NEW BROAD-SPECTRUM CEPHALOSPORINS WITH ANTI-PSEUDOMONAL ACTIVITY

II. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-ACYLAMINO-2-(4-HYDROXYPHENYL)ACETAMIDO]-3-[(1-METHYL-1*H*-TETRAZOL-5-YL)THIOMETHYL]CEPH-3-EM-4-CARBOXYLIC ACIDS

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The influence of the chirality of the 7-acyl side chain and of various *N*-acyl moieties (A–CO–) on the *in vitro* activity of 7β-[2-acylamino-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acids (**6**) was investigated. A cephalosporin having a 7-acyl side chain of *S*-configuration (**6r**) was only weakly active against *Staphylococcus aureus* and *Klebsiella pneumoniae* and was inactive against the other species tested. Among the various *N*-acyl moieties in the cephalosporins having a 7-acyl side chain of the *R*-configuration, the 4-hydroxypyridine-3-carbonyl moiety, unsubstituted or substituted with 5-bromo and/or 6-alkyl groups and the 4-hydroxy-1,5-naphthyridine-3-carbonyl moiety, unsubstituted or substituted with a 6-methyl and a 6-methoxy group gave the most active compounds. *N*-Ethylation of the 4-hydroxy-1,5-naphthyridine-3-carbonyl derivative and the 4-hydroxypyridine-3-carbonyl derivative (**6p**, **6q**) resulted in a decrease of the *in vitro* activity.

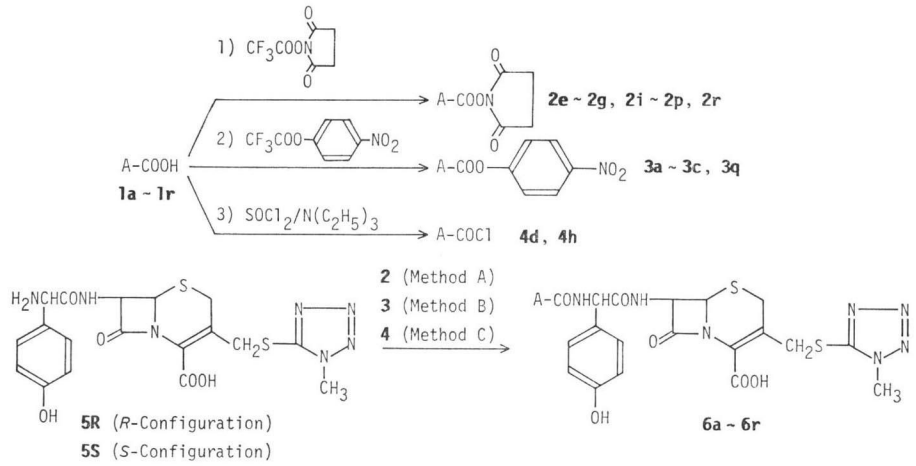
In the preceding paper[1], we reported the synthesis and the antibacterial activity of 7β-[D-2-[(4-hydroxy-1,5-naphthyridine-3-carbonylamino)- and (4-hydroxypyridine-3-carbonylamino)]-2-(4-hydroxyphenyl)acetamido]cephalosporins. The compounds showed high antibacterial activity against a variety of Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. The results showed that the *N*-acyl moieties (A–CO–, see general formulas **6**) and the 3-substituents of the cephalosporins had important effects on the antibacterial activity, spectrum, and pharmacokinetic characteristics of the compounds.

We report here the synthesis and the antibacterial activity of cephalosporins having acyl moieties other than 4-hydroxy-1,5-naphthyridine-3-carbonyl and 4-hydroxypyridine-3-carbonyl residues. In this study, we selected the 1-methyl-1*H*-tetrazol-5-ylthiomethyl group as the 3-substituent because in the preceding study it was the most effective one in increasing the *in vitro* activity of the compounds.

