was used instead of *p*-toluenesulfonyl chloride. Anal. ($C_{14}H_{18}N_3O_7S$) C, H, N, S.

6-(*N*-Ethylamino)-2-[(methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (11). The starting material for the synthesis of compound **11** was 6-(*N*-ethylamino)-2-hydroxy-1*H*-isoindole-1,3-dione (**2a**), which was prepared according to the general procedure used for the synthesis of compound **5**. In this procedure acetaldehyde was used instead of formaldehyde. The crystals of **2a** (2.29 g, 0.008 mol) were suspended in 40 mL of water. $NaHCO_3$ was added until the mixture became basic (pH 8). While this solution was being stirred in an ice bath, methanesulfonyl chloride (0.9 g 0.008 mol) was slowly added. This reaction mixture was continuously stirred for 2 h, and then the residue was filtered and recrystallized from methanol/benzene (1:1). Anal. ($C_{11}H_{12}N_2O_5S$) C, H, N, S.

6-(*N*-Ethylamino)-2-[(isopropylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (12) and 6-(*N*-Ethylamino)-2-(toluenesulfonyloxy)-1*H*-isoindole-1,3-dione (13). The same procedure was used for the synthesis of compound **12** as for compound **11** except that 2-propanesulfonyl chloride was used instead of methanesulfonyl chloride. The general procedure used for the synthesis of compound **9** was followed for the preparation of compound **13** as shown in Scheme II. Compound **12**, anal. ($C_{13}H_{16}N_2O_5S$) C, H, N, S. Compound **13**, anal. ($C_{17}H_{16}N_2O_5S$) C, H, N, S.

2-[(Methylsulfonyl)oxy]-6-nitro-1*H*-isoindole-1,3-dione (14). Compound **1d** (1.66 g, 0.008 mol) was suspended in a 10% $NaHCO_3$ solution (5 mL) until the sodium salt was formed. Methanesulfonyl chloride (1.14 g, 0.01 mol) was added slowly while the mixture was stirred in an ice bath. After stirring for 45 min, the mixture was filtered. The resulting solid was recrystallized from acetone. Anal. ($C_9H_6N_2O_7S$) C, H, N, S.

2-[(Isopropylsulfonyl)oxy]-6-nitro-1*H*-isoindole-1,3-dione (15). The same procedure described above for compound **14** was followed except that 2-propanesulfonyl chloride was used instead of methanesulfonyl chloride. Anal. ($C_{11}H_{10}N_2O_7S$) C, H, N, S.

2-[(Methylsulfonyl)oxy]-1*H*-isoindole-1,3-dione (16). The procedure described for the synthesis of compound **14** was followed except that the starting material used was 2-hydroxy-1*H*-isoindole-1,3-dione instead of compound **1d**. Anal. ($C_9H_7NO_5S$) C, H, N, S.

In Vitro Growth Inhibition Study. The following experiments were performed under sterile conditions. Murine leukemia cell line L1210 grown in media RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Biocell, Compton, CA) (5×10^5 cells/mL) was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 10^{-3} to 10^{-8} M were prepared in phosphate buffer saline (PBS). Each compound was initially solubilized in dimethyl sulfoxide (Me_2SO), however, each final dilution contained less than 1% Me_2SO. Solutions of different concentrations (0.20 mL) were pipeted into separate test tubes (1 × 7.5 cm) in duplicates. Cell culture (1.8 mL) containing a cell population of 6×10^4 cells/mL was pipeted into test tubes. Controls, containing only PBS and Me_2SO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 95% air and 5% carbon dioxide. After 72 h, cells in each test tube was diluted 10 times with saline and counted by using a Coulter counter (Coulter Electronics Inc., Hialeah, FL). The counts were corrected for the dilution.

Chemical Stability Experiment. The stability of compound **7** was investigated. The compound was incubated at 37 °C in medium RMPI 1640, pH 7.4. The sample was analyzed by HPLC (Beckman Model 210) at 1-h intervals for 72 h. A C_{18} column (5 μm, 1.8 × 25 cm) and a variable-wavelength detector set at 268 nm were used in this analysis. The $t_{1/2}$ of compond **7** was determined to be 40 ± 1.3 h. The hydrolyzed product, postulated to be 2-hydroxy-6-(*N*,*N*-dimethylamino)-1*H*-isoindole-1,3-dione (**5**) appeared as an extra peak along with the peak for **7** in the high-pressure liquid chromatogram. Spiking the sample with **5** gave an enhanced peak height, providing further evidence that the hydrolyzed product and **5** were probably the same.

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Registry No. **1**, 105969-84-4; **1a**, 610-27-5; **1b**, 2050-19-3; **1c**, 105970-00-1; **1d**, 105969-98-0; **2**, 105969-85-5; **2a**, 105969-99-1; **3**, 105969-86-6; **4**, 91517-75-8; **5**, 105969-87-7; **6**, 105969-88-8; **7**, 105969-89-9; **8**, 105969-90-2; **9**, 105969-91-3; **10**, 105969-92-4; **11**, 105969-93-5; **12**, 105969-94-6; **13**, 105969-95-7; **14**, 105969-96-8; **15**, 105969-97-9; **16**, 57212-70-1; 2-hydroxy-1*H*-isoindole-1,3-dione, 524-38-9; 3-nitrophthalic acid, 603-11-2; hydroxylamine hydrochloride, 5470-11-1; methanesulfonyl chloride, 124-63-0; 2-propanesulfonyl chloride, 10147-37-2; benzenesulfonyl chloride, 98-09-9; formaldehyde, 50-00-0; *p*-toluenesulfonyl chloride, 98-59-9; *p*-nitrobenzenesulfonyl chloride, 98-74-8; acetaldehyde, 75-07-0.

Synthesis and 3′-Substituent Effects of Some 7α-Methoxy-1-oxacephems on Antibacterial Activity and Alkaline Hydrolysis Rates

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Relationships between intrinsic antibacterial activity and β-lactam chemical reactivity of 7β-(phenylacetamido)-7α-methoxy-1-oxacephems with various 3′-substituents (**1–9**) were studied in order to clarify the effect of the 3′-substituent on the antibacterial activity. The chemical reactivity of the β-lactam ring estimated by pseudo-first-order rate constants log k_{obsd}^{NMR} of alkaline hydrolysis at pD 10.4 and 35.0 °C correlates well linearly with ^{13}C NMR chemical shift differences ($\Delta\delta(4-3)$), infrared stretching frequencies of the β-lactam carbonyl ($\nu_{C=O}$), and σ_I values. Values of log ($1/C_N$), averaged for the MIC values for *Escherichia coli*, *E. coli* NIH JC-2, *E. coli* EC-14, and *Klebsiella pneumoniae* SRC-1, were taken as an estimation of the intrinsic antibacterial activity. The log ($1/C_N$) values of the compounds without good leaving groups (**1**, **2**, **4**, **5**, and **8**) correlated fairly well with log k_{obsd}^{NMR} values. The comparatively high antibacterial activity of compounds with good leaving groups (**6**, **7**, and **9**) may be attributable to the different course of decomposition of these compounds.

β-Lactam antibiotics inhibit the synthesis of bacterial cell walls in bacteria by acylating the active center of the target transpeptidases, which play an important role in constructing the three-dimensional network of the cell

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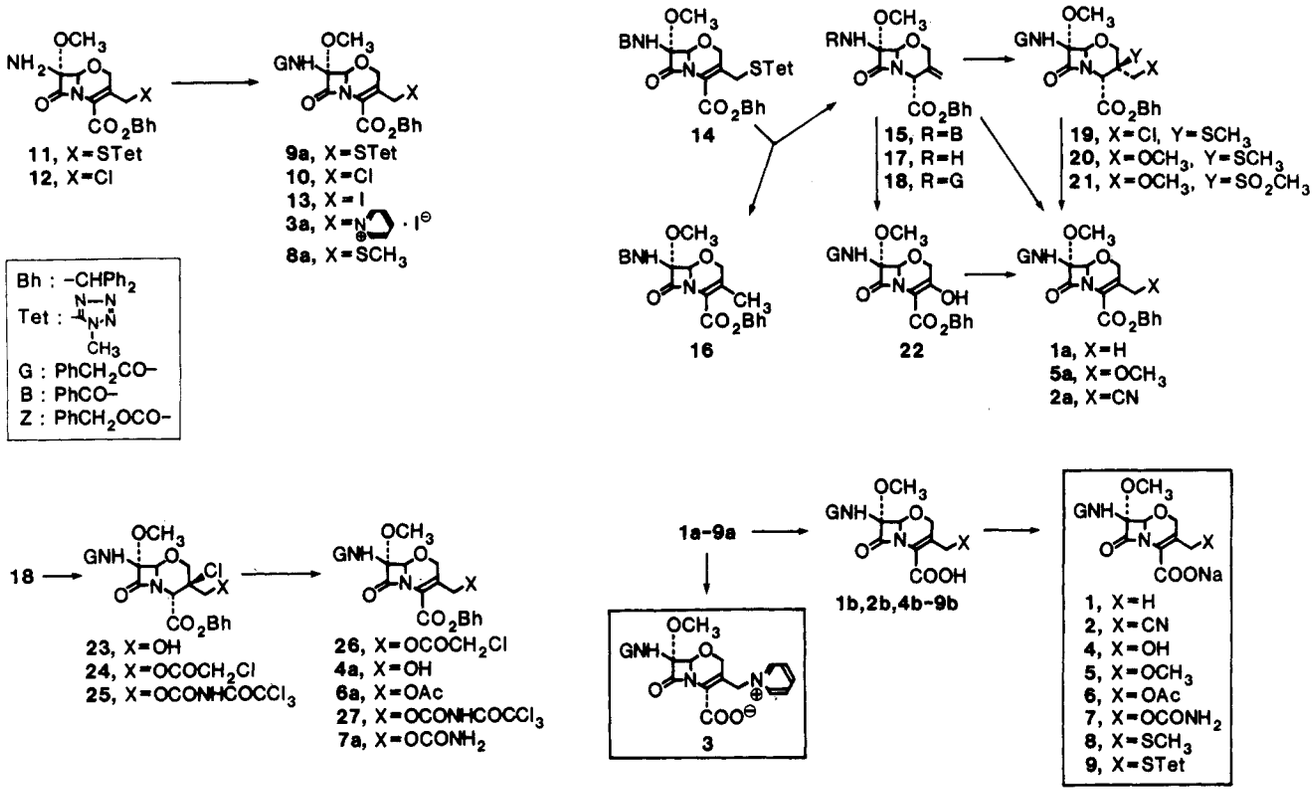
Scheme I



