Studies on Penem and Carbapenem. I. Syntheses and Oral Absorption of Ester-Type Prodrugs of Sodium (5*R*,6*S*)-2-(2-Fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate

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Acyloxyalkyl esters (2a—d), alkyloxycarbonyloxyalkyl esters (2e—g) and (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (2h) of (5*R*,6*S*)-2-(2-fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylic acid (1) were synthesized. Enhanced oral absorption was observed in mice reflecting increased lipophilicity, compared with the parent 1 itself. Among them, the ester 2h showed a prolonged plasma level and a large area under the blood concentration–time curve (*AUC*) in rats. These ester-type prodrugs of penem 1 in phosphate buffer (pH 6.86) were much more stable than those of cephalosporins which easily degraded *via* isomerization to Δ^2 cephalosporins.

Keywords penem; ester; prodrug; oral absorption; lipophilicity; stability

Recently ester-type prodrugs of cephalosporin have been investigated for oral administration. However, chemical instability, which is probably due to the isomerization of the cephem double bond, seems to limit the prodrug approach of cephalosporin.[1] Penem antibiotic without such a moiety to isomerize, on the other hand, may possibly lead to a more stable ester-type prodrug.

Sodium (5*R*,6*S*)-2-(2-fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate (**1**) is a new penem antibiotic that has potent *in vitro* activity against both gram-positive and gram-negative organisms.[2] In particular, it has shown antibacterial activity against the methicillin- and cephem-resistant *Staphylococcus aureus* (MRSA), which has recently been of interest.[3] It is effective, however, only when used by injection and it is not suitable for oral administration. In our research on the oral delivery of penem **1**, esterification of the carboxyl group at the C-3 position was carried out. FCE 22891, a new orally active penem antibiotic reported by the Farmitalia Carlo Erba research group, is also an ester-type prodrug at the C-3 position.[4]

This paper describes the syntheses of acyloxyalkyl esters, alkyloxycarbonyloxyalkyl esters and (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester of penem **1**, and physicochemical properties and biological evaluations in mice and rats.

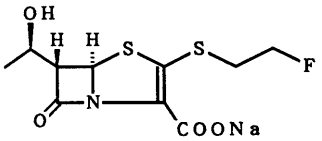
Results and Discussion

Syntheses Penem **1** was treated with acyloxyalkyl or alkyloxycarbonyloxyalkyl iodides in *N*,*N*-dimethylacetamide. Corresponding esters **2a**—**g** were given in moderate yields. Esters **2a**, **2b**, **2d** and **2e**, of low molecular weight, were obtained in a viscous oil. Other esters **2c**, **2f** and **2g** were obtained as a glassy solid but not in a crystalline form.

Ester **2h** was prepared by treating penem **1** with (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl bromide[5] in *N*,*N*-dimethylacetamide. This ester **2h** was obtained as crystals from ethylacetate, which is favorable for purification, stability and handling.

Physicochemical Properties Physicochemical properties, such as lipophilicity, chemical stability and water solubility, are known to affect intestinal absorption.[6] Some of these properties were examined for esters **2f** and **2h**.

Lipophilicity was determined by measuring the partition coefficient (*P*) between *n*-octanol and water. log *P* values of esters **2f** and **2h** were 3.54 and 1.21, respectively. These values are enough for passive absorption through the lipoidal membrane of the small intestine.[6]



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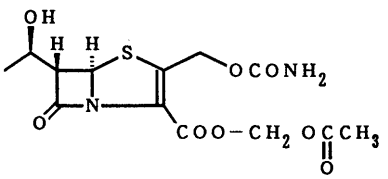
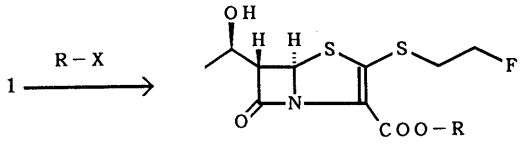


