STUDIES ON β-LACTAM ANTIBIOTICS. III†

SYNTHESIS AND ENZYMATIC STABILITY OF 3-ACYLOXYMETHYL-7β-[(Z)-2-(2-AMINO-4-THIAZOLYL)-2-(METHOXYIMINO)ACETAMIDO]-3-CEPHEM-4-CARBOXYLIC ACIDS

TAKAO TAKAYA*, HISASHI TAKASUGI, TAKEO MURAKAWA and HIROSHI NAKANO

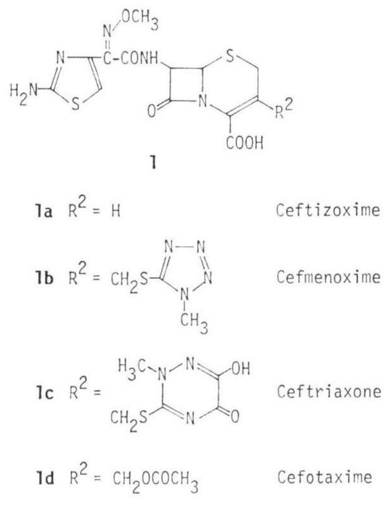
Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan

(Received for publication May 22, 1981)

3-Acyloxymethyl-7β-[(Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino) acetamido]-3-cephem-4-carboxylic acids (**7**) were synthesized. The stability of **7** to enzymatic hydrolysis and their antimicrobial activity were evaluated. **7** showed good antimicrobial activity against a wide range of microorganisms. Cephems (**7b** and **7c**) with sterically more hindered acyl groups such as *t*-butyl and cyclohexyl were most resistant to enzymatic hydrolysis.

Recently, extensive studies have been undertaken on a new family of cephalosporins bearing the oxyiminoacetyl side chain at the C-7 position of a cephem nucleus. Especially, cephalosporins (Fig. 1) with (Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetyl group, such as ceftizoxime,[1,2,3] cefotaxime,[4,5] cefmenoxime[6] and ceftriaxone[7] have been reported to possess excellent antibacterial activity.

Fig. 1.

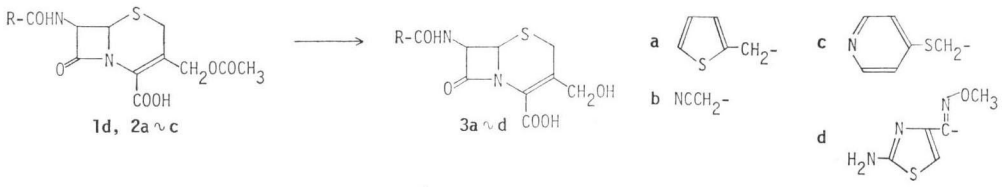


In this series, we became interested in the effect of structural modification of the acyl moiety in the 3-acetoxymethyl group on the stability toward enzymatic hydrolysis, because cephalosporins with the 3-acetoxymethyl group, such as cephalothin,[8] cephacetrile,[9] cephapirin[10] and cefotaxime[11] are known to be metabolized by hydrolysis to the corresponding 3-hydroxymethylcephems (**3a**∼**d**) which are less active than the 3-acetoxymethylcephems (**1d** and **2a**∼**c**) (Scheme 1). In order to overcome this drawback, we intended to change the acetyl radical at the 3-position of the cephem nucleus for a sterically more hindered acyl moiety which can be expected to be resistant to hydrolysis.

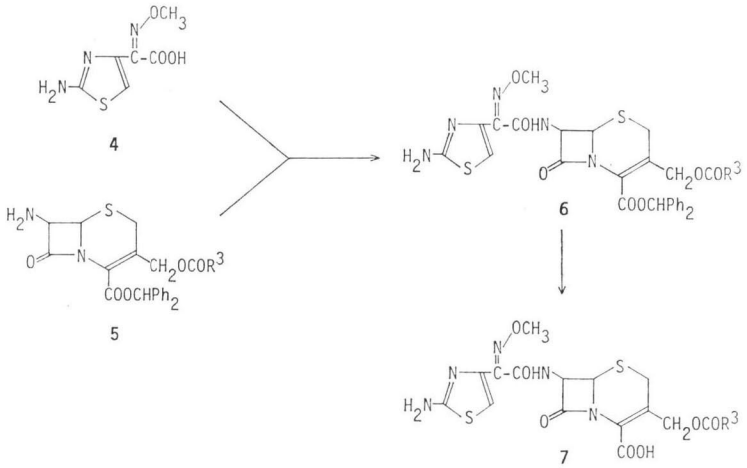
We here report the synthesis (Schemes 2 and 3), stability to enzymatic hydrolysis (Fig. 2), and antimicrobial activity (Table 1) of cephalosporins (**7**) with various 3-acyloxymethyl groups.