walls,[1] and also induce enzymatic self-lysis of the cell walls by interference with the murein metabolism.[2] The following factors of a β-lactam compound may affect the inhibition of the target enzymes that mainly determines its antibacterial activity:[3] (a) its affinity to the target enzymes, (b) its ability to acylate the active center of the target enzymes, (c) the stability of the resulting acylated enzymes, (d) its permeability through bacterial cell membranes, (e) its stability against the β-lactamases, (f) its chemical stability in the culture medium. Factors a–c are concerned with the intrinsic activity of the β-lactam compound, while factors d–f determined its effective concentration around the target enzymes.

Factors a and b may be related to each other, since both are thought to be affected by the pyramidal character of the nitrogen atom of the β-lactam ring. The affinity of the β-lactam moiety for the target enzymes probably depends on the similarity of its geometrical structure to that of the cleaving amide bond moiety of the natural substrate which is believed to assume a pyramidal structure in the transition state of transpeptidation.[1a,1b,4] On the basis of comparison of the antibacterial activity of β-lactam antibiotics of a wide range of structures with the various degrees of the pyramidal character of the lactam nitrogen atom and the rates of alkaline hydrolysis as well as enzymatic hydrolysis, Belgian researchers have concluded that the degree of the pyramidal character substantially determines the antibacterial activity.[5] However, when the problem is limited within a series of β-lactam compounds possessing a definite nucleus with a rather invariable degree of the pyramidal character, studies based on measurements of alkaline hydrolysis rates[6] or molecular orbital treatments[7] of their structure–reactivity relationships have revealed a positive correlation of alkaline hydrolysis rates to antibacterial activity. The antibacterial activity or chemical reactivity of cephalosporins with various 3′-substituents has been correlated with bond characteristics around the β-lactam moiety, which are inferred from the infrared stretching frequencies of the β-lactam carbonyl, $\nu_{C=O}$,[8] or ^{13}C NMR chemical shifts, especially including $\Delta\delta(4-3)$ values.[9] The chemical reactivity has been found

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to correlate well with these values, which vary depending on the σ_I value of the 3′-substituent. However, the antibacterial activity against sensitive Gram-negative bacteria has been found to be only moderately correlated with these values. Only a few studies[5] have been done concerning factor c, although this kind of work should be important for interpreting the behavior of β-lactam antibiotics with respect to the target transpeptidases as well as β-lactamases.

Here we describe the results of an investigation on the relationships among structure, reactivity, and antibacterial activity of 7-α-methoxy-1-oxacephems with various 3′-substituents and the syntheses of these compounds. We found that (1) the reactivity of the β-lactam ring expressed by the logarithms of the alkaline hydrolysis rates, log k_{obsd}^{NMR}, can be estimated by its linear correlation with either the difference values of ^{13}C NMR chemical shifts for C_4 and C_3, $\Delta\delta(4-3)$, or the infrared C=O stretching frequencies of the β-lactam carbonyl, $\nu_{C=O}$; (2) the antibacterial activity (log 1/MIC)) of oxacephems with a poor leaving group at the 3′-position possesses a roughly linear correlation with the log k_{obsd}^{NMR} values, whereas that of oxacephems with a good leaving group deviates from the correlation probably because of the formation of stable acylated transpeptidases; (3) the distance between the β-lactam nitrogen atom and C_4, C_6, C_8 plane (*d*) in three benzhydryl esters appears to be influenced inversely by the σ_I values, implying that a stronger enamine resonance is present within the esters possessing a more electron withdrawing substituent.

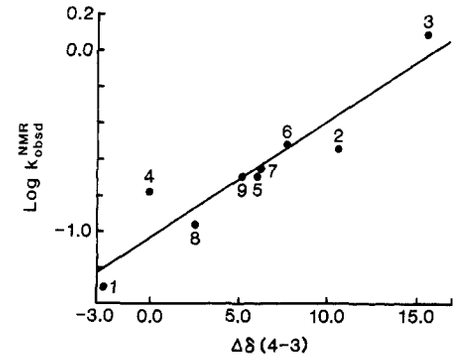

Figure 1. Correlation between hydrolysis rates log k_{obsd}^{NMR} and ^{13}C NMR chemical shift differences $\Delta\delta(4-3)$.

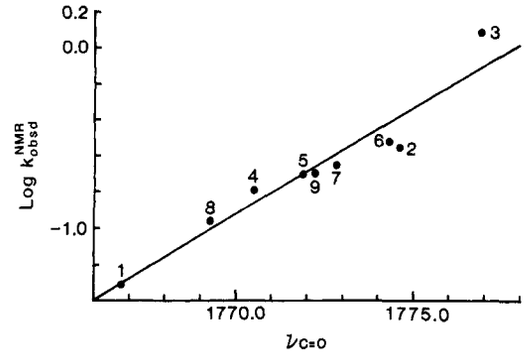

Figure 2. Correlation between hydrolysis rates log k_{obsd}^{NMR} and IR frequencies of lactam carbonyl $\nu_{C=O}$.

Results

Synthesis of 1-Oxacephems. Phenylacetylation of **11**[10] and **12**[11] gave respectively **9a** and **10** (Scheme I). The substitution reaction of **10** with pyridine was rather sluggish, while that of iodide **13** proceeded smoothly, giving **3a** in a satisfactory yield. Treatment of **13** with sodium methyl mercaptide gave **8a**.

The important intermediate **15** was prepared by reduction of **14** with magnesium–acetic acid in methylene chloride. This gave less of byproduct **16** than the combination of zinc and acetic acid. Treatment of **15** with phosphorus pentachloride gave **17**, which upon phenylacetylation produced **18**. Isomerization of the exo methylene double bond of **18** with triethylamine yielded **1a**. Addition of methanesulfenyl chloride to the double bond of **18** gave[12] **19**, which, when treated with methanol in the presence of silver perchlorate and calcium carbonate, yielded[12] **20**, which was oxidized to give **21**. Treatment of **21** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) yielded[12] **5a**. Ozonolysis of **18** gave **22**,[13] which, when subjected to Wittig reaction with (cyanomethylene)triphenylphosphorane at 80 °C, produced **2a**. Treatment of **18** with isocyanuric chloride yielded[14] **23**. Chloroacetylation and trichloroacetylcarbamoylation of **23** produced respectively **24** and **25**, which on treatment with DBU at −70 °C gave[14] respectively **26** and **27**. Deprotection of the chloroacetyl group of **26** with thiourea afforded **4a**, which gave **6a** upon acetylation. Removal of the trichloroacetyl group of **27** by treatment with silica gel yielded **7a**. Deprotection of the benzhydryl ester group of **1a**, **2a**, and **4a–9a** by treatment with aluminum trichloride in anisole[15] yielded respectively **1b**, **2b**, and **4b–9b**. Treatment of the acids with sodium bicarbonate gave respectively the sodium salts **1**, **2**, and **4–9**. Deprotection of **3a** with trifluoroacetic acid and anisole and subsequent reverse-phase column chromatography yielded **3**.

^{13}C NMR Spectra and Infrared Spectra. ^{13}C NMR spectra of **1–9** were measured in D_2O. The important chemical shifts δ, shown in Table I, indicated that only the values for carbons at positions 2, 3, 4, and 3′ are affected significantly by the introduction of the 3′-substituents. The $\Delta\delta(4-3)$ values, shown in Table II, were taken as indicators of the polarization of C_3=C_4 which reflects the inductive effect of the 3′-substituents. Infrared spectra of **1–9** were measured in dry dimethoxy sulfoxide.[16] The

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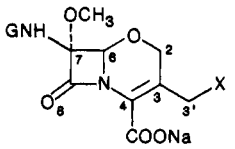
(16) Infrared spectra were measured by Dr. M. Takasuka of these Laboratories.