FCE 22891

Chart 1



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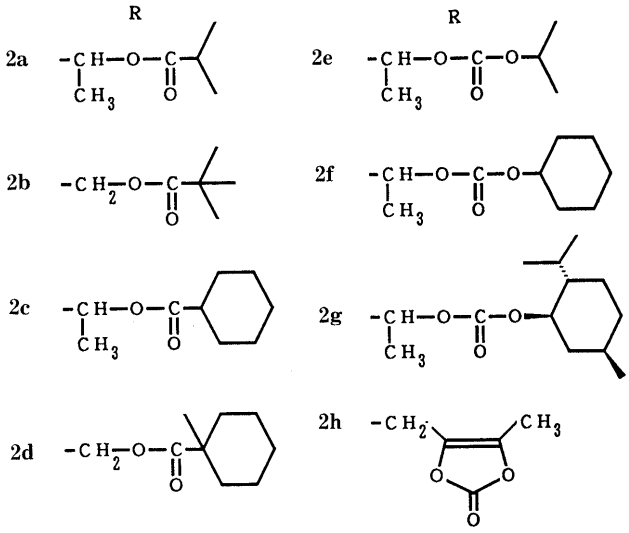

Chart 2

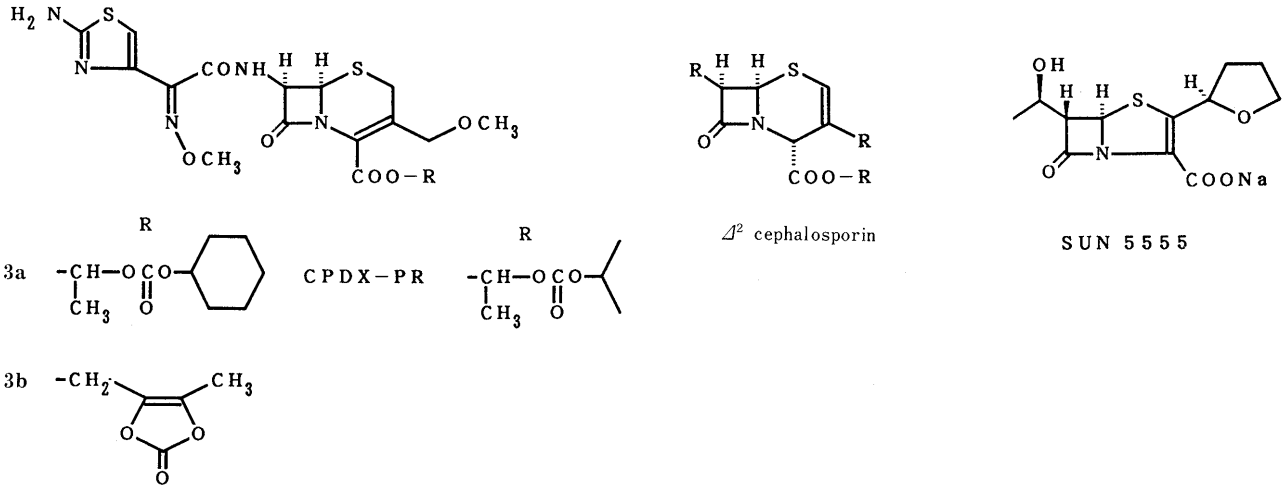

Chart 3

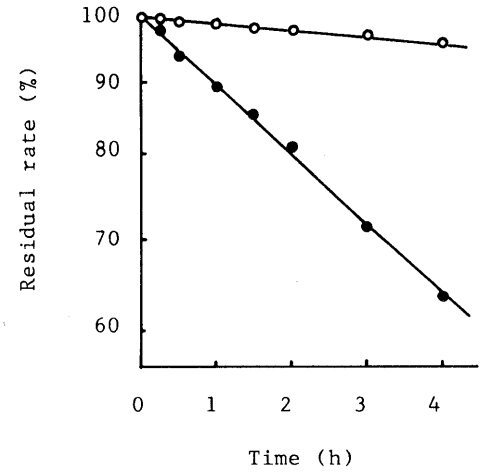

Fig. 1. Chemical Stability of Penem Esters **2f** (—○—) and **2h** (—●—) in Phosphate Buffer Solution

Initial concentration of esters, 50 μg/ml; phosphate buffer, 1/20 M, pH 6.86; temperature, 37 °C.