† Paper II. TAKAYA, T.; H. TAKASUGI, T. MASUGI, H. KOCHI & H. NAKANO: Studies on β-lactam antibiotics. II. Synthesis and structure-activity relationships of α-hydroxyiminoarylacetyl cephalosporins. J. Antibiotics 34: 1290∼1299, 1981

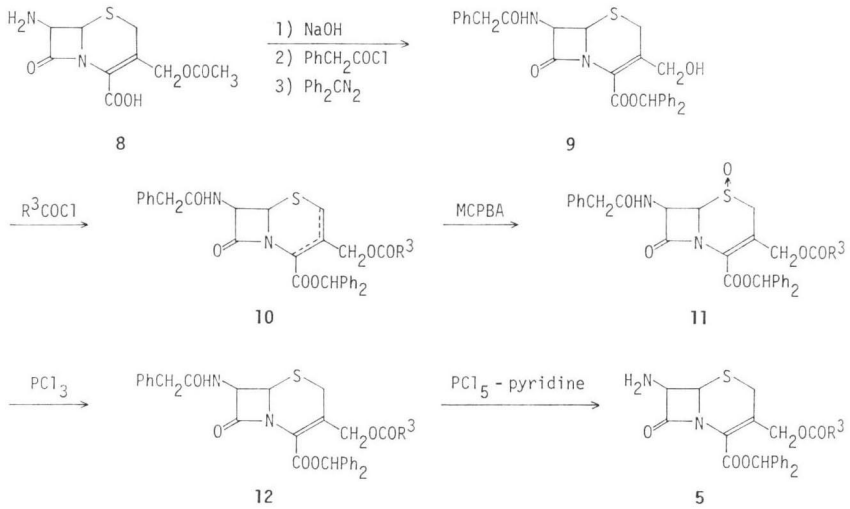
Scheme 1.



Scheme 2.



Scheme 3.

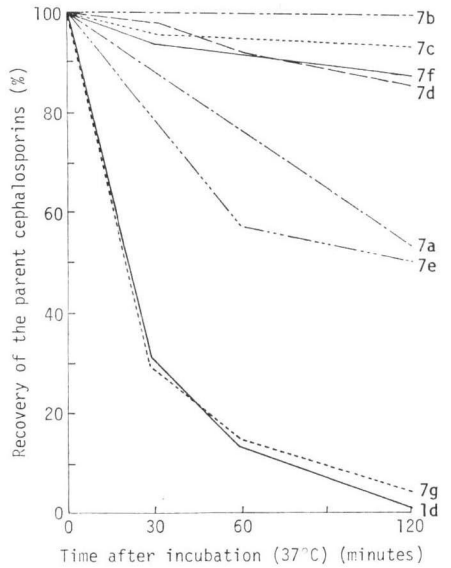


Chemistry

Semisynthetic cephalosporins (**7**) were synthesized by acylation of diphenylmethyl 7β-amino-3-acyloxymethylcephalosporanate (**5**) with (*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetic acid (**4**)[12] followed by subsequent deprotection of the esters (**6**), as outlined in Scheme 2. The 7β-amino-3-acyl-