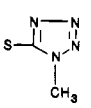
Table I. ^{13}C NMR Chemical Shifts of Nuclear Carbons of Salts 1–9 (in D_2O)



compd	C_2	C_3	C_4	C_6	C_7	C_8	$C_{3'}$
1	67.86	127.89	125.28	83.30	94.94	163.03	14.31
2	66.00	119.00	129.57	83.45	95.12	163.25	17.47
3	64.66	118.40	133.82	83.77	95.45	163.44	58.56
4	65.70	127.90	127.85	83.48	95.06	163.36	58.58
5	65.65	123.43	129.42	83.54	95.20	163.15	68.49
6[a]	65.45	122.26	129.85	83.51	95.23	163.28	61.66
7	65.34	123.08	129.32	83.49	95.20	163.30	61.93
8	66.47	125.41	127.99	83.61	95.10	162.99	31.16
9	65.90	124.38	129.48	83.54	95.05	163.09	32.26

[a] Assignment of the C_3 and C_4 signals was based on the PRFT measurements in which C_4 showed a longer T_1 value. See ref 9i.

Table II. Pseudo-First-Order Hydrolysis Rates and Molecular Parameters[a] of Salts 1–9

compd	X	$\Delta\delta$(4–3),[b] ppm	$\nu_{C=O}$,[c] cm^{-1}	σ_I	log k_{obsd}^{NMR} [d] (eq 1–3)	log $(1/C_N)$[e] (eq 1)
1	H	−2.61	1766.8	0.00	−1.315 (−1.211, −1.284, −1.180)	3.49 (3.34)
2	CN	10.57	1774.6	0.56	−0.540 (−0.348, −0.348, −0.497)	4.19 (4.01)
3		15.42	1776.9	1.09	0.100 (−0.030, −0.072, —)	4.53 (—)
4	OH	−0.05	1770.5	0.25	−0.785 (−1.043, −0.840, −0.875)	3.88 (3.80)
5	OCH_3	5.99	1771.9	0.27	−0.691 (−0.648, −0.672, −0.851)	3.74 (3.89)
6	$OCOCH_3$	7.59	1774.3	0.39	−0.512[f] (−0.543, −0.384, −0.704)	5.36 (—)
7	$OCONH_2$	6.24	1772.8	0.46[g]	−0.642[h] (−0.631, −0.564, −0.619)	5.43 (—)
8	SCH_3	2.58	1769.3	0.23	−0.957 (−0.871, −0.984, −0.899)	3.39 (3.65)
9		5.10	1772.2	0.53	−0.690 (−0.706, −0.636, −0.533)	6.16 (—)

[a] The value in parentheses indicates that calculated by eq 1–3. [b] Difference value for ^{13}C NMR chemical shifts for C_4 and C_3. [c] IR frequency for β-lactam carbonyl. [d] Logarithm of pseudo-first-order rate of alkaline hydrolysis: See following paper in this issue. [e] C_N: geometrical mean of the MICs (M) for the four strains of sensitive Gram-negative bacteria. [f] As the apparent hydrolysis rate, a value of −0.593 (log 0.255) was obtained. [g] σ_I for $OCONMe_2$ is given in place of that for $OCONH_2$. [h] The value of real k_{obsd} (see ref 17).

Table III. Antibacterial Activity of 1–9 against Sensitive Gram-Negative Bacteria

compd	MIC,[a] μg/mL				$10^6 C_N$[b]
	E. coli H	*E. coli* NIHJ JC-2	*E. coli* EC-14	*Kleb. pneumoniae* SRL-1	
1	100	200	100	100	323
2	12.5	50	25	25	63.6
3	12.5	12.5	12.5	12.5	29.5
4	25	100	50	50	130
5	25	200	100	50	178
6	0.78	3.13	3.13	1.56	4.36
7	0.78	3.13	0.78	3.13	3.66
8	50	>400[c]	200	100	406
9	0.1	0.78	0.39	0.39	0.684

[a] Obtained by the gradient plate method. [b] C_N: geometrical mean of the four MICs expressed in M. [c] Value of 800 was used for further calculation.

stretching frequencies for the β-lactam carbonyl are shown in Table II.

Alkaline Hydrolysis Rates. The mechanism of the hydrolysis was subjected to precise examination by ^1H NMR spectroscopy, and the results are described in the accompanying paper.[17] The study revealed that the pseudo-first-order rates, k_{obsd}^{NMR}, obtained for the hydrolysis of 1–9 at 35 °C and pD 10.4 and shown in Table II, were more suitable for the discussion than k_{obsd}^{UV}, which were found to be affected by concomitant side reactions.[17]

Antibacterial Activity. Table III shows minimal inhibitory concentrations (MIC, micrograms per milliliter) of 1–9 against Gram-negative bacteria, determined by the agar dilution method.[18] The values log $(1/C_N)$ were used to estimate the intrinsic activity of 1-oxacephems 1–9 and

(17) Nishikawa, J.; Watanabe, F.; Shudou, M.; Terui, Y.; Narisada, M. *J. Med. Chem.* **1987**, *30*, 523.
(18) MIC measurements were done by K. Motokawa of these Laboratories.

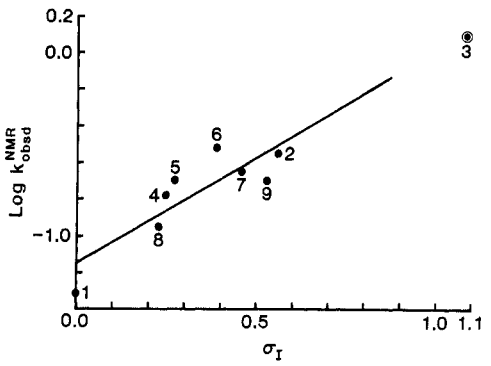


Figure 3. Correlation between hydrolysis rates log k_{obsd}^{NMR} and σ_I.

are shown in Table II, where C_N means the geometric mean of MICs (moles per liter) for *E. coli* H, *E. coli* NIHJ JC-2, *E. coli* EC-14, and *Kleb. pneumoniae* SRL-1.

Discussion

Effects of 3′-Substituent upon Chemical Reactivity of β-Lactam Ring of Oxacephems 1–9. As shown in Figures 1 and 2, $\Delta\delta(4\text{–}3)$ and $\nu_{C=O}$ both show linear correlation with log k_{obsd}^{NMR}. Equations 1 and 2 indicate the regression between log k_{obsd}^{NMR} and the two values.[19]

$$\log k_{obsd}^{NMR} = -1.04 + 0.0655\Delta\delta(4\text{–}3) \quad (1)$$

$$r = 0.927,\ s = 0.142,^{20}\ n = 9$$

$$\log k_{obsd}^{NMR} = -213.3 + 0.120\nu_{C=O} \quad (2)$$

$$r = 0.954,\ s = 0.113,^{20}\ n = 9$$

These facts indicate that the chemical reactivity of the β-lactam ring of an oxacephem may be estimated from either one of the experimental values, $\Delta\delta(4\text{–}3)$ or $\nu_{C=O}$, without tedious measurements of the pseudo-first-order rate k_{obsd}^{NMR} of the alkaline hydrolysis.[17]

The effects of the 3′-substituent on the chemical reactivity may be interpreted on the basis of its inductive effect, since, as shown in Figure 3, a fairly good linear correlation of log k_{obsd}^{NMR} with σ_I is observed. Equation 3 indicates the regression between these values.

$$\log k_{obsd}^{NMR} = -1.18 + 1.22\sigma_I \quad (3)$$

$$r = 0.843,\ s = 0.141,^{20}\ n = 8^{21}$$

Fairly constant ^{13}C NMR chemical shifts of C-8[9] compared with variable $\Delta\delta(4\text{–}3)$ values imply that the 3′-substituent does not significantly affect the character of the β-lactam carbonyl group of oxacephems in the initial state of the reaction coordinate. However, in the nucleophilic attack, delocalization of the charge, which developed over the lactam moiety toward the enamine system polarized by the 3′-substituent, may stabilize the transition state. The degree of polarization is considered to be expressed by the $\Delta\delta(4\text{–}3)$ values. The higher frequency shifts of the $\nu_{C=O}$ values probably reflect the electron-withdrawing character of the enamine group, which seems to destabilize the excited state of the stretching vibration of the β-lactam carbonyl. Thus, these two values are good indices of the chemical reactivity of the β-lactam carbonyl ring toward the nucleophilic attack. In accord with reports published recently,[22] there is little evidence of the contribution of the leavability of the 3′-substituent to the chemical reactivity of the β-lactam ring.

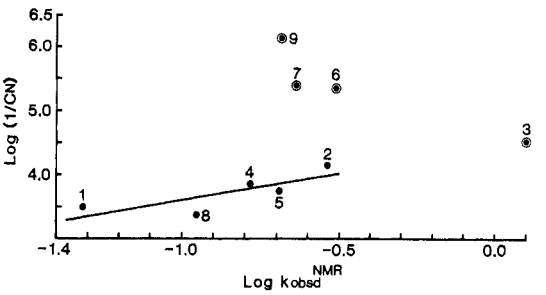


Figure 4. Correlation between antibacterial activity log $(1/C_N)$ and hydrolysis rates log k_{obsd}^{NMR}.