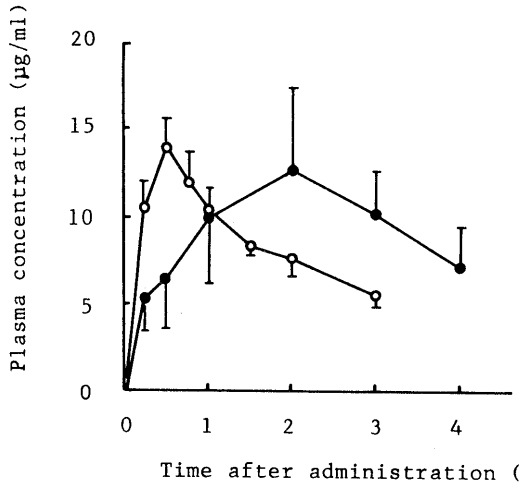

Fig. 2. Plasma Concentrations (μg/ml) of Parent Penem after Oral Administration of Penem Esters **2f** (—○—) and **2h** (—●—) in Rats

Penem esters **2f** and **2h** (dose; 25 mg/kg as parent penem **1**) were orally administered to rats ($n=3$, male, Wister-Imamichi strain). Plasma concentrations of parent penem were determined by bioassay. Mean ± S.E. are given.

TABLE I. Urinary Recovery of Orally Administered Penem Esters **2a**—**h** in Mice[a]

Compound	Urinary recovery (%)
2a	36
2b	22
2c	25
2d	33
2e	21
2f	29
2g	23
2h	41
1	7
1 (s.c.)[b]	43

a) Penem esters **2a**—**h** and parent penem **1** (dose; 50 mg/kg as parent penem) were orally administered to mice ($n=5$, male, slc ddY strain). Recovery as parent penem was determined by bioassay. *b*) Subcutaneous administration.

TABLE II. Therapeutic Effect of Penem **2h** and Other β-Lactam Antibiotics on Experimental Infections in Mice

Bacteria	ED_{50} (mg/kg/dose)			
	2h	SUN 5555	CPDX-PR[a]	CEX[a]
S. aureus Smith	12.0	12.7	6.83	0.12
S. aureus 560[b]	7.36	11.7	16.1	23.9
E. coli 704	30.7	>100	1.54	19.6
E. coli 609[b]	>100	NT	12.5	100

a) Data were taken from reference 7*b*. *b*) β-Lactamase-producing strain.

Chemical stability of esters **2f** and **2h** were examined in phosphate buffer (pH 6.86, 1/20 M) at 37 °C. Degradations were monitored by high performance liquid chromatography (HPLC). Degradation of each derivative followed the first-order kinetic, as shown in Fig. 1, and gave the parent penem **1**. The carbonate-type ester **2f** was more stable than (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester **2h**. Degradation rate constants were 0.011 h^{-1} for **2f** and 0.111 h^{-1} for **2h**. On the other hand, corresponding cephalosporin esters **3a** and **3b** having a methoxymethyl group at the C-3 position (Chart 3) under the same conditions degraded at rate constants of 0.107 and 0.164 h^{-1}, respectively. These results showed that ester-type prodrugs of penem, such as **2f** and **2h**, were more stable than those of cephalosporin, which easily degraded *via* isomerization to Δ^2 cephalosporin.[1)]

Oral Absorption and *in Vivo* Antibacterial Activity Prodrugs **2a**—**h** were administered orally to mice at a dose of 50 mg/kg equivalent to the parent **1**. Oral absorption of these esters was determined from the recovery of parent **1** in urine. These esters exhibited enhanced oral absorption compared with orally administered **1**. Among them ester **2h** showed a high urinary recovery, close to that after a subcutaneous dosage of **1**. Results are listed in Table I.

Next, the plasma levels of **1** after an oral dosage of esters **2f** and **2h** were compared in rats. Both esters of 25 mg/kg equivalent to **1** were administered as a polyethyleneglycol (PEG-400) solution containing 1% hydroxypropylmethylcellose. Ester **2f** gave a high plasma level immediately after administration. Ester **2h**, on the other hand, showed a prolonged plasma level and a large area under the blood concentration–time curve (*AUC*), as shown in Fig. 2. The absorption profiles of the two esters were explicable from the difference in lipophilicity. That is, the highly lipophilic ester **2f** ($\log P = 3.54$) is absorbed faster through the lipoidal membrane of the small intestine, whereas the moderately lipophilic ester **2h** ($\log P = 1.21$) is absorbed gradually and gives a prolonged plasma level.