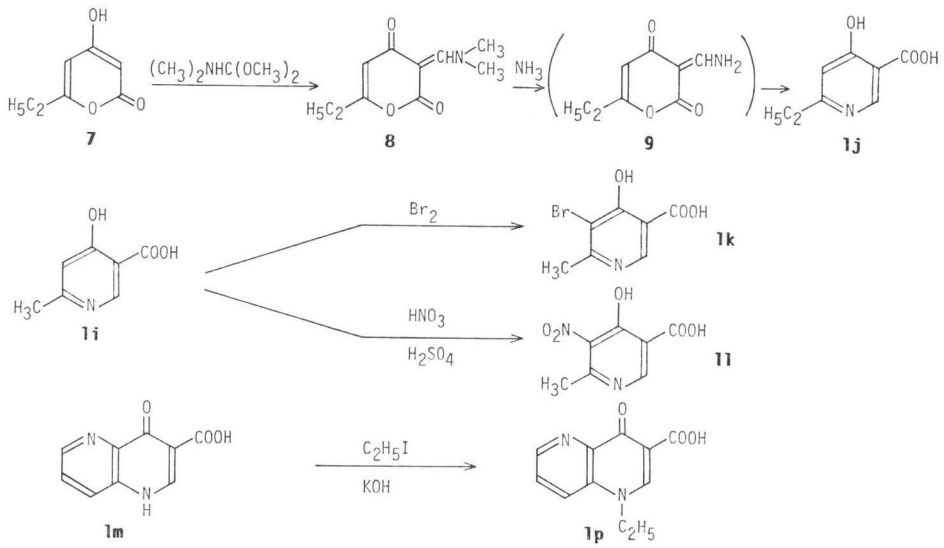
Chemistry

The cephalosporins listed in Table 1 were prepared by one of the methods outlined in Scheme 1. A general procedure for the acylation of **5R** with *N*-hydroxysuccinimide esters (Method A) was described in our preceding paper.[1] The procedure for the acylation of **5R** with *p*-nitrophenyl esters (Method B) was the same as Method A except for using *p*-nitrophenyl esters instead of *N*-hydroxysuccinimide esters. The acylation of **5R** with acid chloride (Method C) was performed as described in the experimental section.

Scheme 1.



Scheme 2.



Active esters used were prepared from *N*-hydroxysuccinimide trifluoroacetate or *p*-nitrophenyl trifluoroacetate[3] and the appropriate carboxylic acids by the general procedure described in previous papers.[4,5]

Compound **5R** used for the preparation of **6a ~ q** was prepared by the method described in the preceding paper.[1] Compound **5S** used for the preparation of **6r** was similarly prepared from (*S*)-*p*-hydroxyphenylglycine. A different synthesis of **5S** has been reported by BREUER *et al.*[2] It was confirmed by HPLC that **6r** was free from contamination by its *R*-epimer **6i**.

Carboxylic acids, **1j ~ l** and **1p**, are new compounds and their methods of preparation are outlined in Scheme 2. 6-Ethyl-4-(1*H*)-pyridone-3-carboxylic acid, **1j**, was prepared in a similar way as the 6-methyl-4-(1*H*)-pyridone-3-carboxylic acid, **1i**.[6] 5-Bromo-6-methyl-4-(1*H*)-pyridone-3-carboxylic acid,

1k, and 6-methyl-5-nitro-4-(1*H*)-pyridone-3-carboxylic acid, **1l**, were prepared by bromination and nitration respectively, of 6-methyl-4-(1*H*)-pyridone-3-carboxylic acid, **1i**. *N*-Ethyl-1,4-dihydro-4-oxo-1,5-naphthyridine-3-carboxylic acid, **1p**, was prepared by ethylation of 1,4-dihydro-4-oxo-1,5-naphthyridine-3-carboxylic acid, **1m**[7], with ethyl iodide in the presence of potassium hydroxide.

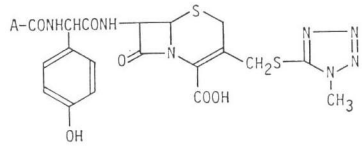
Biological Results and Discussion

The minimum inhibitory concentration (MIC) values of the cephalosporins against three species of Gram-positive bacteria and seven species of Gram-negative bacteria were determined by the serial two-fold agar dilution method. The results are listed in Table 1.

The following structure-activity relationships are derived from Table 1.