Effects of Chemical Reactivity of β-Lactam Ring on Intrinsic Antibacterial Activity of Oxacephems 1–9. Little correlation ($r = 0.137$[20]) was found between log k_{obsd}^{NMR} and log $(1/C_N)$ (or MIC), when all the oxacephems were taken into account. Our accompanying paper shows that the products of alkaline hydrolysis of oxacephems **1–9** may be classified into two groups according to their structures, one with a good leaving group as the 3-substituent and the other without such a substituent. Oxacephems **1**, **2**, **4**, **5**, and **8** fit into the second class. A fairly good correlation was found among these compounds, as shown in Figure 4 and eq 4. The classification is based

$$\log (1/C_N) = 4.47 + 0.856 \log k_{obsd}^{NMR} \quad (4)$$

$$r = 0.717,\ s = 0.222,^{20}\ n = 5$$

on the fact that the low antibacterial activity of **2** may be interpretable only when the poor leavability of the cyano group of **2** is taken into account since this compound shows high reactivity comparable to that for **6**, **7**, and **9**, which possess good leaving groups at the 3′-position.

Although the unexpectedly high antibacterial activity of these compounds may be attributed to several factors including their high chemical reactivity and permeability through the bacterial outer membrane, these compounds may owe their high antibacterial activity to the high stability of the acylated enzymes.[23] This stability, as shown above, is important, since these compounds give the same hydrolysis product while each of **1**, **2**, **4**, **5**, or **8** gives its own hydrolysis product.

Next, we examined the effect of the 3′-substituents upon the geometrical structure of the β-lactam moiety. The distances of the displacement (d) (0.264, 0.233, and 0.220 Å) of the β-lactam nitrogen atom out of the plane C_4, C_6,

(19) (a) It is noteworthy that, for the 7α-unsubstituted 3′-substituted cephalosporins, a widely displaced regression curve with a gradient similar to this has been obtained.[9h] (b) A linear correlation of log k with $\Delta\delta(4\text{–}3)$ has been reported. See: Coene, B.; Schanck, A.; Dereppe, J.-M.; Van Meersche, M. *J. Med. Chem.* **1984**, *27*, 694. Thus, the regression is specific for compounds with a definite nucleus.

(20) The regression was calculated by using MINITAB (Ryan, T. A., Jr.; Joiner, B.; Ryan, B. F., Minitab Project, Statistics Department, 215 Pond Laboratory, The Pennsylvania State University) operated by a Vax 11-780 computer. The correlation coefficient r is adjusted for degrees of freedom.

(21) The point for **3** which has a large influence on the regression is omitted.

(22) (a) Proctor, P.; Gensmental, N. P.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1185. (b) Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144. (c) Page, M. I.; Procter, P. *J. Am. Chem. Soc.* **1984**, *106*, 3820. (d) Grabowski, E. J. J.; Douglas, A. W.; Smith, G. B. *J. Am. Chem. Soc.* **1985**, *107*, 267.

(23) A stable acyl β-lactamase of this type has been described. See: (a) Faraci, W. S.; Pratt, R. F. *Biochemistry* **1985**, *24*, 903. (b) Faraci, W. S.; Pratt, R. F. *Biochemistry* **1986**, *25*, 2934.

and C_8 in three benzhydryl esters, **1a**, **5a**, and **9a**, respectively, were measured by X-ray diffraction analysis[24] as indicators of the pyramidal structure[25] and were compared with their log k_{obsd}^{NMR} values. A roughly inverse correlation was observed, implying that a stronger enamine resonance is present within the esters with the more electron withdrawing substituents. The electronic effects appear to overwhelm the geometrical effects which partly determine the affinity of β-lactam antibiotics to the target transpeptidases. The extraordinarily high reactivity of **3**, even at pH 7.0, can induce chemical decomposition during measurements of the MIC values, which may have caused the unexpectedly low antibacterial activity of **3**.

Conclusion

Studies of the alkaline hydrolysis and physicochemical properties of oxacephems **1–9** and their benzhydryl esters have revealed that, first, log k_{obsd}^{NMR}, measured at pD 10.4 and 35 °C, is linearly correlated with the electron-withdrawing character of the enamine system which can be estimated by $\Delta\delta(4-3)$ or $\nu_{C=O}$ values as well as σ_I values of their 3′-substituents. Second, log $(1/C_N)$ of oxacephems with a 3′-substituent of poor leavability, i.e., **1**, **2**, **4**, **5**, and **8**, can mostly be interpreted on the basis of the chemical reactivity of the β-lactam ring expressed by log k_{obsd}^{NMR}, whereas the antibacterial activity of oxacephems with a 3′-substituent of good leavability, i.e., **6**, **7**, and **8** may be significantly influenced by the probably high stability of the acylated enzymes in addition to the high acylating ability. The third finding was that the degree of the pyramidal structure of the β-lactam nitrogen atom, which is estimated from X-ray analysis data, of **1a**, **5a**, and **9a**, decreases in this order, suggesting that the acylating ability is more important than the pyramidal structure in this case.

Experimental Section

Synthesis. Reactions using anhydrous solvents that had been dried over type 4A molecular sieves were carried out in a nitrogen atmosphere. Melting points were determined on a Yanagimoto apparatus and were not corrected. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer using chloroform as the solvent unless otherwise stated. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian EM-390 spectrometer using deuteriochloroform unless otherwise stated with tetramethylsilane as an internal or external (D_2O) reference. When the sample contains an organic solvent, it was removed by flushing with carbon tetrachloride. Ultraviolet spectra were recorded on a Hitachi 323 spectrometer using methanol as the solvent unless otherwise stated. To dry the organic solution of the extraction, anhydrous magnesium sulfate was used. For column chromatography, silica gel (Merck silica gel 60) deactivated by the adding of 10% water was used.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-1-oxadethia-3-cephem-4-carboxylate (9a). To a solution of methoxy amine **11**[10] (6.00 g, 11.8 mmol) in methylene chloride (30 mL) cooled to −20 °C were added pyridine (1.24 mL, 1.3 × 11.8 mmol) and phenylacetyl chloride (1.72 mL, 1.1 × 11.8 mmol), and the resulting mixture was stirred at −20 °C for 15 min and then at 0 °C for 30 min. The reaction mixture was washed successively with 2 N HCl, 5% $NaHCO_3$, and H_2O and dried, and then the solvent was evaporated in vacuo. The residue was chromatographed on silica gel. Elution with benzene–ethyl acetate (2:1) and crystallization of the main fractions from benzene yielded **9a** as crystals: mp 179–181 °C (6.35 g, 85.9%); UV λ_{max} 282 nm (ε 9700); IR 3410, 1789, 1700, 1630 cm^{-1}; ^{1}H NMR δ 3.43 (s, 3 H), 3.62 (s, 2 H), 3.80 (s, 3 H), 4.23 (s, 2 H), 4.57 (s, 2 H), 4.99 (s, 1 H), 6.15 (s, 1 H), 6.87 (s, 1 H), 7.23–7.57 (m). Anal. Calcd for $C_{32}H_{30}N_6O_6S$: C, 62.80; H, 4.96; N, 12.77; S, 4.87. Found: C, 62.47; H, 4.74; N, 12.36; S, 4.63.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-(chloromethyl)-1-oxadethia-3-cephem-4-carboxylate (10). To a solution of methylene chloride (ca. 100 mL) containing crude **12** that had been prepared[11] from its *N*-benzoyl derivative (10.0 g, 18.28 mmol) were added pyridine (2.2 mL, 1.5 × 18.28 mmol) and phenylacetyl chloride (2.65 mL, 1.1 × 18.28 mmol) at −20 °C. The solution was stirred for 1 h at 0 °C and then worked up in a similar way to that described above, giving **10** as crystals (2.76 g, 27.6%), which were recrystallized from benzene–ether: mp 162–163 °C; UV λ_{max} 275.5 nm (ε 9900); IR 3410, 1790, 1729, 1699, 1636, 1603 cm^{-1}; ^{1}H NMR δ 3.43 (s, 3 H), 3.63 (s, 2 H), 4.45 (s, 2 H), 4.47 (s, 2 H), 5.03 (s, 1 H), 6.25 (s, 1 H), 6.90 (s, 1 H), 7.23–7.55 (m). Anal. Calcd for $C_{30}H_{27}N_2O_6Cl$: C, 65.87; H, 4.98; N, 5.12; Cl, 6.48. Found: C, 65.98; H, 4.92; N, 5.10; Cl, 6.27.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-(pyridiniomethyl)-1-oxadethia-3-cephem-4-carboxylate Iodide (3a). A mixture of **10** (2.50 g, 4.57 mmol) and sodium iodide (1.37 g, 2.0 × 4.57 mmol) in acetone (25 mL) was stirred at room temperature for 1 h. The residue obtained on removal of acetone in vacuo was poured into a mixture of ethyl acetate and H_2O (1:1), and the organic solution was washed with H_2O and dried. The solvent was removed in vacuo, yielding crude **13**. A solution of the crude **13** (1.5 g, 2.35 mmol) in methylene chloride (1.5 mL) was mixed with pyridine (2.5 mL), and the resulting solution was kept at 25 °C for 1 h. Dilution of the reaction solution with ether led to precipitation of **3a** (1.53 g, 84.3%): UV λ_{max} 258.5 nm (ε 9800), 280 nm (ε 6700); IR (Nujol) 3400, 3160, 1788, 1721, 1684, 1631 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 3.51 ns, 3 H), 3.72 (s, 2 H), 4.56, 4.69 (AB q, J = 18 Hz, 2 H), 5.37 (s, 1 H), 5.73, 5.86 (AB q, J = 15 Hz, 2 H), 6.93 (s, 1 H), 7.15–7.65 (m), 8.02 (m, 2 H), 8.30 (s, 1 H), 8.55 (m, 1 H), 9.20 (m, 2 H).