The *in vivo* antimicrobial activity of penem **2h** against *Staphylococcus aureus* and *Escherichia coli* was compared with those of SUN 5555, cefpodoxime proxetil (CPDX-PR) and cephalexin (CEX).[7] Systemic infections were induced by i.p. injection of a bacterial suspension in mice, and antibiotics were orally administered. Results are summarized in Table II. The therapeutic efficacy of penem **2h** against the infection by *S. aureus* Smith was better or equal to that of SUN 55555 and inferior to those of CPDX-PR and CEX. Against infection by β-lactamase-producing strain *S. aureus* 560, however, penem **2h** exerted fairly good activity superior to those of other β-lactams. In this experiment using mice, both penem antibiotics **2h** and SUN 5555 were not very effective against infections by *E. coli* regardless of their high *in vitro* antibacterial activities.

Experimental

General Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a JEOL GX-270 spectrometer using tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a Nicolet FT-IR (5SXC) spectrometer. Ultraviolet (UV) spectra were taken on a Shimadzu UV-3100 spectrometer. HPLC was performed using a Waters chromatography system (6000A pump, 440 absorbance detecter (254 nm)) and a Shimadzu C-R3A Chromatopac.

Preparation of 1-(Isobutyryloxy)ethyl (5*R*,6*S*)-2-(2-Fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate 2a 1-(Isobutyryloxy)ethyl iodide (110 mg) was added to a solution of sodium (5*R*,6*S*)-2-(2-fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate **1** (94 mg) in *N*,*N*-dimethylacetamide (3 ml) and the mixture was stirred under ice-cooling for 3 h. The reaction mixture was poured into ice-water (50 ml) and extracted with EtOAc (50 ml). The extract was washed successively with water (50 ml × 2) and brine (50 ml), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on a silica gel (100 g) column using EtOAc–hexane (1 : 1) as an eluent. Ester **2a** (65 mg) was obtained as a viscous oil. ^{1}H-NMR and IR data are listed in Table III. Esters **2b**—**g** were prepared in a procedure similar to that described above. ^{1}H-NMR and IR data are listed in Table III.

Preparation of (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl (5*R*,6*S*)-2-(2-Fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate (2h) (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl bromide (135 mg) was added to a solution of penem **1** (200 mg) in *N*,*N*-dimethylacetamide (4 ml) under ice-cooling and the mixture was stirred for 24 h. The reaction mixture was diluted with water (50 ml) and extracted with EtOAc (50 ml). The organic layer was washed with brine (50 ml × 2), dried over $MgSO_4$, and concentrated *in vacuo*. Resulting crystals were washed with EtOAc–benzene (1 : 1) and dried under reduced pressure. Ester **2h** (180 mg) was obtained as colorless needles, mp 138—140 °C. ^{1}H-NMR and IR data are listed in Table III.

Lipophilicity Ester was dissolved in *n*-octanol (*ca.* 500 μg/ml). This solution (1 ml) and phosphate buffer (1 ml, 1/20 M, pH 6.86) were shaken at 20 °C for 5 min. The mixture was centrifuged to separate the phases. Concentration of the ester in each phase was determined by HPLC. The organic phase was diluted 10—1000 times with MeOH before analysis. The partition coefficient (*P*) was obtained by dividing the concentration in the organic phase by that in the aqueous phase. HPLC conditions are as follows.

Detection of **2f**: Column; YMC AQ-312. Solvent; 0.2% $AcONH_4$–MeOH (20–80). Flow rate; 1.0 ml/min. Retention time; 6.05 min. Diastereomers were not separated under these conditions.

Detection of **2h**: Column; YMC AQ-312. Solvent; 0.2% $AcONH_4$–MeOH (40–60). Flow rate; 1.0 ml/min. Retention time; 6.10 min.

Chemical Stability A solution of ester (10 mg/ml) in *N*,*N*-dimethylformamide was added to phosphate buffer (1/20 M, pH 6.86) preincubated at 37 °C, and the mixture was stirred at 37 °C. The initial concentration of the ester was about 50 μg/ml. Samples were taken at suitable intervals. The concentration of the ester that remained was determined by HPLC.