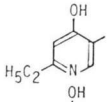
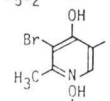
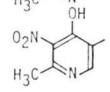
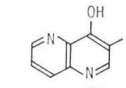
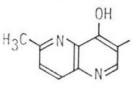
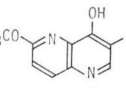
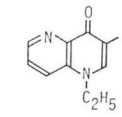
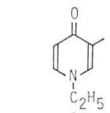
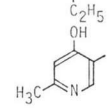
Compound **6a**, which has no hydroxyl substituent at a pyridine-3-carbonyl moiety, had low activity against *Streptococcus faecalis, E. aerogenes, P. aeruginosa*, and *Serratia marcescens*. Comparison of the MIC values of **6a** with those of **6f** indicates that the hydroxyl group or the oxo group on a pyridine ring is essential for significant antibacterial activity against these bacteria. The influence of the position of the hydroxyl group and the carbonyl group on the pyridine ring on *in vitro* activity can be seen by

Table 1. *In vitro* antibacterial activity of 7β-[2-acylamino-2-(4-hydroxyphenyl)acetamido]cephalosporins.



Compound No.	A	MIC (μg/ml)[a]									
		S. a.	*S. e.*	*S. f.*	*E. c.*	*K. p.*	*P. m.*	*P. v.*	*E. a.*	*P. a.*	*S. m.*
6a		0.78	12.5	100	3.13	0.39	50	0.10	>100	>100	>100
6b		0.78	6.25	25	3.13	0.20	25	≤0.025	25	3.13	>100
6c		0.20	3.13	12.5	0.78	0.05	6.25	≤0.025	25	3.13	6.25
6d		0.39	1.56	6.25	1.56	≤0.025	25	≤0.025	12.5	6.25	100
6e		0.39	3.13	12.5	1.56	0.20	12.5	0.05	12.5	3.13	12.5
6f		0.20	1.56	3.13	1.56	≤0.025	6.25	≤0.025	1.56	1.56	12.5
6g		3.13	12.5	>100	6.25	0.20	6.25	≤0.025	3.13	1.56	25
6h		0.39	3.13	25	1.56	0.10	12.5	0.10	6.25	6.25	25
6i		0.39	3.13	6.25	0.39	0.05	3.13	0.05	1.56	0.78	6.25

Table. 1. (Continued).

Compound No.	A	MIC (μg/ml)[a]								
		S. a.	*S. e.*	*S. f.*	*E. c.*	*K. p.*	*P. m.*	*P. v.*	*E. a.*	*P. a.*	*S. m.*
6j		0.39	3.13	12.5	0.39	≤0.025	3.13	≤0.025	1.56	0.78	3.13
6k		0.39	1.56	3.13	0.20	≤0.025	3.13	≤0.025	3.13	1.56	3.13
6l		0.39	6.25	6.25	0.78	≤0.025	3.13	≤0.025	6.25	3.13	12.5
6m		0.39	1.56	6.25	0.025	≤0.025	1.56	≤0.025	0.39	0.20	0.39
6n		0.78	3.13	12.5	0.025	≤0.025	0.78	≤0.025	0.39	0.39	0.78
6o		1.56	3.13	12.5	0.025	≤0.025	3.13	≤0.025	0.39	0.39	3.13
6p		0.78	3.13	6.25	0.05	≤0.025	6.25	≤0.025	1.56	0.78	1.56
6q		0.78	3.13	6.25	1.56	0.20	50	0.20	25	1.56	100
6r[b]		6.25	50	>100	>100	6.25	>100	>100	>100	>100	>100

[a] The MIC's were determined by the serial two-fold agar dilution method.[16]
Test organisms and abbreviations: *S. a.*, *Staphylococcus aureus* 209 P; *S. e.*, *Staphylococcus epidermidis* IAM 1296; *S. f.*, *Streptococcus faecalis* NCTC 8213; *E. c.*, *Escherichia coli* NIHJ JC-2; *K. p.*, *Klebsiella pneumoniae* ATCC 10031; *P. m.*, *Proteus mirabilis* GN 2425; *P. v.*, *Proteus vulgaris* OX-19; *E. a.*, *Enterobacter aerogenes* ATCC 13048; *P. a.*, *Pseudomonas aeruginosa* IFO 3451; *S. m.*, *Serratia marcescens* X100.