oxymethylcephems (**5**) were prepared according to the improved reaction sequence (Scheme 3) which was accomplished by a combination of the synthetic procedures described by KUKOLJA,[13] VAN HEYNINGEN[14] and the Glaxo group.[15] A 3-hydroxymethyl derivative (**9**) was prepared in a one-pot process from alkaline hydrolysis[16] of 7-aminocephalosporanic acids (7-ACA) followed by subsequent phenylacetylation, and esterification. Acylation of the 3-hydroxymethyl function of **9** with acyl chloride gave a mixture (**10**) of Δ^2- and Δ^3-acyloxymethylcephems. The Δ^3-3-acyloxymethylcephems (**12**) were prepared from the above mixture by utilizing the oxidative-reductive process (**10**→**11**→**12**) for isomerization of the double bond. Cleavage for the phenylacetyl side chain of **12** with PCl_5 gave the 7β-amino-3-acyloxymethylcephems (**5**). A cephem ester (**6**) was obtained by coupling of **5** with an activated acid which was prepared by treatment of the acid (**4**) with VILSMEIER-like reagent ($POCl_3$-DMF) without protection of the 2-amino function. Deprotection of **6** with CF_3COOH-anisole gave the 3-acyloxymethylcephem (**7**).

Fig. 2. Enzymatic hydrolysis of the 3-acyloxymethyl cephalosporins (**7**).



The structure of all compounds (**5**, **6**, **7**, **11** and **12**) were supported by their IR and NMR spectra. Their yields and spectral data are summarized in Tables 2, 3, 4, 5 and 6.

Biological Results

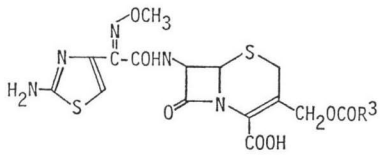
Enzymatic cleavage of the various 3-acyloxymethylcephems (**7**) by rat liver homogenates was examined. Results are pictured in Fig. 2. The 3-acetoxymethyl analog (**1d**) and **7g** ($R^3 = -CH_2O-C_6H_4-OCH_3$) were easily hydrolyzed by rat liver homogenates to a similar extent. In contrast, cephems (**7b** and **7c**) with sterically more hindered acyl groups such as *t*-butyl and cyclohexyl were the most resistant to enzymatic hydrolysis. Especially **7b** was virtually stable under these experimental conditions. In general, the more bulky the 3-acyloxymethyl group in the cephems (**7**) is, the more resistant it can be to undergo metabolic conversion to the less active 3-hydroxymethyl cephems (**3d**), as is expected.

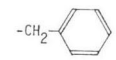
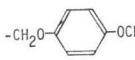
The *in vitro* activity of the above various acyloxymethylcephems (**7a**～**7g**) against selected Gram-positive and Gram-negative organisms is shown in Table 1. Their antibacterial activity except for **7b**, **e**, **g** tends to be enhanced (*ca.* 2～4 times) against Gram-positive bacteria and indole-negative *Proteus* species such as *Proteus vulgaris* 2 (*ca.* 2～8 times), but to be relatively reduced against other Gram-negative bacteria as compared to that of the 3-acetoxymethylcephem (**1d**). However, they still retain good antibacterial activity and a broad spectrum of activity.

Experimental

NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a JEOL-MH 100 NMR spectrometer using Me_4Si as an internal standard. IR spectra were taken on

Table 1. Antibacterial activity of the 3-acyloxymethyl cephalosporins (**7**).



	Compounds R^3	MIC (mcg/ml)						
		S. aureus 209 P JC 1	*B. subtilis* ATCC 6633	*E. coli* 28	*K. pneumoniae* 20	*P. mirabilis* 1	*P. vulgaris* 2	*P. aeruginosa* NCTC 10490
1d	$-CH_3$	3.13	6.25	0.20	0.05	0.05	1.56	6.25
7a	$-(CH_2)_4CH_3$	1.56	0.78	0.78	0.20	0.39	1.56	6.25
7b	$-C(CH_3)_3$	12.5	3.13	0.78	0.78	1.56	0.78	50
7c		3.13	3.13	0.78	0.78	0.78	0.78	25
7d		1.56	1.56	0.39	0.39	0.39	0.39	12.5
7e		6.25	3.13	0.78	0.78	0.20	0.78	50
7f		1.56	0.78	0.39	0.20	0.39	0.20	25
7g		25	6.25	1.56	0.39	0.20	0.78	800

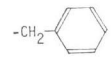
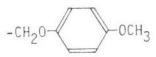
a Hitachi 260-10 spectrophotometer or Shimadzu IR-420 spectrophotometer.