Oral Absorption a) Recovery in Mice: Ester was dissolved in

Table III. ^{1}H-NMR and IR Spectral Data for Penem Esters **2a**—**h**

No.	^{1}H-NMR[a]										IR[c]			
	5-CH (d)	6-CH (dd)	9-CH (m)	10-CH_3 (d)	FCH_2---CH_2S– (dt)	(m)	Ester moiety							
2a	5.66, 5.65	3.73, 3.72	4.20—4.31	1.37, 1.35	4.62	3.13—3.40	6.97, 6.95	1.55, 1.54	2.57, 2.54	1.19, 1.18, 1.16	1792	1749	1699	1500
	(1.5)	(6.6, 1.5)		(6.2)	(46.5, 6.6)		(1H, q, 5.5)	(3H, d, 5.5)	(1H, sept, 7.0)	(6H, d, 7.0)	1367	1326	1064	(B)
2b	5.67	3.72	4.20—4.30	1.35	4.62	3.09—3.43	5.83, 5.92	1.22			1795	1750	1710	1505
	(1.5)	(6.8, 1.5)		(6.0)	(46.5, 6.6)		(2H, ABq, 5.5)	(9H, s)			1335	1095		(D)
2c	5.66, 5.65	3.73, 3.72	4.20—4.31	1.37, 1.35	4.62	3.13—3.40	6.96, 6.94	1.54, 1.53	2.24—2.38	1.20—1.95	1792	1747	1698	1500
	(1.5)	(6.9, 1.5)		(6.2)	(46.5, 6.6)		(1H, q, 5.5)	(3H, d, 5.5)	(1H, m)	(10H, m)	1369	1328	1070	(B)
2d	5.67	3.73	4.20—4.30	1.35	4.62	3.13—3.40	5.92, 5.86	1.18	1.19—2.06		1793	1749	1704	1498
	(1.5)	(6.8, 1.5)		(6.3)	(46.5, 6.6)		(2H, ABq, 5.4)	(3H, s)	(10H, m)		1335	1092		(C)
2e	5.66, 5.65	3.73, 3.72	4.20—4.32	1.37, 1.35	4.62	3.12—3.40	6.87. 6.86	1.59, 1.57	4.84—5.01	1.30. 1.31	1793	1756	1698	1500
	(1.5)	(6.6, 1.5)		(6.2)	(46.5, 6.6)		(1H, q, 5.5)	(3H, d, 5.5)	(1H, m)	(6H, d, 6.2)	1368	1327	1073	(B)
2f	5.66, 5.65	3.73, 3.72	4.19—4.31	1.37, 1.35	4.62	3.11—3.48	6.88, 6.87	1.59, 1.57	4.59—4.72	1.25—2.00	1794	1755	1698	1502
	(1.5)	(6.6, 1.5)		(6.2)	(46.5, 6.6)		(1H, q, 5.5)	(3H, d, 5.5)	(1H, m)	(10H, m)	1730	1329	1075	(A)
2g	5.65	3.73, 3.72	4.18—4.32	1.37, 1.35	4.62	3.11—3.40	6.86, 6.85	1.59, 1.57	4.60—4.48	0.77—2.17	1795	1756	1370	1328
	(1.5)	(7.0, 1.5)		(6.2)	(46.5, 6.6)		(1H, q, 5.5)	(3H, d, 5.5)	(1H, m)	(18H, m)	1266	1074		(A)
2h[b]	5.5765	3.83	4.18—4.32	1.35	4.65	3.20—3.48	5.09, 5.05	2.17	5.20		1821	1772	1685	1487
	(1.5)	(5.9, 1.5)		(6.3)	(46.9, 5.9)		(2H, ABq, 14.2)	(3H, s)	(1H, d, 4.9)		1337	1188	1122	(A)

a) Spectra were measured in $CDCl_3$ at 270 MHz with tetramethylsilane as an internal standard. Chemical shifts are given in δ (ppm). Coupling patterns and constants (Hz) are given in parentheses. *b*) Measured in DMSO-d_6. *c*) Spectra were taken in a KBr pellet (A), liquid film (B), Nujol (C) or $CHCl_3$ solution (D). Characteristic absorption bands are given in wave number (cm^{-1}).

polyethyleneglycol (PEG-400) and diluted with an equal volume of water. The solution was administered orally to slc ddY mice (male, $n=5$, 50 mg/kg as a parental penem **1**). Mice were allowed free access to water but were fasted overnight before administration. Excretion of the parent penem **1** into urine and feces after administration (0—24 h) was determined by bioassay using *Bacillus subtilis* ATCC 6633 as a test strain.

b) Plasma Level in Rats: Ester (10 mg) was dissolved in polyethyleneglycol (PEG-400) (1 ml) with hydroxypropylmethylcellose (10 mg). The solution was administered orally to rats (male, Wister–Imamichi strain, $n=3$, 25 mg/kg as parent penem **1**). Blood (0.1 ml) was taken from the tail vein, mixed with 10% trichloroacetic acid (0.1 ml) and centrifuged at $1000 \times g$ for 10 min. The concentration of parent penem **1** was determined by bioassay.

