^{1}H NMR Study of Degradation Mechanisms of Oxacephem Derivatives with Various 3′-Substituents in Alkaline Solution

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The degradation process of oxacephems with various 3′-substituents (1–9) in alkaline solution was examined by ^{1}H NMR spectroscopy, and the structures of two types of degradation products were determined: the hydrolysis products 10–14 having the cleaved β-lactam ring and the remaining 3′-substituents, and the *exo*-methylene compounds 15 and 16 having the cleaved β-lactam ring and the expelled 3′-substituents. The oxacephems were found to decompose, giving the former compounds that subsequently decomposed to the latter compound. Although the ratios of the formation of the *exo*-methylene compound 15 relative to the other degradation products depended on the leavability of the 3′-substituents, there was little correlation between the relative yields and the β-lactam reactivity. Thus, the expulsion of the leaving group at the 3′-position was concluded to be not involved in the nucleophilic attack on the β-lactam carbonyl.

In order to understand the factors affecting the antibacterial activity of β-lactam antibiotics, many studies have been done on the cleavage reactions of their β-lactam ring in alkaline aqueous solution and on those catalyzed by β-lactamases with use of a variety of analytical methods, such as iodometry, pH–stat titration, UV spectroscopy, and microbiological assay.[1] However, these methods are mostly concerned with the disappearance of the β-lactam antibiotics and accordingly, have not provided precise information about the structure of the degradation products as well as the effects of the leavability of the 3′-substituents on the degradation rates of cephalosporins.[2] Whether or not the leavability of the 3′-substituents affects the rates of the β-lactam cleavage has not been determined (see, Scheme I).

The concurrent occurrence of β-lactam ring cleavage, release of the leaving groups, and loss of the antibacterial activity of 3′-azido- and 3′-pyridiniocephalosporins have been reported, implying that degradation occurs without formation of an intermediate possessing both the cleaved β-lactam and the 3′-leaving groups.[3] Also, the importance of the leavability of the 3′-substituent in determining the β-lactam reactivity has been suggested on the basis of molecular orbital calculations.[4] On the other hand, good correlations of the β-lactam reactivity only with either the σ_I value[5] of the 3′-substituents or the polarization of the C_3–C_4 double bond, indicated by the ^{13}C NMR chemical shift differences,[1e,2] suggest that the expulsion of the 3′-substituents does not occur in the transition state of the alkaline hydrolysis of cephem compounds. Also, observation of the consecutive reaction during β-lactamase decomposition of cephaloridine and its derivative had led to the conclusion that there exists an intermediate with the remaining 3′-leaving group.[6] The aminolysis rates are independent of the expulsion of the leaving groups at the 3′-position of some cephalosporins.[7] Recently, intervention of the intermediate during aminolysis of cephamycin and a consecutive reaction mechanism have been suggested on the basis of a ^{13}C NMR study.[8] This method is very useful for determining the structures of these kinds of degradation products, although it is less accurate than the ^{1}H NMR method[9] for quantitative analysis.

Scheme I

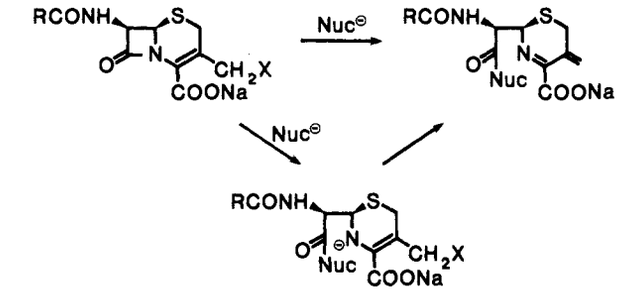
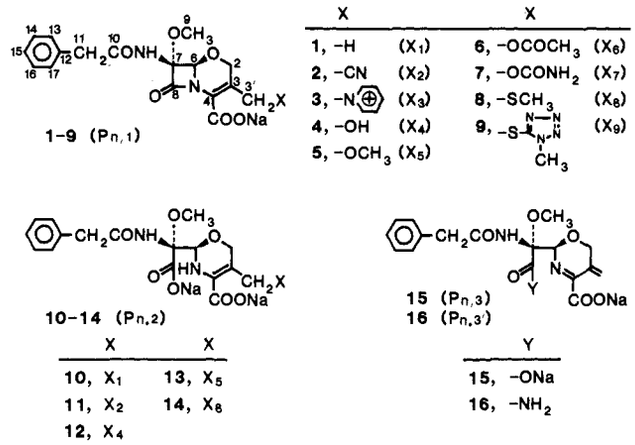