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-[(methylthio)methyl]-1-oxadethia-3-cephem-4-carboxylate (8a). To a solution of crude **13** (1.5 g, 2.35 mmol) in DMF (15 mL) was added an aqueous solution of sodium mercaptide (15%, 1.08 mL, 0.98 × 2.35 mmol) at −45 °C, and the resulting solution was stirred at −45 °C for 15 min. The reaction solution was mixed with 2 N HCl (4.0 mL) and then ice water and extracted with ethyl acetate. The organic solution was washed successively with 5% $NaHCO_3$ and H_2O and dried. Removal of the solvent in vacuo and subsequent chromatography of the residue on silica gel using benzene–ethyl acetate (4:1) as the eluant yielded **8a** as a foam (1.1 g, 83.8%): UV λ_{max} 275 nm (ε 8700); IR 3425, 1787, 1726, 1700, 1632, 1607 cm^{-1}; ^{1}H NMR δ 1.87 (s, 3 H), 3.45 (s, 3 H), 3.53 (br s, 2 H), 3.62 (s, 2 H), 4.48 (s, 2 H), 5.05 (s, 1 H), 6.28 (s, 1 H), 6.87 (s, 1 H), 7.30–7.53 (m).

Magnesium–Acetic Acid Reduction of Diphenylmethyl 7β-Benzamido-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)-thio]methyl]-1-oxadethia-3-cephem-4-carboxylate (14). To a stirred solution of **14** (3.0 g, 5.8 mmol) in a mixture of methylene chloride (30 mL) and acetic acid (30 mL) was added at 20 °C magnesium turnings (1.2 g, 8.6 × 5.8 mmol) in four portions at 3-h intervals. The resulting solution was washed successively with 5% $NaHCO_3$ and H_2O and dried. The crystalline residue obtained by evaporation of the solvent in vacuo was washed with ether to give an *exo*-methylene compound **15** (1.25 g, 43.0%): mp 191–193 °C; UV λ_{max} 219 nm (ε 22 000); IR 3420, 1779, 1747, 1687, 1604, 1584 cm^{-1}; ^{1}H NMR δ 3.55 (s, 3 H), 4.25 (s, 2 H), 5.17 (s, 1 H), 5.19 (s, 1 H), 5.29 (s, 1 H), 5.47 (s, 1 H), 6.87 (s, 1 H), 6.94 (s, 1 H), 7.22–7.87 (m). Anal. Calcd for $C_{29}H_{26}N_2O_6 \cdot 0.25(C_2H_5)_2O$: C, 69.68; H, 5.56; N, 5.42. Found: C, 69.48; H, 5.30; N, 5.45.

The mother liquors and washings were concentrated in vacuo to give a mixture of crude **15** and **16**, which was dissolved in methylene chloride (20 mL) and treated with triethylamine (0.30 mL) at 0 °C for 1 h. The reaction solution was washed successively with 2 N HCl, 5% $NaHCO_3$, and H_2O, dried, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution

(24) X-ray analyses of **1a**, **5a**, and **9a** were carried out by Dr. M. Shiro and H. Nakai of these laboratories. We are grateful for their kindly supplying us the data prior to their publication. For **1a** and **5a**, data to be published. For **9a**, see: Shiro, M.; Nakai, H.; Onoue, H.; Narisada, M. *Acta Crystallogr., Sec. B* **1980**, *B36*, 3137.

(25) Some other averaged bond lengths for cephalosporins with and without 3′-leaving groups have been described. See: Boyd, D. B. *J. Org. Chem.* **1985**, *50*, 886.

with benzene–ethyl acetate (4:1) yielded **16** as a foam (0.82 g, 28.4%): IR 3445, 1790, 1732, 1692 cm^{-1}; ^1H NMR δ 1.97 (s, 3 H), 3.61 (s, 3 H), 4.26 (s, 2 H), 5.18 (s, 1 H), 6.91 (s, 1 H), 7.08 (s, 1 H), 7.25–7.92 (m).

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-methylene-1-oxadethia-3-cepham-4α-carboxylate (18). To a solution of **15** (13.6 g, 27.3 mmol) in methylene chloride (140 mL) cooled at −20 °C were added successively pyridine (4.84 mL, 2.2 × 27.3 mmol) and phosphorus pentachloride (11.25 g, 1.98 × 27.3 mmol), and the resulting mixture was stirred at room temperature for 1.5 h. To the resulting mixture cooled to −20 °C was added methanol (280 mL) which had been cooled to −30 °C. After the resulting solution was stirred at 0 °C for 2.5 h, diethylamine (22.6 mL, 8 × 27.3 mmol) was introduced to the reaction solution, which after 5 min of stirring was poured into water. The separated organic solution was successively washed with 2 N H_3PO_4, 5% $NaHCO_3$, and H_2O, dried, and concentrated in vacuo to a volume of about 130 mL to give a concentrate containing crude **17**. Phenylacetylation of the concentrate containing crude **17** in a way similar to that for **11** and subsequent silica gel chromatography and then crystallization of the product yielded **18** as crystals: mp 129–131 °C (7.53 g, 53.8%). IR 3415, 1780, 1747, 1698, 1603 cm^{-1}; ^1H NMR δ 3.40 (s, 3 H), 3.63 (s, 2 H), 4.22 (s, 2 H), 5.13 (s, 1 H), 5.24 (s, 1 H), 5.33 (s, 2 H), 6.17 (br, 1 H), 6.88 (s, 1 H), 7.32 (m, 15 H). Anal. Calcd for $C_{30}H_{28}N_2O_6$: C, 70.30; H, 5.51; N, 5.47. Found: C, 70.13; H, 5.33; N, 5.30.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-methyl-1-oxadethia-3-cephem-4-carboxylate (1a). Isomerization of **18** was carried out in a way similar to that described above for the mixture of **15** and **16**. Crystallization of the product from ether yielded **1a** as crystals, mp 182–184 °C, in quantitative yield. Recrystallization from benzene gave a pure sample: UV λ_{max} 269 nm (ϵ 7400); IR 3415, 1781, 1722, 1697, 1642, 1602 cm^{-1}; ^1H NMR δ 1.98 (s, 3 H), 3.44 (s, 3 H), 3.62 (s, 2 H), 4.23 (s, 2 H), 5.02 (s, 1 H), 6.20 (s, 1 H), 6.86 (s, 1 H), 7.22–7.55 (m). Anal. Calcd for $C_{30}H_{28}N_2O_6 \cdot 0.8C_6H_6$: C, 72.68; H, 5.75; N, 4.87. Found: C, 72.47; H, 5.83, N, 4.64.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-(methoxymethyl)-1-oxadethia-3-cephem-4-carboxylate (5a). To a stirred, ice-cold solution of methyl disulfide (0.175 mL, 1.95 mmol) in carbon tetrachloride (2.0 mL) was added a carbon tetrachloride solution of chlorine (1.6 M, 1.20 mL, 1.95 mmol), and the resulting solution was further stirred at 0 °C for 15 min. To the resulting solution containing methanesulfenyl chloride was added a solution of **18** (1.0 g, 1.95 mmol) in methylene chloride (5.0 mL) and ethyl acetate (5.0 mL), and the resulting solution was stirred at 0 °C for 2.0 h. The reaction solution was washed successively with 5% $Na_2S_2O_3$, 5% $NaHCO_3$, and H_2O and dried. Evaporation of the solvent in vacuo yielded **19** as a foam in quantitative yield: IR 3400, 1795, 1752, 1710 cm^{-1}; ^1H NMR δ 2.00 (s, 3 H), 3.21, 3.51 (AB q, J = 12 Hz, 2 H), 3.42 (s, 3 H), 3.64 (s, 2 H), 3.79, 4.13 (AB q, J = 13.5 Hz, 2 H), 4.50 (s, 1 H), 5.40 (s, 1 H), 6.62 (s, 1 H), 6.91 (s, 1 H), 7.34 (m).

To a solution of the crude foam of **19** (1.16 g, 1.95 mmol) in methanol (20 mL) were added precipitated calcium carbonate (780 mg, 4.0 × 1.95 mmol) and silver perchlorate (808 mg, 2.0 × 1.95 mmol), and the resulting mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered to remove inorganic salts and the filterate was concentrated in vacuo. The residue dissolved in ethyl acetate was washed with H_2O, dried, and concentrated in vacuo. The residue was chromatographed on silica gel and eluted with benzene–ethyl acetate (2:1). The main fractions were combined to give **20** as a foam in quantitative yield: IR 3400, 1778, 1738, 1690 cm^{-1}; ^1H NMR δ 2.01 (s, 3 H), 2.83 (s, 3 H), 3.20, 3.32 (AB q, J = 9 Hz, 2 H), 3.40 (s, 1 H), 3.63 (s, 2 H), 3.68, 4.30 (AB q, J = 13.5 Hz, 2 H), 4.43 (s, 1 H), 5.40 (s, 1 H), 6.40 (s, 1 H), 6.87 (s, 1 H), 7.32 (m).