Preparation of Diphenylmethyl 3-Hydroxymethyl-7β-phenylacetamido-3-cephem-4-carboxylate (**9**)

To a stirred suspension of 7-ACA (50 g, 0.183 mole) in H_2O (200 ml) was added 20% NaOH solution (85 ml, 0.402 mole) during 5 minutes to keep the reaction temperature between 2~5°C under cooling in an ice-salt bath. After stirring for a further 5 minutes at this temperature, the reaction solution was adjusted to pH 8.5 with AcOH and diluted with acetone (150 ml). To the solution was dropwise added a solution of phenylacetyl chloride (34.1 g, 0.22 mole) in acetone (35 ml) at 0~5°C, keeping the pH between 7.5~8.5 with Et_3N. The reaction mixture was stirred for one hour at 0~5°C, and then concentrated *in vacuo* to remove the organic solvent. The resulting solution was overlayered with ethyl acetate (AcOEt) (700 ml), and acidified to pH 3.0 with 17.5% HCl. The aqueous layer was further extracted with AcOEt (300 ml). The combined organic layers were washed with brine, dried ($MgSO_4$) and filtered. To the obtained solution was added an AcOEt solution (*ca.* 0.7~0.8 mole) of diphenyldiazomethane under stirring until the color of diphenyldiazomethane persisted. The reaction solution was concentrated to a volume of *ca.* 400 ml and then cooled to 5~10°C in a refrigerator. The precipitate was collected by filtration and washed with AcOEt to give 45.8 g (48.5%) of **9**; mp 178~179°C (Ref. 17, mp 176.5°C); IR (Nujol) 3500, 3280, 1760, 1710, 1660 cm^{-1}; NMR (DMSO-d_6) δ 3.60 (2H, s), 3.63 (2H, s), 4.27 (2H, d, J=6Hz), 5.12 (1H, d, J=6Hz), 5.73 (1H, dd, J=5,8Hz), 6.93 (1H, s), 7.10~7.73 (15H,m), 9.10 (1H, d, J=8Hz).

General Preparation of Diphenylmethyl 3-Acyloxymethyl-7β-phenylacetamido-3-cephem-4-carboxylate 1-Oxide (**11**)

Table 2. Yields of **5**, **6**, **7**, **11** and **12**.

	Compounds	*O*-Acylation & oxidation	Reduction	Cleavage	*N*-Acylation	Deprotection
	R^3	**11** (%)	**12** (%)	**5** (%)	**6** (%)	**7** (%)
a	$-(CH_2)_4CH_3$	58.4	95.0	72.2	75.6	58.8
b	$-C(CH_3)_3$	50.8	84.7	77.3	66.3	60.0
c		64.7	98.5	63.2	73.5	55.3
d		71.5	95.5	75.7	77.8	74.9
e		66.4	95.4	88.7	71.0	59.7
f		72.0	92.6	68.3	82.9	78.7
g		72.8	92.0	93.3	80.0	71.8

Acyl chloride (0.04 mole) was added to the solution of **9** (0.02 mole) and Et_3N (0.04 mole) in tetrahydrofuran (200 ml), and the mixture was stirred at 50~60°C for 2~5 hours. After removing the solvent *in vacuo*, the residue was dissolved in a mixture of AcOEt (200~300 ml) and H_2O (100 ml). The separated AcOEt layer was washed with 10% HCl, saturated $NaHCO_3$ solution, and brine. The AcOEt layer was dried ($MgSO_4$) and filtered.

To the above filtrate was dropwise added a solution of *m*-chloroperbenzoic acid (0.02 mole) in AcOEt (40 ml) under ice-cooling, and the mixture was stirred at similar temperature for 1~3 hours. After addition of AcOEt (100~300 ml) to the reaction mixture, the resulting solution was washed successively with saturated aqueous $NaHCO_3$, brine, and dried ($MgSO_4$). The solution was concentrated *in vacuo* to a volume of *ca.* 100~150 ml, and then cooled to 5~10°C in a refrigerator. The precipitate was collected by filtration and washed with AcOEt or diethyl ether to afford **11** as crystals.