Chart I



(1) (A) Alkaline degradation: (a) Indelicato, J. M.; Norvilas, T. T.; Pfeiffer, R. R.; Wheeler, W. J.; Wilham, W. L. *J. Med. Chem.* **1974**, *17*, 523. (b) Everett, J. R.; Jennings, D. R.; Woodnutt, G.; Buckingham, M. J. *J. Chem. Soc., Chem. Commun.* **1984**, 894. (c) Yamana, T.; Tsuji, A. *J. Pharm. Sci.* **1976**, *65*, 1563. (d) Narisada, M.; Yoshida, T.; Ohtani, M.; Ezumi, K.; Takasuka, M. *J. Med. Chem.* **1983**, *26*, 1577. (e) Nishikawa, J.; Tori, K.; Takasuka, M.; Onoue, H.; Narisada, M. *J. Antibiot.* **1982**, *35*, 1723. (f) Konecny, J.; Felber, E.; Gruner, J. *J. Antibiot.* **1973**, *26*, 135. (g) Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144. (h) Proctor, P.; Gensmantel, N. P.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1185. (i) Morris, J. J.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1980**, 212. (B) Enzymatic hydrolysis: (j) Frere, J.-M.; Kelly, J. A.; Klein, D.; Ghuysen, J.-M. *Biochem. J.* **1982**, *203*, 223. (k) Anderson, E. G.; Pratt, R. F. *J. Biol. Chem.* **1981**, *25*, 11401. (l) Anderson, E. G.; Pratt, R. F. *J. Biol. Chem.* **1983**, *2*, 13120. (m) Murakami, K.; Takasuka, M.; Motokawa, K.; Yoshida, T. *J. Med. Chem.* **1981**, *24*, 88.

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(3) O'Callaghan, C. H.; Kirby, S. M.; Morris, A.; Waller, R. E.; Duncombe, R. E. *J. Bacteriol.* **1972**, *110*, 988.

(4) (a) Boyd, D. B.; Herron, D. K.; Lunn, W. H. W.; Spitzer, W. A. *J. Am. Chem. Soc.* **1980**, *102*, 1812. (b) Boyd, D. B. *J. Med. Chem.* **1983**, *26*, 1010.

(5) Boyd, D. B. *J. Med. Chem.* **1984**, *27*, 63.

(6) Faraci, W. S.; Pratt, R. F. *J. Am. Chem. Soc.* **1984**, *106*, 1489.

(7) Page, M. I.; Proctor, P. *J. Am. Chem. Soc.* **1984**, *106*, 3820.

(8) Grabowski, E. J.; Douglas, A. W.; Smith, G. B. *J. Am. Chem. Soc.* **1985**, *107*, 267.

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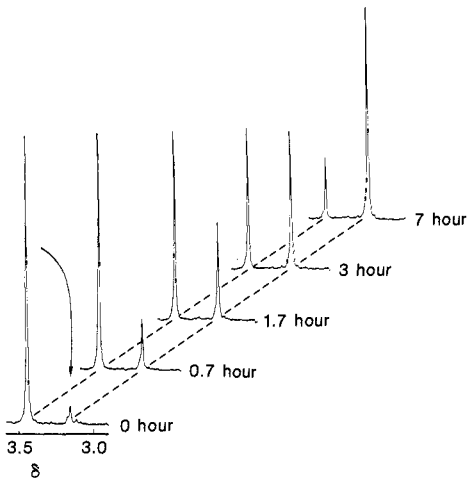


Figure 1. Change in intensities of ^{1}H NMR signals during degradation of **9** in deuteriated carbonate buffer solution (pD 10.4, 35 °C). Upfield shifts of signals of methoxy at δ 3.47 to those at δ 3.17 are indicated.

Since the ^{1}H NMR method yields detailed information about degradation processes of the β-lactam antibiotics by their kinetic analysis, we tried to estimate the effects of the leavability of the 3′-substituents on the reactivity of the β-lactam ring by studying the alkaline hydrolysis of oxacephems with various 3′-substituents (**1–9**). Here we describe our results of our ^{1}H NMR study on the rate constants of the alkaline degradation of oxacephems **1–9** (Chart I), the determination of the structures of the degradation products, i.e., the hydrolysis products **10–14** and the *exo*-methylene compounds **15** and **16**, and the consecutive mechanism of their formation.

Results and Discussion

Determination of Rate Constants of β-Lactam Cleavage by UV and ^{1}H NMR Spectroscopies. The rate constants k_{obsd}^{UV} and k_{obsd}^{NMR} of the β-lactam ring cleavage of oxacephems **1–9** in alkaline aqueous solution were determined from the disappearance of the β-lactam ring from the UV and the ^{1}H NMR spectra, respectively. For example, the time course of degradation of **9** indicated by the change in intensities of the typical signals of the ^{1}H NMR spectra is shown in Figure 1. The peaks at δ 3.47 and 3.17 correspond to the 7α-methoxy carbons of **9** and its degradation products, respectively. The upfield shifts of the methoxy group (from δ ca. 3.2 to ca. 3.5), which are generally observed for any β-lactam cleavage, are remarkable. The resulting k_{obsd}^{UV} and k_{obsd}^{NMR} values for the oxacephems **1–9** can be compared in Table I.