[b] The chirality of the asymmetric carbon of 7-acyl side chain in **6r** is *S*-configuration.

the comparison of MIC values of **6d**, **6e** and **6f**. It appears that the 4-hydroxypyridine-3-carbonyl derivative, **6f**, was more active than the 3-hydroxypyridine-2-carbonyl derivative, **6d**, as well as the 2-hydroxypyridine-3-carbonyl derivative **6e**. Similarly, the 4-hydroxy-6-methylpyridine-3-carbonyl derivative, **6i**, was more active than its 2-hydroxyl isomer, **6h**.

The *in vitro* activity of the 4-hydroxypyrimidine-3-carbonyl derivative **6b** was clearly inferior, and that of the 3-hydroxypyridazine-4-carbonyl derivative, **6c**, except for *E. aerogenes*, of the same order as that of the 4-hydroxypyridine-3-carbonyl derivative, **6f**.

Introduction of a 6-methyl (**6i**) and a 6-ethyl substituent (**6j**) into the 4-hydroxypyridine-3-carbonyl moiety exerted no significant influence on the activity, whereas a 6-hydroxyl substituent (**6g**) resulted in a significant decrease in activity against Gram-positive bacteria. The influence of a 5-substituent on the

4-hydroxy-6-methylpyridine-3-carbonyl moiety on the *in vitro* activity was also investigated. Introduction of a 5-bromo substituent (**6k**) did not significantly influence the activity, but a 5-nitro substituent (**6l**) slightly decreased the activity against *E. aerogenes* and *P. aeruginosa*.

In the 4-hydroxy-1,5-naphthyridine-3-carbonyl derivative (**6m**), introduction of a 6-methyl substituent (**6n**) exerted no significant influence on the activity, while introduction of a 6-methoxy substituent (**6o**) slightly decreased it against *Staphylococcus aureus* and *S. marcescens*.

N-Ethylation of the 4-hydroxy-1,5-naphthyridine-3-carbonyl moiety and the 4-hydroxypyridine-3-carbonyl moiety leads to compounds, **6p** and **6q**, with lower activities than those of the parent compounds (**6m** and **6f**).

Compound **6r**, the epimer of **6i**, was only weakly active against *S. aureus*, *Staphylococcus epidermidis*, and *Klebsiella pneumoniae* and was inactive against the other species tested.

Of the cephalosporins described in this study, eight compounds (**6f**, **6i**, **6j**, **6k**, **6m**, **6n**, **6o** and **6p**) emerged as those tentatively worthy of further consideration because of their high activity against a variety of Gram-positive and Gram-negative bacteria.

Experimental

Infrared spectra were recorded on a Hitachi model EPI-G3 spectrophotometer. NMR spectra were recorded on a JEOL FX-90Q (90 MHz) spectrometer or a Varian T-60 (60 MHz) spectrometer using TMS as an internal standard; all chemical shifts are reported in δ values. Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Empirical formulas and IR data, and NMR data of the cephalosporins synthesized are shown in Tables 2 and 3, respectively.

Materials

Compounds **1a**, **1d** and **1h** were obtained commercially.

Compounds **1b**[8], **1c**[9], **1e**[10], **1f**[11], **1g**[12], **1i**[6], **1m**[7], **1n**[5], **1o**[2] and **1q**[13] were prepared as reported in the reference.

Compounds **6f** and **6m** were reported in the preceding paper.[1]

Table 2. Synthetic methods, empirical formulas, and IR data of the cephalosporins.