An ice-cold mixture of crude **20** (1.15 g, 1.95 mmol) in methylene chloride (15.0 mL) containing *m*-chloroperbenzoic acid (purity 80%, 841 mg, 2 × 1.95 mmol) was stirred for 1.5 h. Precipitates were removed by filtration and the filtrate was washed successively with 5% $Na_2S_2O_3$, 5% $NaHCO_3$, and H_2O and dried. Removal of the solvent in vacuo gave **21** as a foam in quantitative yield: IR 3400, 1786, 1740, 1692, 1601, 1312, 1148 cm^{-1}; ^1H NMR δ 2.82 (s, 3 H), 3.16, 3.37 (AB q, J = 10.5 Hz, 2 H), 3.40 (s, 3 H), 3.57 (s, 2 H), 4.15, 4.36 (AB q, J = 13.5 Hz, 2 H), 5.02 (s, 1 H), 5.36 (s, 1 H), 6.74 (s, 1 H), 6.90 (s, 1 H), 7.30 (m).

To a solution of crude **21** (1.214 g, 1.95 mmol) in methylene chloride (12.0 mL) cooled at −50 °C was added DBU (0.320 mL, 1.1 × 1.95 mmol), and the resulting solution was stirred at −50 °C for 30 min. The reaction solution, after quenching with acetic acid (2.0 mL), was washed successively with 2 N HCl, 5% $NaHCO_3$, and H_2O and dried. The residue obtained on removal of the solvent in vacuo was chromatographed on silica gel. Elution with benzene–ethyl acetate (2:1) gave a crystalline residue (947 mg), which was recrystallized from ether to give **5a** as crystals: mp 147–148 °C (813 mg, 76.8% from **18**); UV λ_{max} 270 nm (ϵ 8600); IR 3410, 1787, 1721, 1698, 1631, 1603 cm^{-1}; ^1H NMR δ 3.23 (s, 3 H), 3.45 (s, 3 H), 3.63 (s, 2 H), 4.38 (s, 2 H), 4.48 (s, 2 H), 5.00 (s, 1 H), 6.18 (br s, 1 H), 6.84 (s, 1 H), 7.30–7.58 (m). Anal. Calcd for $C_{31}H_{30}N_2O_7$: C, 68.62; H, 5.57; N, 5.16. Found: C, 68.74; H, 5.42; N, 5.14.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-hydroxy-1-oxadethia-3-cephem-4-carboxylate (22). Ozone was introduced by gentle bubbling for 10 min to a solution of **18** (3.00 g) in a mixture of methylene chloride (75 mL) and MeOH (30 mL) cooled at −70 °C. The resulting solution was mixed with acetic acid (52 mL) and methylene chloride (22 mL) and treated with activated zinc powder (7.5 g). The mixture was further stirred at room temperature for 1 h. Zinc was removed by filtration, and the filtrate and washings were combined, washed four times with H_2O, and dried. Evaporation of the solvent in vacuo quantitatively yielded crude **22**, which was used without further purification.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-(cyanomethyl)-1-oxadethia-3-cephem-4-carboxylate (2a). A stirred solution of the crude **22** (3.0 g, 5.85 mmol) and (cyanomethylene)triphenylphosphorane (2.60 g, 1.5 × 5.85 mmol) in toluene (90 mL) was heated at 80 °C for 40 min. The solvent was removed in vacuo and the residue was chromatographed on silica gel. Elution with benzene–ethyl acetate (2:1) and crystallization of the main fractions from benzene–ether gave **2a** as crystals: mp 158–159 °C (1.96 g, 62.3% from **18**); UV λ_{max} 272 nm (ϵ 7900); IR 3415, 2250, 1792, 1724, 1700, 1643, 1602 cm^{-1}; ^1H NMR δ 3.45 (s, 3 H), 3.63 (s, 4 H), 4.42 (s, 2 H), 5.03 (s, 1 H), 6.24 (s, 1 H), 6.87 (s, 1 H), 7.23–7.55 (m). Anal. Calcd for $C_{31}H_{27}N_3O_6$: C, 69.26; H, 5.06; N, 7.82. Found: C, 69.62; H, 5.30; N, 7.82.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-(hydroxymethyl)-1-oxadethia-3-cephem-4-carboxylate (4a). A mixture of **18** (3.00 g, 5.85 mmol), acetone (30 mL), H_2O (3.0 mL), and isocyanuric chloride (680 mg, 0.5 × 5.85 mmol) containing 60% $HClO_4$ (21 μL, 0.05 × 5.85 mmol) was stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with methylene chloride. The organic solution was washed with 10% $Na_2S_2O_3$ and H_2O, dried, and concentrated in vacuo to give crude **23**. To a solution of the crude **23** in methylene chloride (35 mL) cooled at −20 °C, pyridine (0.95 mL, 2.0 × 5.85 mmol), and chloroacetyl chloride (0.66 mL, 1.5 × 5.85 mmol) were added successively, and the resulting mixture was stirred at −10 to −5 °C for 20 min and then at 0 °C for 5 min. Excess reagent was decomposed by adding ice and the organic layer was separated. The organic solution was washed successively with 2 N HCl, 5% $NaHCO_3$, and H_2O and then dried and concentrated in vacuo, giving crude **24** as a foam (3.75 g, quantitative yield): ^1H NMR δ 3.42 (s, 3 H), 3.67–3.85 (m, 6 H), 4.17 (s, 2 H), 4.87 (s, 1 H), 5.37 (s, 1 H), 6.45 (s, 1 H), 6.92 (s, 1 H), 7.33 (m).

To a solution of the crude foam of **24** (3.75 g, 5.85 mmol) in methylene chloride (35 mL) cooled to −70 °C was added DBU (1.31 mL, 1.5 × 5.85 mmol) and the resulting solution was stirred at −70 °C for 2.5 h. Acetic acid (1.0 mL) was added to the reaction solution, which was then poured into H_2O. The organic solution was washed successively with 2 N HCl, 5% $NaHCO_3$, and H_2O, dried, and concentrated in vacuo. The residue was chromatographed on silica gel, and elution with benzene–ethyl acetate (4:1) gave crude **26** as a foam (2.6 g, 73.5%): ^1H NMR δ 3.46 (s, 3 H), 3.65 (s, 2 H), 3.96 (s, 2 H), 4.45 (s, 2 H), 5.03 (s, 1 H), 5.10, 5.22 (AB q, J = 15 Hz, 2 H), 6.20 (s, 1 H), 6.89 (s, 1 H), 7.25–7.56 (m).

The crude **26** (2.6 g, 4.3 mmol) was kept in ethanol (100 mL) and thiourea (1.3 g, 4.0 × 4.3 mmol) at room temperature overnight. The reaction solution was poured into H_2O and extracted with ethyl acetate. The organic solution was washed with H_2O and dried, and the solvent was evaporated in vacuo. Chromatography of the residue on silica gel and elution with benzene–ethyl

acetate (2:1) yielded **4a** as a foam (1.16 g, 51.0%): UV λ_{max} 270 nm (ε 7500); IR 3560 (br), 3415, 1788, 1701, 1637, 1602 cm^{-1}; ^1H NMR δ 3.00 (br, 1 H), 3.43 (s, 3 H), 3.58 (s, 2 H), 4.05–4.44 (m, 4 H), 4.97 (s, 1 H), 6.50 (s, 1 H), 6.85 (s, 1 H), 7.25–7.55 (m). Anal. Calcd for $C_{30}H_{28}N_2O_7 \cdot 0.5H_2O$: C, 67.03; H, 5.44; N, 5.21. Found: C, 67.23; H, 5.28 ; N, 4.97.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-(acetoxymethyl)-1-oxadethia-3-cephem-4-carboxylate (6a). Acetylation of **4a** (1.5 g) at 0 °C using pyridine (1.58 mL) and acetyl chloride (0.23 mL) yielded **6a** as a foam (1.13 g, 74.7%): UV λ_{max} 272 nm (ε 8700); IR 3415, 1790, 1740, 1700, 1638, 1605 cm^{-1}; ^1H NMR δ 2.00 (s, 3 H), 3.44 (s, 3 H), 3.63 (s, 2 H), 4.41 (s, 2 H), 4.95, 5.09 (AB q, J = 15 Hz, 2 H), 5.02 (s, 1 H), 6.23 (s, 1 H), 6.88 (s, 1 H), 7.23–7.54 (m). Anal. Calcd for $C_{32}H_{30}N_2O_8 \cdot 0.25H_2O$: C, 66.83; H, 5.35; N, 4.87. Found: C, 66.95; H, 5.28; N, 4.77.