Determination of Structure of Degradation Products. Degradations of **1–9** in NMR tubes, followed at pD 10.4 and 35 °C, yielded two types of alkaline degradation products: the hydrolysis products **10–14** and the *exo*-methylene compounds **15** and **16**. The structures of compound **15** and **16** were confirmed by identification of their ^{1}H NMR spectra in the buffer solution with those of the corresponding authentic samples, which were prepared separately in a pure form and characterized by ^{1}H NMR, ^{13}C NMR, IR, and UV spectroscopies and elemental analysis. The other degradation products were not isolated and their structures were assigned from the ^{1}H NMR spectra of the reaction mixtures. The chemical shifts and assignments of the ^{1}H NMR signals are listed in Table II. Typical ^{1}H NMR spectra of degradation products of **5** are shown in Figure 2. Compounds **12–14** gradually became degraded further to the secondary degradation products at pD 10.4 and 35 °C, although compounds **10** and **11** were sufficiently stable under the same conditions. The structure was determined as **15** for only one of the sec-

Table I. Pseudo-First-Order Rate Constants, k_{obsd},[a] of β-Lactam Cleavage of Oxacephems **1–9** in Alkaline Solution Determined by UV and ^{1}H NMR Spectroscopies

compd	$k_{obsd}^{NMR} \times 10^2$ h^{-1}	$k_{obsd}^{UV} \times 10^2$ h^{-1}
1	4.84	3.46
2	28.86	23.44
3	125.9	138.9
4	16.41	13.9
5	20.38	14.45
6	25.5,[b] 30.8[c]	28.55[b]
7	45.0,[d] 22.8[c]	23.00
8	11.04	10.09
9	20.43	19.20

[a] The rate constants for β-lactam cleavage were determined in 0.008 M carbonate buffer solution (pH 10.0, μ = 0.1, 35 °C) by UV and in 0.2 M deuteriated carbonate buffer solution (pD 10.4, μ = 0.5, and 35 °C by ^{1}H NMR. [b] With this compound, β-lactam cleavage by OH^- ion and deacetylation took place. The k_{obsd}^{UV} and k_{obsd}^{NMR} values were calculated from the apparent pseudo-first-order kinetics observed up to 3 h. [c] Value is corrected for parallel reactions. See text. [d] For this compound, degradations by both the OH^- ion and ammonia produced by the parallel reaction took place (see text). Thus, the value varies depending on the initial concentration of **7**. The data was obtained at a concentration of 1 × 10^{-2} M.

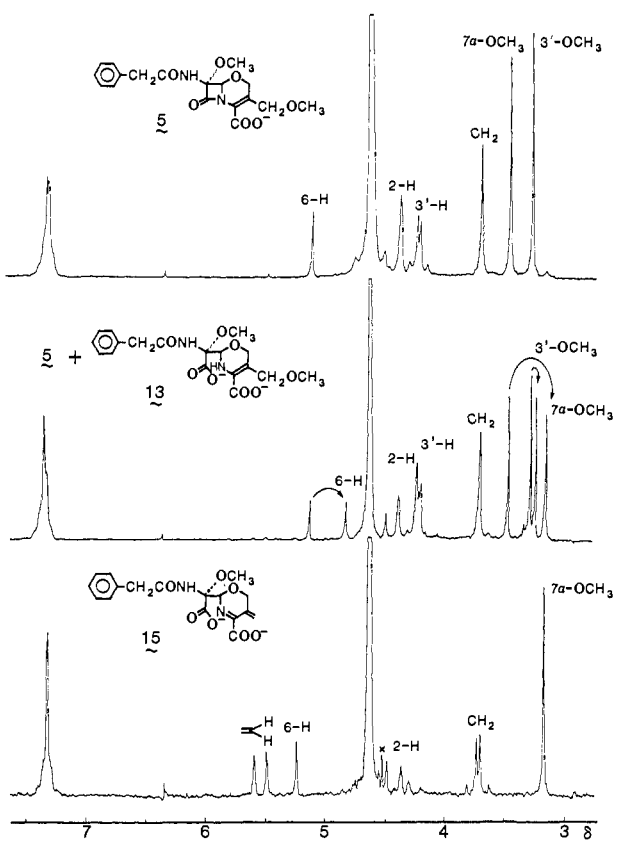


Figure 2. ^{1}H NMR spectra of **5** and its degradation products **13** and **15** in D_2O.