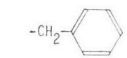
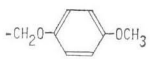
General Preparation of Diphenylmethyl 3-Acyloxymethyl-7β-phenylacetamido-3-cephem-4-carboxylate (**12**)

Phosphorus trichloride (0.02 mole) was added to a solution of **11** (0.01 mole) in DMF (80~100 ml) at −40°C and the mixture was stirred at −40~−10°C for 1~2 hours. The reaction mixture was poured into cold water (400 ml). The precipitate was collected by filtration, washed with H_2O, and dried (P_2O_5) to afford **12** as crystals or powder.

General Preparation of Diphenylmethyl 3-Acyloxymethyl-7β-amino-3-cephem-4-carboxylate (**5**)

Pyridine (0.015 mole) was added to a suspension of PCl_5 (0.015 mole) in methylene chloride (30~40 ml) at 5°C, and the mixture was stirred at room temperature for 30 minutes. **12** (0.015 mole) was added to the above mixture at 5°C and stirred at 5~8°C for 1~2 hours. Then, MeOH (0.25 mole) was added to reaction mixture at −30°C and the resulting solution was stirred at −15~0°C for 1~2 hours. After removing the solvent *in vacuo*, the residue was added to a mixture of H_2O and diethyl ether. The separated aqueous layer was adjusted to pH 7.5 with 20% aqueous Na_2CO_3, and extracted with AcOEt. The AcOEt layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was triturated with diethyl ether or diisopropyl ether to afford **5** as a powder.

Table 3. IR spectral data of **5**, **6**, **7**, **11** and **12**.

	Compounds	IR ν_{max}^{Nujol} (cm^{-1})													
		11			**12**			**5**		**6**			**7**		
	R^3	β-Lactam	Ester	CONH	β-Lactam	Ester	CONH	β-Lactam	Ester	β-Lactam	Ester	CONH	β-Lactam	Ester	CONH
a	$-(CH_2)_4CH_3$	1780	1720	1650	1780	1730	1665	1770	1730	1780	1725	1670	1780	1705	1660
b	$-C(CH_3)_3$	1780	1728	1650	1780	1720 1710	1680	1780*	1720*	1780	1725	1680	1780	1720	1675
c		1780	1720	1650	1770	1720	1670	1800	1730	1780	1730	1690	1780	1730	1675
d		1780	1725 1710	1650	1770	1720	1685	1780	1720	1770	1710	1670	1780	1720	1680
e		1780	1715 1705	1650	1770	1720 1690	1680	1770	1710	1770	1710	1670	1780	1710	1680
f		1785	1730 1725	1650	1780	1725	1670	1770	1730	1780	1720	1660	1780	1710	1660
g		1780	1740	1650	1775	1745 1725	1650	1780	1720	1780	1725	1680	1780	1740 (sh)	1680

* Film.
sh=shoulder

Table 4. NMR spectral data of **5**, **11** and **12**.

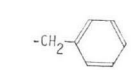
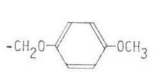
Compounds		NMR (DMSO-d_6, δ)											
		11				**12**				**5**			
	R^3	CONH d J=8Hz	C_7-H dd J=5,8Hz	C_6-H d J=5Hz	C_3-CH_2 AB, q J=13Hz	CONH d J=8Hz	C_7-H dd J=5,8Hz	C_6-H d J=5Hz	C_3-CH_2 AB, q J=13Hz	C_7-H	C_6-H	C_3-CH_2	C_2-H_2 br.s
a	$-(CH_2)_4CH_3$	8.40	5.93	4.97	4.60 5.13	9.17	5.83	5.17	4.65 5.00		4.60~5.17 4H, m		3.60
b	$-C(CH_3)_3$	8.42	6.00	5.00	4.65 5.18	9.23	5.87	5.22	4.73 5.03		4.60~5.17 4H, m		3.57
c		8.40	5.90	4.94	4.64 5.16	9.17	5.83	5.20	4.68 4.98		4.66~5.33 4H, m		3.58
d		8.44	5.96	4.98	4.90 5.40	9.22	5.87	5.23	4.93 5.23		4.88~5.44 4H, m		3.82
e		8.42	5.95	4.98	4.83 5.38	9.10	5.75	5.11	4.90 5.18	5.12 d J=5Hz	4.90 d J=5Hz	5.03 dd J=13Hz	3.68
f		8.45	5.95	4.95	4.65 5.17	9.12	5.78	5.13	4.68 4.98	5.03 d J=5Hz	4.85 d J=5Hz	4.80 dd J=13Hz	3.50
g		8.41	5.93	4.92	4.90 5.20	9.00	5.72	5.07	4.70 4.97		4.60~5.10 4H, m		3.52