Compound No.	Method	Formula[a]	IR(KBr) β-lactam (cm^{-1})	Compound No.	Method	Formula[a]	IR(KBr) β-lactam (cm^{-1})
6a	B	$C_{24}H_{22}N_8O_9S_2\cdot 2.5H_2O$[b]	1778	**6j**	A	$C_{26}H_{26}N_8O_7S_2\cdot 1.5H_2O$[f]	1775
6b	B	$C_{23}H_{21}N_9O_7S_2\cdot 2.5H_2O$	1773	**6k**	A	$C_{25}H_{23}BrN_8O_7S_2\cdot 3.5H_2O$[g]	1773
6c	B	$C_{23}H_{21}N_9O_7S_2\cdot 1.5H_2O$[c]	1775	**6l**	A	$C_{25}H_{23}N_9O_9S_2\cdot 3H_2O$	1779
6d	C	$C_{24}H_{22}N_8O_7S_2\cdot 1.5H_2O$	1778	**6m**	A	$C_{27}H_{23}N_9O_7S_2\cdot 3H_2O$	1777
6e	A	$C_{24}H_{22}N_8O_7S_2\cdot H_2O$	1778	**6n**	A	$C_{28}H_{25}N_9O_7S_2\cdot 2H_2O$	1772
6f	A	$C_{24}H_{22}N_8O_7S_2\cdot 1.5H_2O$	1770	**6o**	A	$C_{28}H_{25}N_9O_8S_2\cdot 2H_2O$[h]	1775
6g	A	$C_{24}H_{22}N_8O_8S_2\cdot 2.5H_2O$[d]	1775	**6p**	A	$C_{29}H_{27}N_9O_7S_2\cdot 2H_2O$	1774
6h	C	$C_{25}H_{24}N_8O_7S_2\cdot 2H_2O$[e]	1780	**6q**	B	$C_{26}H_{26}N_8O_7S_2\cdot 1.5H_2O$	1778
6i	A	$C_{25}H_{24}N_8O_7S_2\cdot 2.5H_2O$	1770	**6r**	A	$C_{25}H_{24}N_8O_7S_2\cdot 3H_2O$	1775

[a] Compounds were analyzed for C, H and N. Unless otherwise indicated, analyses are within $\pm 0.4\%$ of the theoretical values.

[b] N: calcd. 17.85; found 17.14.

[c] N: calcd. 20.12; found 19.05.

[d] N: calcd. 16.99; found 16.21.

[e] N: calcd. 17.28; found 16.49.

[f] N: calcd. 17.14; found 16.44.

[g] H: calcd. 4.01; found 3.38.

[h] N: calcd. 17.61; found 16.71.

General Procedure for the Acylation of Cephalosporin **5R** with an Acid Chloride (Method C)

Synthesis of Cephalosporins **6d** and **6h**: To an ice-cooled solution of 3-hydroxypicolinic acid (3.38 mmole) or 2-hydroxy-6-methylnicotinic acid (3.38 mmole) and triethylamine (3.72 mmole) in 10 ml of dichloromethane, thionyl chloride (3.51 mmole) was added dropwise, and the mixture was stirred at 0°C to 3°C for 1 hour. Compound **5R** (trifluoroacetic acid salt) (3.38 mmole) and triethylamine (10.1 mmole) were added to the reaction mixture at 0°C to 3°C. After stirring for 1.5 hours at the same temperature and for 1 hour without external cooling, the dichloromethane was evaporated *in vacuo* and the residue was dissolved in 30 ml of water containing 0.6 g of sodium bicarbonate. After removal of insoluble material by filtration, the solution was cooled in an ice-bath and adjusted to pH 2~3 by addition of 2 N HCl. The precipitate formed was collected, washed with water and dried *in vacuo* over phosphorus pentoxide.

The crude products were purified by preparative liquid chromatography on a reverse phase column of LiChroprep RP-8 with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8) and MeOH (**6d**, 67% Buffer - 33% MeOH; **6h**, 77% Buffer - 23% MeOH by volume).

The fractions containing the product were concentrated *in vacuo* to a small volume and adjusted to pH 2 at 0~5°C. The precipitate was collected, washed with water, and dried *in vacuo* over phosphorus pentoxide.

7β-[L-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid Trifluoroacetic Acid Salt (**5S**)

1. L-2-(4-Methoxybenzyloxycarbonylamino)-2-(4-hydroxyphenyl)acetic acid was prepared in a manner similar to that described for the preparation of D-isomer.[1]

mp 136~137°C (dec.); $[\alpha]_D^{27}$ +105.1° (*c* 1, DMF); IR (Nujol) 1740, 1665, 1615, 1520 cm^{-1}; NMR (DMSO-d_6) δ 3.68 (s, 3H, OCH_3), 4.93 (s, 2H, CH_2), 5.02 (d, 1H, J=8Hz, CH), 6.60~7.28 (m, 8H, phenyl protons), 7.66 (d, 1H, J=8Hz, NH).

Anal. Calcd. for $C_{17}H_{17}NO_6$: C 61.63, H 5.17, N 4.23.
Found: C 61.57, H 5.14, N 4.16.