(9) (A) A semiquantitative analysis of the activity of the β-lactamases using iodometry and ^{1}H NMR spectroscopy has been reported: (a) Kono, M.; Ohara, K.; Shiomi, Y. *Microbios Lett.* **1981**, *17*, 67. (b) Kono, M.; Ohara, K.; Shiomi, Y. *Antimicrob. Agents Chemother.* **1980**, *17*, 16. (B) ^{1}H NMR studies of the degradation of penicillin G in acidic and aqueous solutions have been reported: (c) Mitsumori, F.; Arata, Y.; Fujiwara, S.; Muranaka, M.; Horiuchi, Y. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 3164. (d) Degelaen, J. P.; Loukas, S. L.; Feeney, J.; Roberts, G. C. K.; Burgen, A. S. V. *J. Chem. Soc., Perkin Trans 2* **1979**, 86.

Table II. ^1H Chemical Shifts[a] and Assignments of Various 3′-Substituted Oxacephems 1–9 and Their Degradation Products 11–16 (in D_2O)

compd	2-H	6-H	3′-CH_2	OCH_3	$PhCH_2$	Ph	others
1	4.27 d, 4.31 d (J = 17.6 Hz)	5.10	1.81	3.47	3.71	7.3–7.5	
2	4.37 d, 4.45 d (J = 17.6 Hz)	5.15	3.61	3.48	3.71	7.3–7.5	
3	4.13 d, 4.42 d (J = 17.6 Hz)	5.18	5.20 d, 5.69 d (J = 14.6 Hz)	3.51	3.67	7.29	8.06, 8.58, 8.93
4	4.44	5.13	4.26 d, 4.29 d (J = 13.7 Hz)	3.47	3.71	7.3–7.5	
5	4.38 d, 4.42 d (J = 17.5 Hz)	5.15	4.21 d, 4.28 d (J = 12.6 Hz)	3.47	3.71	7.3–7.5	3.29
6	4.39 d, 4.41 d (J = 17.6 Hz)	5.13	4.75 d, 4.91 d (J = 12.9 Hz)	3.48	3.71	7.3–7.5	2.08
7	4.38 d, 4.41 d (J = 17.5 Hz)	5.13	4.72 d, 4.89 d (J = 13.0 Hz)	3.47	3.71	7.3–7.5	
8	4.35 d, 4.58 d (J = 17.3 Hz)	5.16	3.26 d, 3.76 d (J = 13.5 Hz)	3.47	3.71	7.3–7.5	2.02
9	4.41 d, 4.54 d (J = 17.4 Hz)	5.10	4.01 d, 4.21 d (J = 13.6 Hz)	3.46	3.70	7.3–7.5	4.02
10[b]	4.04 d, 4.14 d (J = 16.4 Hz)	4.72	1.74	3.17	3.69 d, 3.75 d (J = 15.8 Hz)	7.3–7.5	
11[b]	4.19 d, 4.28 d (J = 15.8 Hz)	4.85	*c*	3.17	3.70 d, 3.75 d (J = 16.0 Hz)	7.3–7.5	
12[b]	(4.26)	4.81	(4.19)	3.17	(3.71)	7.3–7.5	
13[b]	(4.25)	4.84	(4.21)	3.17	(3.71)	7.3–7.5	3.25
14[b]	4.25 d, 4.32 d (J = 15.5 Hz)	4.83	3.50 d, 3.63 d (J = 13.0 Hz)	3.17	3.70 d, 3.75 d (J = 16.0 Hz)	7.3–7.5	2.01
15	4.35 d, 4.53 d (J = 13.8 Hz)	5.23	5.50, 5.60	3.17	3.68 d, 3.77 d (J = 15.7 Hz)	7.3–7.5	
16	4.41 d, 4.57 d (J = 13.8 Hz)	5.26	5.57, 5.66	3.24	3.72 d, 3.80 d (J = 15.3 Hz)	7.3–7.5	

[a] ^1H NMR spectra were recorded on a Varian XL-200 NMR spectrometer (200.057 MHz) at ordinary probe temperature (23 °C) in 5-mm spinning tubes in D_2O (internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate reference, δ 0.0). The concentration was about 1×10^{-2} M. Typical FT NMR measurement parameters were follows: spectral width, 2401.0 Hz; pulse width, 6 μs (flipping angle 45°); acquisition time, 5 s; number of data points, 24008. The chemical shifts of 1–9, 15, and 16 measured in a carbonate buffer solution (pD 10.4, 35 °C) were identical with those in D_2O solution. [b] ^1H NMR chemical shifts of 10–14 were estimated from the spectra of mixtures containing these compounds. The chemical shifts shown in parentheses are not accurate. [c] The 3′-methylene protons appear to be deuteriated in the buffer solution: the ^1H NMR peaks were not observed.