Therapeutic Effect on Systemic Mouse Infections Overnight cultures of organisms grown at 37 °C in trypto-soy broth (Eiken-Chemical Co., Ltd., Tokyo, Japan) were diluted according to their virulence. The diluted cultures, if necessary, were mixed with the same amount of 10% gastric mucin (Tokyokasei-Kogyo Co., Ltd., Tokyo, Japan). Ten male ddY mice in each group were infected intraperitoneally with 0.2 ml portions of those bacterial mixtures. β-Lactam antibiotics were administered orally at 0 and 4 h after infection. The effective doses for 50% of the mice (ED_{50}s) were calculted by the Probit method according to the survival rate after 5 d.

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References and Notes

1) *a*) M. Miyauchi, K. Sasahara, K. Fujimoto, I. Kawamoto, J. Ide and H. Nakao, *Chem. Pharm. Bull.*, **37**, 2369 (1989); *b*) M. Miyauchi, T. Hirota, K. Fujimoto and J. Ide, *ibid.*, **37**, 3272 (1989); *c*) A. N. Saab, L. W. Dittert and A. A. Hussain, *J. Pharm. Sci.*, **77**, 906 (1988).
2) *a*) I. Kawamoto, R. Endo and S. Sugawara, *J. Antibiot.*, **39**, 1551 (1986); *b*) I. Kawamoto, R. Endo, K. Suzuki and T. Hata, *Heterocycles*, **25**, 123 (1987).
3) Penem **1** showed the minimum inhibitory concentration (MIC) of 3.1 μg/ml against *S. aureus* 535 (MRSA), whereas Flomoxef showed 12.5 μg/ml.
4) *a*) G. Franceschi, M. Foglio, M. Alpegiani, C. Battistini, A Bedeschi, E. Perrone, F. Zarini, F. Arcamone, C. Della and A. Safilioppo, *J. Antibiot.*, **36**, 938 (1983); *b*) G. Franceschi, E. Perrone, M. Alpegiani, A. Bedeschi, C. Battistini, F. Zarini and C. Della Bruna, *J. Antimicrob. Chemother.*, **23** (Suppl C), 1 (1989).
5) *a*) F. Sakamoto, S. Ikeda and G. Tsukamoto, *Chem. Pharm. Bull.*, **32**, 2241 (1984); *b*) S. Ikeda, Y. Takebe, R. Hirayama, F. Sakamoto, K. Iuchi and G. Tsukamoto, *ibid.*, **36**, 394 (1988).
6) A. Tsuji and T. Yamana, "β-Lactam Antibiotics," ed. by S. Mitsuhashi, Japan Sci. Soc. Press, Tokyo, 1981, p. 235; S. H. Yalkowsky and W. Morozowich, "Drug Design," Vol. IX, ed . by E. J. Ariens, Academic Press, New York, 1980, p. 121 (Chapter 3).
7) *a*) SUN 5555: M. Ishiguro, H. Iwata, T. Nakatsuka, R. Tanaka, Y. Maeda, T. Nishihara, T. Noguchi and T. Nishino, *J. Antibiot.*, **41**, 1685 (1988); T. Nishino, Y. Maeda, E. Ohtsu and S. Koizumi, *ibid.*, **42**, 977 (1989); *b*) CPDX-PR and CEX: H. Yasuda, T. Magaribuchi, I. Igarashi, M, Katsuta, T. Takenouchi, T. Tanii, Y. Ajiki, S. Yurugi, N. Sekine, M. Kasahara and M. Iwata, *Chemotherapy*, **36** (S-1), 159 (1988); K. Fujimoto, S. Ishihara, H. Yanagisawa, J. Ide, E. Nakayama, H. Nakao, S. Sugawara and M. Iwata, *J. Antibiot.*, **40**, 370 (1987).