2. 7-[L-2-(4-Methoxybenzyloxycarbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid was prepared as discribed for the D isomer[1] and purified as the diethylamine salt from dimethylformamide - acetone.

mp 128~132°C (dec.); IR (Nujol) 1770, 1690, 1650, 1615, 1580, 1520 cm^{-1}; NMR (DMSO-d_6) δ 1.17 [t, 6H, $(-CH_2-CH_3)_2$], 2.87 [q, 4H, $(-CH_2-CH_3)_2$], 3.53 (broad s, 2H, C_2-H_2), 3.73 (s, 3H, OCH_3), 3.90 (s, 3H, N-CH_3), 4.32 (broad s, 2H, C_3-CH_2), 4.95 (s, 2H, $-CH_2$-Ph), 4.90~5.62 [m, 3H, C_6-H, C_7-H, $-CH$(NH)-], 6.68, 7.22 (each d, 4H, J=9Hz, phenyl protons), 6.85, 7.27 (each d, 4H, J=9Hz, phenyl protons), 7.52 (d, 1H, -CONH-), 9.02 (d, 1H, -CONH-).

Anal. Calcd. for $C_{31}H_{33}N_5O_8S_2 \cdot H_2O$: C 50.81, H 5.50, N 15.29.
Found: C 51.14, H 5.60, N 14.89.

3. Preparation of **5S**.

A solution of 3 g of the diethylamine salt described above in trifluoroacetic acid (15 ml) and anisole (3 ml) was stirred at room temperature for 20 minutes. The solution was added to ethyl ether (200 ml) with stirring. The resulting precipitate was filtered off and washed with ethyl ether to give 2.43 g of **5S**. IR (Nujol) 1775, 1675, 1615, 1525 cm^{-1}; NMR (CF_3COOH) δ 3.80 (broad s, 2H, C_2-H_2), 4.13 (s, 3H, N-CH_3), 4.34, 4.68 (ABq, 2H, J=14Hz, C_3-CH_2), 5.23 (d, 1H, J=5Hz, C_6-H), 5.43~5.82 [m, 2H, C_7-H & $-CH$(NH)-], 7.02, 7.42 (each d, 4H, phenyl protons).

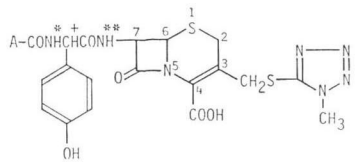
The product was used in subsequent steps without further purification.

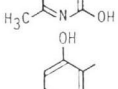
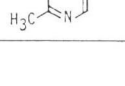
3-(Dimethylaminomethylene)-4-oxo-6-ethyl-2-pyrone (**8**)

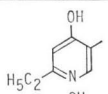
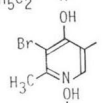
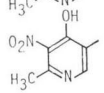
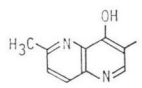
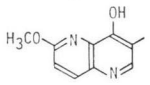
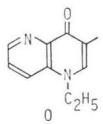
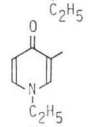
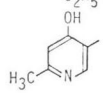
To a solution of *N*,*N*-dimethylformamide dimethyl acetal (20 g, 0.168 mole) in 25 ml of dioxane was added 4-hydroxy-6-ethyl-2-pyrone[14] (8.4 g, 0.06 mole). After stirring at room temperature for 30 minutes, the reaction mixture was cooled in an ice-water bath and stirred for 1 hour. The precipitate was collected, washed with cold dioxane, and dried *in vacuo*: yield 6.60 g (56.3%). An analytical sample was recrystallized from 2-propanol.

mp 109~111°C; IR (KBr) 1700, 1655, 1613, 1575 cm^{-1}; NMR (DMSO-d_6) δ 1.10 (t, 3H, C-CH_3),

Table 3. ^{1}H NMR data of the cephalosporins **6**[a].