Table III. Patterns of Degradations and Classification of 1–9

class	compd	rel molar concn of degradation products,[a] % $P_{n,2}$	15	$P_{n,4}$[b]
1	3	0	100	0
	6	*c*	76[d]	*c*
	7	0	100[e]	0
	9	0	100	0
2	4	9	32	59
	5	30	27	43
	8	37	26	37
3	1	100	0	0
	2	100	0	0

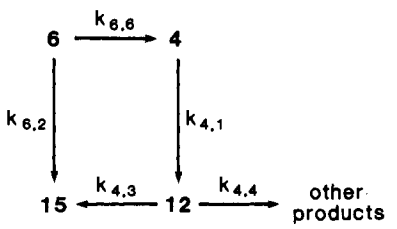
[a] The values were determined as the relative molar concentration of the degradation products when the β-lactam antibiotics ($P_{n,1}$) could not be observed on ^1H NMR spectra. [b] By difference, the value was calculated which corresponds to the total concentration of various unidentified secondary degradation products. [c] Combined yield of $P_{4,2}$ and $P_{4,4}$ was about 24%. [d] Parallel formation of 4 may decrease this value. [e] A mixture of 15 and 16.

ondary degradation products.

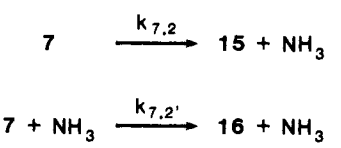
The degradation patterns of 1–9 are shown in Table III. Since the extent of the formation of the *exo*-methylene compound 15 appears to vary with the leavability of the 3′-substituents, the examined oxacephems 1–9 may be classified into the three classes given in the table.

Examination of Parallel Reactions. ^1H NMR spectroscopy afforded an efficient method for following the time courses of the degradation reactions and showed that 6 and 7 undergo parallel reactions during their degradations. The change in the peak intensities of the representative signals of the reaction mixtures of 6 led to the conclusion that the parallel reactions shown in Scheme II occur. The concentration changes of 4, 6, and 15 were determined from the peak intensities of acetylmethyl in

Scheme II



Scheme III



6 (δ 2.08), those of *exo*-methylene and 6α-H in 15 (δ 5.50, 5.60, and 5.23, respectively), those of 6α-H in 4 and 6 (both at δ 5.13), and those of 7α-methoxy in 4 and 6 (both at δ 3.47). The concentration change of 12 could not be determined because the signal of 6α-H in 12 overlapped with those of the 3-methylene in 6. The time courses found for the formation of 4 and 15 from 6 are shown in Figure 3.

Compound 7 was degraded to give the two *exo*-methylene compounds 15 and 16. The presence of 15 in the reaction solution was confirmed by comparison of its ^1H NMR spectrum with that of an authentic sample. Formation of the second *exo*-methylene compound 16 was accelerated by an increase in the initial concentration of 7. Compound 16 was presumed to be formed by cleavage of the β-lactam ring with ammonia, which had been formed from the cleaved 3′-carbamoyloxy group. The structure of 16 was verified by comparing its ^1H NMR spectrum with that of an authentic sample of 16 synthesized by aminolysis

Table IV. Rate Constants for Degradations of 4–7

compd	rate constants × 10^2 h^{-1} $k_{n,1}$	$k_{n,2}$ ($k_{n,2'}$)	$k_{n,3}$	$k_{n,4}$	$k_{n,6}$	measd peaks of compds[a]
4	15.3		4.6	8.4		4, 10, 15
5	19.9	0.1	3.2	5.2		5, 11, 15
6		30.8			12.3	6, 15, 4
7		22.8 (6840)[b]				7, 15, 16

[a] Time courses of relative molar concentrations of the compounds described were measured by ^1H NMR spectroscopy. Kinetic analyses of the time courses of the degradations afforded the rate constants. [b] Second order rate constants (L/mol·h).

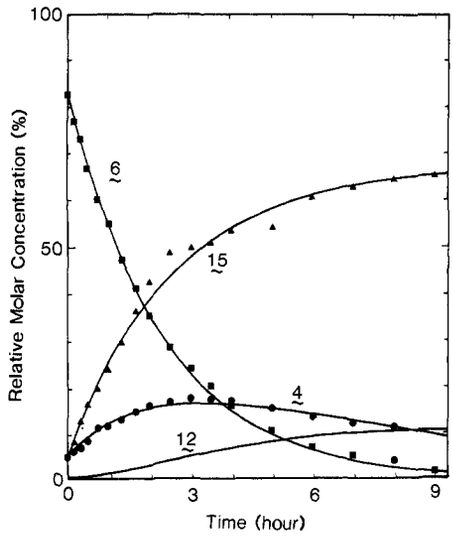


Figure 3. Time course of the alkaline degradation of **6** in deuteriated carbonate buffer solution (pD 10.4, 35 °C): (■, ●, ▲) experimental, (—) calculated.