Table 5. NMR spectral data of **6**.

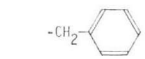
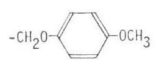
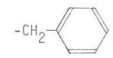
	Compounds	NMR (DMSO-d_6, δ)									
	R^3	CONH 1H, d J=8Hz	$\phi\rangle C\langle\phi$ 10H, m	$\phi\rangle CH-\phi$ 1H, s	Thiazole C_5-H 1H, s	C_7-H 1H, dd J=5,8Hz	C_6-H 1H, d J=5Hz	C_3-CH_2 2H, dd J=13Hz	C_2-H_2 2H, br.s	=N-OCH_3 3H, s	R^3
a	$-(CH_2)_4CH_3$	9.67	7.1 ~7.70	6.97	6.80	5.93	5.25	4.83	3.63	3.87	2.27 2H, t, J=7Hz 1.0~1.67 6H, m 0.83 3H, t, J=6Hz
b	$-C(CH_3)_3$	9.73	7.12~7.68	6.98	6.80	5.96	5.26	4.86	3.62	3.88	1.13 9H, s
c		9.80	7.1 ~7.58	7.00	6.83	5.83	5.20	4.83	3.60	3.97	3.07~3.33 1H, m 1.0~2.09 10H, m
d		9.62	7.1 ~7.60	7.00	6.77	5.83	5.20	5.17	3.73	3.87	7.45~7.73 3H, m 7.87~8.13 2H, m
e		9.67	7.1 ~7.67	6.97	6.77	5.93	5.27	5.03	3.73	3.87	7.71~8.1 2H, m 7.1~7.67 1H, m
f		9.73	7.1 ~7.70	7.00	6.83	5.96	5.25	4.88	3.68	3.90	3.63 2H, s 7.1~7.7 5H, m
g		9.64	7.07~7.64	6.95	6.78	5.92	5.22	4.91	3.58	3.87	4.70 2H, s 3.70 3H, s 6.88 4H, s

Table 6. NMR spectral data of **7**.

	Compounds R^3	NMR (DMSO-d_6, δ)							
		CONH	Thiazole C_5-H	C_7-H	C_6-H	C_3-CH_2	C_2-H_2	=N-OCH_3	R^3
		1H,d J=8Hz	1H,s	1H,dd J=5,8Hz	1H,d J=5Hz	2H,dd J=13Hz	2H,br.s	3H,s	
a	$-(CH_2)_4CH_3$	9.63	6.78	5.81	5.17	4.88	3.56	3.84	2.32 2H,t,J=7Hz 1.0~1.72 6H,m 0.86 3H,t,J=6Hz
b	$-C(CH_3)_3$	9.58	6.75	5.76	5.13	4.83	3.50	3.80	1.14 9H,s
c		9.62	6.77	5.82	5.18	4.88	3.53	3.87	1.0~2.03 10H,m 3.83 1H,m
d		9.63	6.75	5.83	5.19	5.15	3.72	3.83	7.45~7.75 3H,m 7.87~8.1 2H,m
e		9.72	6.85	5.83	5.22	5.15	3.70	3.92	7.27 1H,m 8.12~7.83 2H,m
f		9.58	6.73	5.77	5.12	4.88	3.70	3.84	3.47 2H,s 7.18 5H,s
g		9.80	6.90	5.78	5.14	4.96	3.53	3.93	3.68 3H,s 4.74 2H,s 6.86 4H,s

