δ^2 ester in which the 3′-methylene substituent is directed to the same "β-face" as the proton at the C-4 position and/or there is large steric repulsion

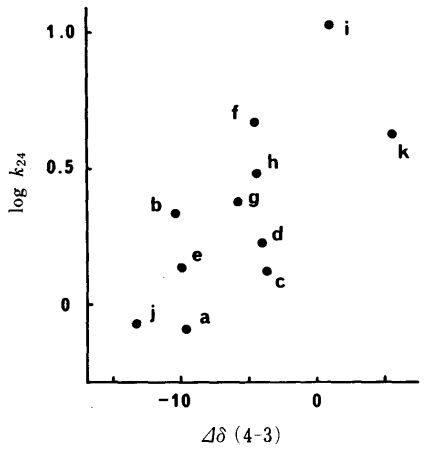


Fig. 5. Relation between $\log k_{24}$ and $\Delta\delta$ (4–3)
k_{24} (h^{-1}) is the rate constant of hydrolysis from the Δ^2 ester II to the Δ^2 acid IV.

between the bulky 3-substituent and the C-4 ester group in the Δ^3 isomer compared to that in the Δ^2 isomer, which thermodynamically favors the formation of the Δ^2 isomer from the intermediate V (see Chart 4).

$$\log k_{21} = 0.809 - 4.068\,\mathrm{MV}/100 \quad (3)$$
$$(\pm 0.909)(\pm 0.994)$$
$$n = 11,\ s = 0.535,\ r = 0.951$$

3-Substituent Effect on the Other Rates k_{13} and k_{24} The hydrolysis rate to the Δ^3 acid from the Δ^3 ester, k_{13}, had a linear correlation with the electronic parameters, $\Delta\delta$(4–3), but the hydrolysis rate to the Δ^2 acid from the Δ^2 ester, k_{24}, did not (Figs. 4 and 5).

The rate constants k_{12} and k_{13} for the process from the Δ^3 ester were strongly affected by the electronic properties of the 3-substituent. The contribution of the steric factor to this process was negligible. On the other hand, the electronic effect of the 3-substituent on the rate constants k_{21} and k_{24} for the process from the Δ^2 ester was weak because the 3-substituent in the Δ^2 ester lacks direct conjugation with the 4′-ester carbonyl function.

Conclusions

These results indicate that the 3-substituent of cephalosporin esters has a significant effect on the stability to chemical hydrolysis *via* the isomerization from the Δ^3 to the Δ^2 ester. The effect depended mainly on the electron-inductive property of the substituent. Decrease of the inductive property would make cephalosporin esters more stable to isomerization but concomitantly diminish the reactivity of the parent β-lactams.[9] The methoxymethyl group ($-CH_2OCH_3$) of cefpodoxime proxetil (CPDX-PR in Chart 1), which contributes to both the stability of the ester and the reactivity of the β-lactam, is a well balanced 3-substituent for an ester-type prodrug of cephalosporin.[10] The effect of the 3-substituent on gastrointestinal absorption will be reported elsewhere.

Experimental

Materials Cephalosporin POM esters employed in this study are listed in Chart 1. They are the same as those used in the preceding paper.[1]

NMR ^{13}C-NMR spectra were determined on a JEOL GX-270 spectrometer using tetramethylsilane as an internal standard.

Calculations The MNDO and TRIBBLE computations were carried out using a VAX 11/780 computer.

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