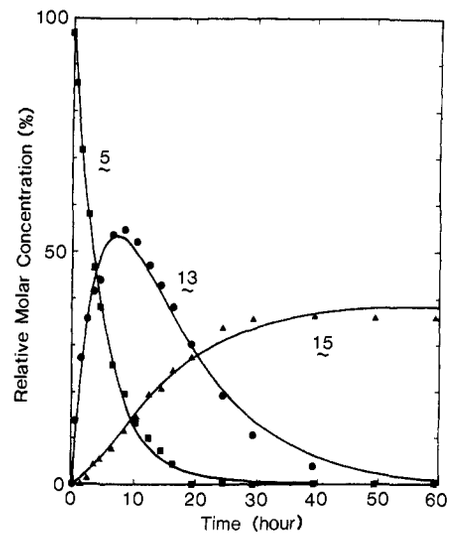
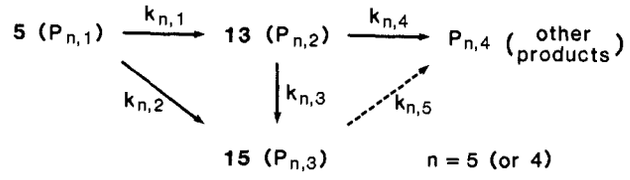


Figure 4. Time course of the alkaline degradation of **5** in deuteriated carbonate buffer solution (pD 10.4, 35 °C): (■, ●, ▲) experimental, (—) calculated.

Scheme IV



of **7**. This revealed the parallel reactions shown in Scheme III. The rate constants calculated from kinetic analyses of the degradations of **6** and **7** are shown in Table IV. The value of $k_{6,2}$ is believed to be more accurate than that obtained by the UV method (k_{obsd}^{UV}), where the effects of parallel reactions could not be eliminated. As expected, the $k_{7,2}$ value, which represents the contribution of the OH^- ion to the rate of the β-lactam cleavage, proved to be similar to the k_{obsd}^{UV} value that was determined in a highly diluted solution (7.5×10^{-5} M), where the effect of the second-order aminolysis is negligibly small. After these corrections a good correlation ($k_{obsd}^{NMR} = 1.92 + 1.03 \times k_{obsd}^{UV}$; $n = 8$, $r = 0.96$, $s = 2.33$) was established between these values.

Examination of the Degradation Process of Various 3′-Substituted Oxacephem Derivatives. Degradations of oxacephems **4** and **5** of class 2 were examined in detail. The rates of the formation of **13** and **15** were determined by measuring the intensities of the peaks of the 7α-methoxy group at δ 3.47 in **5**, those of 6-H at δ 4.84 in **13**, and those of the *exo*-methylene group and 6-H at δ 5.50 and 5.60 and δ 5.23 in **15** relative to the intensities of the 7α-methoxy group in **5**. The reaction mechanism of the degradation of **5** was analyzed by assuming the mechanism shown in Scheme IV. Further degradations of **15** were neglected, because it was sufficiently stable under these conditions. The resulting time course of a representative case of **5** is shown in Figure 4.

The rate constants for **4** and **5** are also shown in Table IV. The direct process from **5** to **15** (concurrent reaction) was concluded not to take place since the rate constant $k_{5,2}$ obtained was negligibly small. The time course of the oxacephem **5** may be explained by only three processes, i.e., from **5** to **13**, from **13** to **15**, and from **13** to other secondary degradation products, shown in Scheme IV.

The degradation processes of class 3 oxacephems are the most simple, with the degradation practically stopping at the $P_{n,2}$ stage. The degradation processes of class 1 oxacephems are considered to be similar in nature to those of class 2 oxacephems, although the measurements afforded only $k_{n,2}$ and implied the direct conversion of $P_{n,1}$ into $P_{n,3}$. As described in the accompanying paper,[8] the logarithms of the real rate constants (k_{obsd}^{NMR} and in some cases $k_{n,1}$ or $k_{n,2}$) for the cleavage of the β-lactam ring of **1–9** have been found to be linearly correlated with the σ_I values of the 3′-substituents. These findings suggest that the leavability of the 3′-substituents may not influence the real rate constants of the β-lactam cleavage.