General Preparation of Diphenylmethyl 3-Acyloxymethyl-7β-[(Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate (**6**)

A mixture of (Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetic acid (**4**) (0.011 mole) and $POCl_3$ (0.012 mole) in AcOEt (20~40 ml) was stirred at 2~6°C for 30 minutes. Then $POCl_3$ (0.012 mole) was added to the reaction mixture, and stirred at 4~6°C for 30 minutes. Then DMF (0.012 mole) was added all at once to the above reaction mixture and stirred at same temperature for an hour to produce an activated acid solution of **4**. To the solution of **5** (0.01 mole) and *N*-(trimethylsilyl)acetamide (0.06 mole) in AcOEt (30~40 ml) was added the above activated acid solution at −10~−5°C for one hour. AcOEt and H_2O were added to the above mixture. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and then evaporated *in vacuo*. The residue was treated with Et_2O to afford diphenylmethyl 3-acyloxymethyl-7β-[(Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate (**6**) as a powder.

General Preparation of 3-Acyloxymethyl-7β-[(Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**7**)

Trifluoroacetic acid (0.1 mole) was added to a mixture of **6** (0.005 mole) in anisole (0.02 mole) and methylene chloride (10~20 ml) under ice-cooling, and the mixture was stirred at room temperature for 30~60 minutes. After removing the solvent *in vacuo*, the residue was added to a mixture of H_2O and AcOEt, and adjusted to pH 7.5 with saturated aqueous $NaHCO_3$. The separated aqueous solution was adjusted to pH 5.5 with 10% HCl, and washed with AcOEt. The separated aqueous solution was evaporated *in vacuo* to remove the organic solvent, and then acidified to pH 3.0 with 10% HCl under ice-cooling. The precipitate was collected by filtration, and dried (P_2O_5) to afford 3-acyloxymethyl-7β-[(Z)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylic acid (**7**).

Antibiotic Susceptibility

All the *in vitro* antibacterial activity data are given as the minimum inhibitory concentration (MIC)

in mcg/ml required to prevent growth of the bacterial culture. MIC was determined by agar dilution method using heart infusion agar (Difco) after incubation at 37°C for 20 hours and the inoculum size was about 10^8 C.F.U./ml. *Escherichia coli* 28 is a cephalosporin-resistant strain.

Enzymatic Deacylation of **7**

The enzymatic deacylation of the derivatives (**7**) was determined with the deacylase prepared from rat liver. Six weeks old Sprague Dawley rats were exsanguinated and the liver was removed, washed with 0.9% saline and blotted with filter paper. The livers were pooled and homogenized with a Polytron homogenizer after addition of 2 ml of 1/15M phosphate buffer (pH 7) per g of the liver. The homogenate was centrifuged at 100,000×*g* for 60 minutes. The 6-fold dilution of the supernatant fluid with 1/15M phosphate buffer was used as the deacylase preparation. The reaction mixture consisted of 4 ml of a drug solution (300 μg/ml) in 1/15M phosphate buffer (pH 7) and 1 ml of the enzyme solution, and was incubated at 37°C, 0.5 ml of the reaction mixture being sampled at the specified intervals. The enzymatic activity was stopped by addition of 1 ml of 99% EtOH, and the solution was centrifuged at 10,000×*g* for 10 minutes. The resulting supernatant fluid was filtered through a membrane filter (pore size: 0.45 μ, Millipore Corp.), and the drug concentrations in the filtrate was determined by high performance liquid chromatography (HPLC). HPLC was carried out using a Waters Model 6000 A pump with a Waters Model U6K injector. Chromatography was monitored by UV detector, Waters Model 440 at 254 nm. For analytical purpose, a steel column (4.0×30.5 mm) packed with a Bondapak C_{18} (Waters Assoc.) was used at a flow rate of 1.0 ml/minute. Mobile phase consisted of a mixture of methanol and 1% aqueous acetic acid (70: 30).

Acknowledgement

We are indebted to Dr. M. NISHIDA and his coworkers for providing the biological data.