Diphenylmethyl 7β-(Phenylacetamido)-7α-methoxy-3-[(carbamoyloxy)methyl]-1-oxadethia-3-cephem-4-carboxylate (7a). To an ice-cold solution of the crude **23** (1.88 g, 3.3 mmol) in methylene chloride (10 mL), prepared in a way similar to ester **4a**, was added trichloroacetyl isocyanate (0.79 mL, 2.0 × 3.3 mmol), and the resulting mixture was stirred at 0 °C for 2 h. The reaction solution was poured into ice water and extracted with methylene chloride. The organic solution was washed with H_2O, dried, and concentrated in vacuo, giving crude **25** as a foam (2.48 g, quantitative yield): ^1H NMR δ 3.40 (s, 3 H), 3.65 (s, 2 H), 3.82, 4.18 (AB q, J = 15 Hz, 2 H), 4.20 (s, 2 H), 4.92 (s, 1 H), 5.33 (s, 1 H), 6.52 (s, 1 H), 6.89 (s, 1 H), 7.30 (s), 8.53 (s, 1 H).

To a solution of the crude **25** (2.40 g, 3.3 mmol) in methylene chloride (30 mL) and tetrahydrofuran (5.0 mL) cooled at −50 °C was added DBU (0.59 mL, 1.2 × 3.3 mmol), and the resulting mixture was stirred at −50 °C. After 1.5 and 4.0 h, additional amounts of DBU (0.15 mL each, 0.3 × 3.3 mmol) were introduced, and the solution was stirred for another hour. Next, acetic acid (2.0 mL) was added and then the solution was poured into H_2O. The organic portion was washed successively with 2 N HCl, 5% $NaHCO_3$, and H_2O, dried, and concentrated in vacuo. The residue (mainly crude **27**) dissolved in benzene was left overnight to allow absorption onto silica gel in a column, which was then eluted with ethyl acetate, yielding **7a** as a foam (1.4 g, 74.0%): UV λ_{max} 271 nm (ε 8100); IR 3550, 3435, 1790, 1736, 1700, 1636, 1585 cm^{-1}; ^1H NMR δ 3.43 (s, 3 H), 3.62 (s, 2 H), 4.43 (s, 2 H), 4.70 (s, 2 H), 4.93, 5.10 (AB q, J = 15 Hz, 2 H), 5.00 (s, 1 H), 6.37 (s, 1 H), 6.87 (s, 1 H), 7.21–7.53 (m).

Deprotection of the Benzhydryl Ester Group. General Procedure. To a stirred solution of aluminum trichloride (2.5 × 2.0 mmol) in a mixture of anisole (6.0 mL) and nitromethane (6.0 mL) cooled at −40 °C was added a solution of a benzhydryl ester (2.0 mmol) in methylene chloride (6.0 mL) and stirring was continued at −40 °C for 40 min. The reaction mixture was poured into a vigorously stirred mixture of acetone, H_2O, and 2 N HCl at 0 °C. The mixture was salted out with NaCl and separated. The organic solution was extracted with an ice-cold solution of 5% $NaHCO_3$ and the aqueous solution was made acid with 2 N HCl at 0 °C, while the liberated acid was continuously extracted with ethyl acetate. The aqueous solution was extracted three times with ethyl acetate. The organic solutions were washed with a saturated solution of NaCl, dried, and concentrated in vacuo. The residue was rinsed with a suitable solvent and crystallized from an appropriate solvent, if possible. This method was used to prepare the following acids.

7β-(Phenylacetamido)-7α-methoxy-3-methyl-1-oxadethia-3-cephem-4-carboxylic acid (1b): crystallized from ethyl acetate–ether, mp 170–171 °C dec; UV λ_{max} 263 nm (ε 8300); IR (KBr) 3340, 3310, 2625, 1774, 1760, 1705, 1630, 1542, 1513, 1496 cm^{-1}; ^1H NMR (MeOH-d_4) δ 1.95 (s, 3 H), 3.43 (s, 3 H), 3.57 (s, 2 H), 4.25 (s, 2 H), 4.97 (s, 1 H), 7.26 (s, 5 H). Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.95; H, 5.24; N, 8.09. Found: C, 58.69; H, 5.24;, N, 7.84.

7β-(Phenylacetamido)-7α-methoxy-3-(cyanomethyl)-1-oxadethia-3-cephem-4-carboxylic acid (2b): a foam; UV λ_{max} 266 nm (ε 6900), 375 (1500); IR (KBr) 3440 (sh), 3320, 2560, 2250, 1780, 1706 (sh), 1682, 1640 (sh), 1520; ^1H NMR (acetone-d_6) δ 3.43 (s, 3 H), 3.67 (s, 2 H), 3.77 (s, 2 H), 4.52 (s, 2 H), 5.11 (s, 1 H), 5.23 (br s, 1 H), 7.13–7.45 (m, 5 H), 8.03 (br s, 1 H).

7β-(Phenylacetamido)-7α-methoxy-3-(hydroxymethyl)-1-oxadethia-3-cephem-4-carboxylic acid (4b): a foam; UV λ_{max} 265 (ε 5600); IR (KBr) 3330, 2600, 1780, 1675, 1514 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.40 (s, 2 H), 3.65 (s, 2 H), 4.10 (br s, 1 H), 4.47 (s, 2 H), 4.53 (s, 2 H), 4.98 (s, 1 H), 7.17–7.36 (m), 7.90 (br s, 1 H).

7β-(Phenylacetamido)-7α-methoxy-3-(methoxymethyl)-1-oxadethia-3-cephem-4-carboxylic acid (5b): precipitated from ethyl acetate, powder, mp 76–78 °C dec; UV λ_{max} 265 nm (ε 6900); IR (KBr) 3450 (br), 3320, 1780, 1716, 1702, 1657, 1534 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.27 (s, 3 H), 3.39 (s, 3 H), 3.64 (s, 2 H), 4.36 (s, 2 H), 4.45 (s, 2 H), 5.02 (s, 1 H), 7.20–7.35 (m), 7.92 (br s, 1 H). Anal. Calcd for $C_{18}H_{20}N_2O_7 \cdot CH_3COOC_2H_5$: C, 56.89, H, 6.08; N, 6.03. Found: C, 56.60; H, 6.06; N, 6.17.

7β-(Phenylacetamido)-7α-methoxy-3-(acetoxymethyl)-1-oxadethia-3-cephem-4-carboxylic acid (6b): powder; UV λ_{max} 266 nm (ε 6600); IR (KBr) 3300, 2580, 1783, 1732, 1680, 1511 cm^{-1}; ^1H NMR (acetone-d_6) δ 2.02 (s, 3 H), 3.42 (s, 3 H), 3.67 (s, 2 H), 4.48 (s, 2 H), 4.94, 5.09 (AB q, J = 13.5 Hz, 2 H), 5.05 (s, 1 H), 5.77 (br s, 1 H), 7.30 (m, 5 H), 8.00 (br s, 1 H). Anal. Calcd for $C_{19}H_{20}N_2O_8$: C, 56.43; H, 4.99; N, 6.93. Found: C, 56.22; H, 5.21, N, 6.37.

7β-(Phenylacetamido)-7α-methoxy-3-[(carbamoyloxy)methyl]-1-oxadethia-3-cephem-4-carboxylic acid (7b): UV λ_{max} 266 nm (ε 5900); IR (KBr) 3360, 2560, 1782, 1716, 1678 (br), 1511 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.42 (s, 3 H), 3.67 (s, 2 H), 4.47 (s, 2 H), 4.92, 5.04 (AB q, J = 15 Hz, 2 H), 5.04 (s, 1 H), 5.52 (br s, 3 H), 5.96 (br s, 1 H), 7.20–7.38 (m, 5 H), 8.00 (br s, 1 H).

7β-(Phenylacetamido)-7α-methoxy-3-[(methylthio)methyl]-1-oxadethia-3-cephem-4-carboxylic acid (8b): ^1H NMR (acetone-d_6) δ 2.04 (s, 3 H), 3.41 (s, 3 H), 3.54, 3.74 (AB q, J = 14 Hz, 2 H), 3.66 (s, 2 H), 4.53 (s, 2 H), 4.55 (br s, 1 H), 5.07 (s, 1 H), 7.20–7.37 (m), 7.98 (br s, 1 H).

7β-(Phenylacetamido)-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-1-oxadethia-3-cephem-4-carboxylic acid (9b): precipitated from ether, powder, mp 173–175 °C dec; UV λ_{max} 275 nm (ε 10 900); IR (KBr) 3410, 3270, 1784, 1770, 1712, 1666, 1630 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 3.32 (s, 3 H), 3.53 (s, 2 H), 3.91 (s, 3 H), 4.19 (s, 2 H), 4.49 (s, 2 H), 5.02 (s, 1 H), 7.25 (s, 5 H), 9.08 (s, 1 H). Anal. Calcd for $C_{19}H_{20}N_6O_6S$: C, 49.55; H, 4.38; N, 18.25; S, 6.96. Found: C, 49.19; H, 4.27; N, 18.17; S, 6.92.