Conclusion

We conclude that, in the courses of alkaline degradations of all the oxacephems examined, the reaction rate of the β-lactam ring cleavage does not depend on the leavability but mainly on the electron-withdrawing character of the 3′-substituents. Actually, oxacephem **2** with the 3′-CN group possesses high chemical reactivity, although the CN group does not leave during the β-lactam cleavage. These observations agree with the recent reports that the leavability of the 3′-substituents of cephalosporins does not affect the β-lactam reactivity. However, the leavability of the 3′-substituent may influence the antibacterial activity, because the antibacterial activity of the oxacephem **2** with the 3′-cyano group has been shown to be rather low in spite of its high β-lactam reactivity.[10]

Experimental Section

Synthesis of β-Lactam Compounds. The synthesis of the various 3′-substituted oxacephem derivatives **1–9** used in this work is reported in the accompanying paper.[10]

Synthesis of Degradation Products. Infrared spectra (KBr disk) were recorded on a Hitachi 215 spectrometer. Ultraviolet spectra (aqueous solution) were recorded on a Hitachi 323 spectrometer. Carbon-13 NMR spectra (D_2O solution) were recorded on a Varian XL-100-12A spectrometer with dioxane as the internal reference (δ 67.4). The products were purified by reverse-phase chromatography using styrene–divinylbenzene copolymer (Diaion, HP-20). HPLC was carried out with Nucleosil 10 C18 as a stationary phase, a 55:44 mixture of N-TBA in a phosphate buffer solution and methanol as a mobile phase, and a UV detector (at 254 nm).

(*R*,*R*)-2-[(Phenylacetamido)methoxycarboxymethyl]-5,6-dihydro-5-methylene-2*H*-1,3-oxazine-4-carboxylic Acid Disodium Salt (15). A mixture of a solution of **9** (1.00 g, 2.07

(10) Narisada, M.; Nishikawa, J.; Watanabe, F.; Terui, Y. *J. Med. Chem.* **1987**, *30*, 514.

mmol) in water (4.00 mL) and a 1 N solution of sodium hydroxide (6.2 mL, 3.0 × 2.07 mmol) was stirred at room temperature for 45 min. An acid ion-exchange resin (AG50WX2, RSO_3H type) was added to the stirred mixture until the pH reached 8.0. The resin was removed by filtration and the filtrate was purified by chromatography using HP-20 (200 mL) and cold water as the eluant. The fast-running fractions containing sodium *N*-methyltetrazol-5-yl mercaptide were removed and further cautious elution afforded the desired fractions, which were freeze-dried to obtain **15** as a hygroscopic white powder (435 mg, 51.7%): UV λ_{max} (H_2O) 225 nm (ϵ 12 000), 293 (600); IR (KBr) 3415, 1655 (inflex), 1620 (br), 1494 cm^{-1}; ^{13}C NMR δ 43.5 (C-11), 52.6 (C-9), 67.2 (C-2), 88.3 (C-7), 89.5 (C-6), 121.0 (C-3′), 128.2 (C-15), 129.8 (C-14 and C-16), 130.2 (C-13 and C-17), 131.3 (C-3), 135.7 (C-12), 166.2 (C-4), 172.8 and 173.2 (4-COO^- and C-8), 174.9 (C-10). Anal. Calcd for $C_{17}H_{16}N_2O_7Na_2 \cdot 1.2H_2O$: C, 47.71; H, 4.33; N, 6.55; Na, 10.75. Found: C, 47.66; H, 4.99; N, 6.88; Na, 10.51.

(*R*,*R*)-2-[(Phenylacetamido)methoxycarbamoylmethyl]-5,6-dihydro-5-methylene-2*H*-1,3-oxazine-4-carboxylic Acid Sodium Salt (16). To a solution of **7** (50 mg, 0.12 mmol) in water (2.00 mL) was added a 25% solution of NH_4OH (36 μL, 4.0 × 0.12 mmol) and the resulting mixture was stirred at room temperature for 1 h. The HPL chromatogram of the reaction solution showed only a single peak and freeze-drying of the solution gave a white powder (43 mg, 93%). The powder was chromatographed on a HP-20 column (10 mL) and cautious elution with cold water afforded pure fractions, which were collected and freeze-dried to obtain **16** as a hygroscopic white powder (11 mg, 24%): UV λ_{max} (H_2O) 225 nm (ϵ 8400); IR (KBr disk) 3400 (br), 1676, 1605, 1495, 1400 cm^{-1}; ^{13}C NMR 42.9 (C-11), 52.6 (C-9), 67.2 (C-2), 87.0 (C-7), 88.0 (C-6), 121.9 (C-3′), 128.3 (C-15), 129.8 (C-14 and C-16), 130.2 (C-13 and C-17), 130.9 (C-3), 135.3 (C-12), 167.1 (C-4), 172.3 and 173.0 (4-COO^- and C-8), 175.6 (C-10). Anal. Calcd for $C_{17}H_{18}N_3O_6Na \cdot 3.6H_2O$: C, 45.55; H, 5.67; N, 9.38. Found: C, 45.42; H, 5.28; N, 8.99.