References

1) TAKAYA, T.; T. KAMIMURA, H. KOJO, Y. MINE, S. NISHIDA, S. GOTO & S. KUWAHARA: Ceftizoxime (FK 749), a new parenteral cephalosporin: *in vitro* and *in vivo* antibacterial activities. Current Chemotherapy and Infectious Disease, Vol. 1, p. 255, The American Society for Microbiology, Washington, DC, 1980
2) TAKAYA, T.; H. TAKASUGI, T. MASUGI, T. CHIBA, K. TSUJI, H. KOCHI & H. NAKANO: Structure-activity relationships of ceftizoxime (FK 749) and its related compounds. 2nd Symposium on Medicinal Chemistry, Abstract 81~84, Tokyo, Oct. 31, 1980
3) TAKAYA, T.; H. TAKASUGI, T. MASUGI, T. CHIBA, H. KOCHI, T. TAKANO & H. NAKANO: Structure-activity relationships of sodium 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate (ceftizoxime) and its related compounds. Nippon Kagaku Kaishi 1981: 785~804, 1981
4) BUCOURT, R.; R. HEYMES, A. LUTZ, L. PENASSE & J. PERRONT: Propriétés antibiotiques inattendues dans le domaine des cephalosporines. C. R. Acad. Sc. Paris, Série D, 284: 1847~1849, 1977
5) HEYMES, R.; A. LUTZ & E. SCHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative: Pre-clinical study. Infection 5: 259~260, 1977
6) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977
7) REINER, R.; U. WELSS, U. BROMBACHER, P. LANZ, M. MONTAVON, A. FURLENMEIER, P. ANGEHRN & P. J. PROBST: Ro 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiotics 33: 783~786, 1980
8) SULLIVAN, H. R.; R. E. MCMAHON & R. E. HEINEY: The fate of parenteral cephalothin and related antibiotics in the rat. J. Antibiotics 24: 375~382, 1971
9) NAKAI, Y.; Y. KANAI, T. FUGONO & S. TANAYAMA: Metabolic fate of cephacetrile after parenteral administration in rats and rabbits. The nature of a minor long-lived metabolite. J. Antibiotics 26: 81~90, 1976
10) CABANA, B. E.; D. R. HARKEN & G. H. HOTTENDORF: Comparative pharmacokinetics and metabolism of cephapirin in laboratory animals and humans. Antimicr. Agents & Chemoth. 10: 307~317, 1976
11) SCHRINNER, E.; M. LIMBERT, L. PENASSE & A. LUTZ: Antibacterial activity of cefotaxime and other newer cephalosporins. (*in vitro* and *in vivo*) J. Antimicr. Chemoth. 6 Suppl. A: 25~30, 1980
12) TAKAYA, T.; T. MASUGI, H. TAKASUGI & H. KOCHI: 3,7-Disubstituted *syn*-isomers of 3,7-cephem-4-car-

boxylic acid derivatives. Belg. 852,427, Sep. 14, 1977 (Chem. Abst. 89: 59892h, 1978)
13) KUKOLJA, S.: Chemistry of cephalosporin antibiotics. XX. Synthesis and biological properties of 3-acyloxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic acid and related derivatives. J. Med. Chem. 13: 1114~1117, 1970
14) HEYNINGEN, E. VAN: The chemistry of cephalosporin antibiotics. III. Acylation of cephalosporadesates. J. Med. Chem. 8: 22~25, 1965
15) SOMERFIELD, G. A.; H. WYCOMBE & D. CHAGOURI: Cephemoic acids and process for preparing same. U. S. Patent 3,532,694, Oct. 6, 1970
16) NOMURA, H.; Y. FUGONO, T. HITAKA, I. MINAMI, T. AZUMA, S. MORIMOTO & T. MATSUDA: Semisynthetic β-lactam antibiotics. 6. Sulfocephalosporins and their antipseudomonal activities. J. Med. Chem. 17: 1312~1315, 1974
17) BICKEL, H. & P. HEINRICH: 3-Formyl-7-acylamino-3-cephem-4-carboxylic acid derivatives. Ger. Offen. 2,128,605, Dec. 23, 1971 (Chem. Abst. 76: 72534u, 1972)