Preparation of Sodium Salts. General Procedure. To a solution of a free acid in H_2O containing 0.9 equiv of $NaCHCO_3$ was added a dilute $NaHCO_3$ solution until the solution reached pH 6.4. The reaction solution was freeze-dried to obtain an amorphous powder of the corresponding sodium salt. The procedure was used to prepare the following sodium salts. The sodium salts are hygroscopic and prone to absorb water.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-methyl-1-oxadethia-3-cephem-4-carboxylate (1): IR (KBr) 3425, 1762, 1678, 1598, 1524, 1496 cm^{-1}; ^1H NMR (D_2O) δ 2.25 (s, 3 H), 3.91 (s, 3 H), 4.14 (s, 2 H), 4.69 (s, 2 H), 5.53 (s, 1 H), 7.82 (s, 5 H). Anal. Calcd for $C_{17}H_{17}N_2O_6Na \cdot 0.5H_2O$: C, 54.11; H, 4.81; N, 7.42. Found: C, 54.24; H, 4.76; N, 7.62.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-(cyanomethyl)-1-oxadethia-3-cephem-4-carboxylate (2): UV λ_{max} (H_2O) 260 nm (ε 8100); IR (KBr) 3395, 2250, 1769, 1685, 1616, 1499 cm^{-1}; ^1H NMR (D_2O) δ 3.92 (s, 3 H), 4.03 (s, 2 H), 4.13 (s, 2 H), 4.82 (s, 2 H), 5.57 (s, 1 H), 7.80 (s, 5 H). Anal. Calcd for $C_{18}H_{16}N_3O_6Na \cdot 0.7H_2O$: C, 53.25; H, 4.32; N, 10.35. Found: C, 53.06; H, 4.52; N, 10.46.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-(hydroxymethyl)-1-oxadethia-3-cephem-4-carboxylate (4): UV λ_{max} (H_2O) 260 nm (ε 7400); IR (KBr) 3395, 3270, 1765, 1678, 1603, 1519, 1497 cm^{-1}; ^1H NMR (D_2O) δ 3.91 (s, 3 H), 4.13 (s, 2 H), 4.70 (s, 2 H), 4.87 (s, 2 H), 5.55 (s, 1 H), 7.82 (s, 5 H). Anal. Calcd for $C_{17}H_{17}N_2O_7Na \cdot 0.6H_2O$: C, 51.67; H, 4.64; N, 7.09. Found: C, 51.46; H, 4.60; N, 7.16.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-(methoxymethyl)-1-oxadethia-3-cephem-4-carboxylate (5): UV (H_2O) λ_{max} 260 nm (ε 8500); IR (KBr) 3415, 1769, 1678, 1606, 1517, 1496 cm^{-1}; ^1H NMR (D_2O) δ 3.75 (s, 3 H), 3.93 (s, 3 H), 4.16 (s, 2 H), 4.70 (s, 2 H), 4.85 (s, 2 H), 5.60 (s, 1 H), 7.85 (s, 5 H). Anal. Calcd for $C_{18}H_{19}N_2O_7 \cdot H_2O$: C, 51.92; H, 5.08; N, 6.73. Found: C, 51.99; H, 5.17; N, 6.94.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-(acetoxymethyl)-1-oxadethia-3-cephem-4-carboxylate (6): IR (KBr) 3400, 3280 (sh), 1770, 1738, 1682, 1612, 1520, 1497 cm^{-1}; ^1H NMR (D_2O) δ 2.50 (s, 3 H), 3.90 (s, 3 H), 4.13 (s, 2 H), 4.82 (s, 2 H), 5.19, 5.35 (AB q, J = 13.5 Hz, 2 H), 5.55 (s, 1 H), 7.81 (s, 5 H). Anal. Calcd for $C_{19}H_{19}N_2O_8N \cdot 0.8H_2O$: C, 51.77; H, 4.71; N, 6.36. Found: C, 51.63; H, 4.58; N, 6.47.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-[(carbamoyloxy)methyl]-1-oxadethia-3-cephem-4-carboxylate (7): UV (H_2O) λ_{max} 260.5 nm (ε 8300); IR (KBr) 3420, 1770, 1705 (br), 1610, 1519, 1498 cm^{-1}; ^1H NMR (D_2O) δ 3.91 (s, 3 H), 4.13 (s, 2 H), 4.82 (s, 2 H), 5.16, 5.31 (AB q, J = 13 Hz, 2 H), 5.56 (s, 1 H), 7.82 (s, 5 H). Anal. Calcd for $C_{18}H_{18}N_3O_8Na \cdot 1.8H_2O$: C, 47.02; H, 4.74; N, 9.14. Found: C, 47.21; H, 4.60; N, 8.88.

Sodium 7β-(phenylacetamido)-7α-methoxy-3-[(methylthio)methyl]-1-oxadethia-3-cephem-4-carboxylate (8): UV (H_2O) λ_{max} 264 nm (ε 8200); IR (KBr) 3400, 1765, 1675, 1604, 1497 cm^{-1}; ^1H NMR (D_2O) δ 2.49 (s, 3 H), 3.72, 4.19 (AB q, J = 13.5 Hz, 2 H), 4.16 (s, 2 H), 4.89, 5.00 (AB q, J = 17 Hz, 2 H), 5.60, (s, 1 H), 7.84 (s, 5 H).

Sodium 7β-(phenylacetamido)-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-1-oxadethia-3-cephem-4-carboxylate (9): UV (H_2O) λ_{max} 270 nm (ε 11 100); IR (KBr) 3400, 1766, 1682, 1608, 1497 cm^{-1}; ^1H NMR (D_2O) δ 3.90 (s, 3 H), 4.08 (s, 2 H), 4.35 (s, 2 H), 4.46, 4.54 (AB q, J = 13.5 Hz, 2 H), 4.78, 4.89 (AB q, J = 17.5 Hz, 2 H), 5.50 (s, 1 H), 7.71 (s, 5 H). Anal. Calcd for $C_{19}H_{19}N_6O_6SNa \cdot H_2O$: C, 45.60; H, 4.23; N, 16.79; S, 6.41. Found: C, 45.75; H, 4.32; N, 16.93; S, 6.56.

7β-(Phenylacetamido)-7α-methoxy-3-(pyridiniomethyl)-1-oxadethia-3-cephem-4-carboxylate (3). A mixture of **3a** (1.3 g), anisole (2.0 mL), and trifluoroacetic acid (3.0 mL) was stirred at 0 °C for 30 min. The reaction solution was concentrated in vacuo and the residue was dissolved in 5% $NaHCO_3$. The aqueous solution was washed with ethyl acetate, made acid by adding 2 N HCl, and chromatographed on HP-20. Elution with 30–50% aqueous MeOH afforded the desired fractions. MeOH was removed in vacuo, and the aqueous solution was freeze-dried to obtain a crystalline residue (533 mg), which was recrystallized from MeOH to give **3**: mp 159 °C dec (451 mg, 58.8%); UV (H_2O) λ_{max} 230 nm (ε 8800), 259 (9900); IR (KBr) 3405, 1777, 1681, 1611, 1490 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 3.30 (s, 3 H), 3.42, 3.55 (AB q, J = 14 Hz, 2 H), 4.03, 4.31 (AB q, J = 17 Hz, 2 H), 4.97 (s, 1 H), 5.07, 5.81 (AB q, J = 14 Hz, 2 H), 7.22 (s, 5 H), 8.12 (m, 2 H), 8.59 (m, 1 H), 9.05 (s, 1 H), 9.47 (m, 2 H). Anal. Calcd for $C_{22}H_{21}N_3O_6 \cdot 2.5H_2O$: C, 56.40; H, 5.59; N, 8.97. Found: C, 56.32; H, 5.31; N, 9.04.

Measurement of ^{13}C FT NMR Spectra and Infrared Spectra. ^{13}C FT NMR was recorded on a Varian XL-100-12A NMR spectrometer (25.16 MHz) at ordinary probe temperature (31 °C) in 10- and/or 5-mm spinning tubes in D_2O (internal dioxane reference, δ 67.4). The concentrations were fixed between 0.1 and 0.2 mmol/mL because ^{13}C chemical shifts of cephalosporin analogues are influenced slightly by the concentration. Typical FT NMR measurement parameters were as follows: spectral width, 6016 Hz; pulse width, 7 μs (flipping angle 17°); acquisition time, 0.8 s; number of data points, 9625. ^{13}C NMR signals were assigned by using single-frequency and noise off-resonance decoupling and ^1H nondecoupling with NOE in the gated mode and by comparison of ^{13}C relaxation time T_1 and of the chemical shifts with those of related compounds.

IR spectra were recorded on a JASCO DS-403G grating spectrometer calibrated for the rotational bands of vapor. Oxacephem esters were dissolved in $CHCl_3$ at ca. 0.0025 M (cell length 0.5 cm) and sodium salts were dissolved under a nitrogen stream in dry dimethyl sulfoxide at ca. 0.02 M (cell length 0.025 cm). The accuracy of the $\nu_{C=O}$ value was ±1.0 cm^{-1}.

Determination of Antibacterial Activity. MICs were determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/mL with the same broth. One loopful of this suspension was inoculated with an inoculating device onto agar containing serial twofold dilutions of an antibiotic. Organisms were incubated at 37 °C for 18–20 h. The MIC of an antibiotic was defined as the lowest concentration that inhibited visible growth. The values log $(1/C_N)$ are believed to be reproducible within 0.20.

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