Kinetic Methods. Method I. The loss of the characteristic UV absorbance at ca. 260 nm of each 3-oxacephem derivative in a 0.008 M carbonate buffer solution (pH 10.0, μ = 0.1, 35.0 ± 0.2 °C) was followed as a function of time with a Hitachi UV 320 automatic recording spectrometer. The initial concentrations of 3-oxacephem derivatives were about 7.5×10^{-5} M. The values of the pseudo-first-order rate constants, k_{obsd}^{UV}, for the β-lactam cleavage were determined by the method reported by Guggenheim.[11]

Method II. Each oxacephem derivative was dissolved in 0.2 M deuteriated carbonate buffer solution (pD 10.4, μ = 0.5). ^1H NMR spectra of the solution were recorded at proper intervals on a Varian XL-200 NMR spectrometer (200.057 MHz) in a 5-mm spinning tube. Typical FT NMR measurement parameters were as follows: spectral width, 2401 Hz; pulse width, 6 μs (flipping angle 45°); acquisition time, 3 s; number of data points, 14 404; number of acquisitions, 64. The sample temperature was maintained at 35 ± 0.5 °C throughout the experiments. The initial concentrations of oxacephem derivatives were about 1×10^{-2} M. The pseudo-first-order rate constants, k_{obsd}^{NMR}, of the β-lactam cleavage were determined from the residual ratio of the oxacephem (α) at each time t (hour) by analysis of the regression of the following eq:

$$-\ln \alpha = -\ln ([A]/([A] + [B])) = k_{obsd}^{NMR} t \qquad (1)$$

where [A] and [B] are defined as the intensities of the peaks at δ 3.47 and 3.17, respectively. Standard deviations for k_{obsd}^{NMR} are around 0.4×10^{-2} h^{-1}.

Kinetic Analysis. General Method for Compounds 4 and 5. The kinetics of **4** and **5** were analyzed assuming the reaction mechanism shown in Scheme IV and the following rate equations:

$$d[P_{n,1}]/dt = -(k_{n,1} + k_{n,2})[P_{n,1}]$$

$$d[P_{n,2}]/dt = k_{n,1}[P_{n,1}] - (k_{n,3} + k_{n,4})[P_{n,2}]$$

$$d[P_{n,3}]/dt = k_{n,2}[P_{n,1}] + k_{n,3}[P_{n,2}] \qquad (n = 4, 5)$$

where $[P_{n,1}]$, $[P_{n,2}]$, and $[P_{n,3}]$ are the relative molar concentrations (%) of compounds $P_{n,1}$, $P_{n,2}$, and $P_{n,3}$ (= **15**), respectively. With use of the NONLIN program,[11] the rate constants $k_{n,1}$–$k_{n,4}$ were determined by minimizing $S(\theta)$:

$$S(\theta) = \sum_j^n \sum_i^m w_i[y_{ji} - f_i(t_j,\theta)]^2$$

where y_{ji} is the jth observation of $P_{n,i}$, $f_i(t_j,\theta)$ is the predicted value corresponding to y_{ji}, θ expresses the rate constants $k_{n,1}$–$k_{n,4}$, and w_i is the appropriate weight, which was set equal to unity in the present study. The rate constant $k_{n,5}$ was not considered.

Method for Compound 6. The rate equations for the reaction mechanism shown in Scheme II were assumed to be as follows:

$$d[6]/dt = -(k_{6,2} + k_{6,6})[6]$$

$$d[4]/dt = k_{6,6}[6] - k_{4,1}[4]$$

$$d[12]/dt = k_{4,1}[4] - (k_{4,3} + k_{4,4})[12]$$

$$d[15]/dt = k_{6,2}[6] + k_{4,3}[12]$$

The rate constants $k_{4,1}$, $k_{4,3}$, and $k_{4,4}$ obtained for **4** were utilized for the calculations.

Method for Compound 7. The rate equations for the reaction mechanism shown in Scheme III were assumed to be as follows:

$$d[7]/dt = -k_{7,2}[7] - k_{7,2'}[7][NH_3]$$

$$d[15]/dt = k_{7,2}[7]$$

$$d[16]/dt = k_{7,2'}[7][NH_3]$$

where $[NH_3]$ was assumed to be equal to [15], and $k_{7,2'}$ is the second-order rate constant (L/mol·h) of the aminolysis.

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(12) Metzler, C. M.; Elfring, G. L.; McEwen, A. T. *A User's Manual for* NONLIN *and Associated Programs*; Upjohn: Kalamazoo, MI, 1974.