Compound No.	A	NMR δ value (DMSO-d_6)								
		–CONH*– 1H, d J=7~8 Hz	–CONH**– 1H, d J=8~9 Hz	C_7-H, –CH$^+$– 2H, m	C_6-H 1H, d J=5 Hz	C_3-CH_2 2H, ABq J=13 Hz	C_2-H_2 2H, br	>N–CH_3 3H, s	Phenyl proton J=8~9 Hz	A ring proton
6a		9.08~ 9.37[ov]	9.08~ 9.37[ov]	5.57~5.80	5.00	4.20 4.32	[b]	3.90	6.40 (d, 2H) 7.27 (d, 2H)	7.61 (dd, 1H, J=8.6, 5.7 Hz, 5-H) 8.39 (m, 1H, 4-H) 8.74 (dd, 1H, 6-H) 9.06 (d, 1H, 2-H)
6b		9.94	9.29	5.59~5.74	4.96	4.18 4.30	3.56	3.90	6.66 7.17	8.56 (s, 1H) 8.39 (s, 1H)
6c		10.31	9.31	5.54~5.77	4.97	4.20 4.31	3.57	3.89	6.69 7.22	8.00 (d, 1H) 8.11 (d, 1H)
6d		9.40 or	8.94	5.60~5.80	5.00	4.26 (br)	3.60	3.89	6.69 7.26	7.49 (m, 2H, 4-H, 5-H) 8.15 (m, 1H, 6-H)
6e		10.51	9.29	5.60~5.77	4.97	4.20 4.31	3.60	3.91	6.69 7.20	6.43 (t, 1H, 5-H) 7.69 (m, 1H, 6-H) 8.29 (dd, 1H, 4-H)
6g		9.27 or	8.98	5.57~5.79	4.99	4.20 4.31	3.60	3.91	6.69 7.20	5.60 (s, 1H, 5-H) 8.05 (s, 1H, 2-H)
6h		10.42	9.26	5.60~5.76	4.94	4.20 4.31	3.57	3.91	6.66 7.17	2.29 (s, 3H, CH_3), 6.24 (d, 1H, 5-H) 8.14 (d, 1H, 4-H)
6i		11.06	9.27	5.60~5.75	4.98	4.20 4.31	3.59	3.92	6.69 7.22	2.25 (s, 3H, CH_3), 6.25 (s, 1H, 5-H) 8.28 (br, 1H, 2-H)

6j		11.07	9.25	5.60~5.83	4.96	4.30 (br)	3.62	3.93	6.72 7.27	1.19 (t, 3H, CH_3), 2.57 (q, $-CH_2$) 6.27 (s, 1H, 5-H), 8.28 (s, 1H, 2-H)
6k		10.83	9.26	5.57~5.77	4.97	4.20 4.31	3.57	3.91	6.69 7.20	2.46 (s, CH_3) 8.29 (s, 1H, 2-H)
6l		10.42	9.31	5.63~5.80	5.00	4.29 (br)	3.57	3.91	6.69 7.20	2.36 (s, 3H, CH_3) 8.45 (s, 1H, 2-H)
6n		10.91	9.33	5.66~5.83	5.00	4.23 4.33	3.60	3.91	6.71 7.31	2.63 (s, 3H, CH_3), 7.63 (d, 1H, 7-H) 8.04 (d, 1H, 8-H), 8.69 (s, 1H, 2-H)
6o		10.97	9.31	5.66~5.77	5.00	4.22 4.33	3.60	3.91	6.71 7.20~ 7.31	3.97 (s, 3H, OCH_3), 7.20~7.31 (m, 7-H) 8.06 (d, 1H, 8-H), 8.65 (s, 1H, 2-H)
6p		10.80	9.37	5.70~5.86	5.01	4.29 (br)	3.63	3.91	6.74 7.29	1.37 (t, 3H, CH_3), 4.54 (q, 2H, CH_2) 7.86 (dd,1H,7-H), 8.46 (dd,1H,8-H) 8.83~8.91 (m, 2H, 2-H, 6-H)
6q		11.06	9.29	5.63~5.80	4.97	4.21 4.31	3.60	3.91	6.70 7.23	1.31 (t, 3H, CH_3), 4.06 (q, 2H, CH_2) 6.43 (d,1H,5-H), 7.89 (dd,1H,6-H) 8.49 (d, 1H, 2-H)
6r		11.03	9.26	5.51~5.64	5.03	4.24 4.35	3.67	3.93	6.70 7.23	2.25 (s,3H,CH_3), 6.24 (s,1H,5-H) 8.23 (m, 1H, 2-H)

a) In NMR descriptions, s=singlet, d=doublet, dd=double doublet, m=multiplet, q=quartet, t=triplet, ABq=AB quartet, br=broad singlet, $^{ov)}$ signals overlapped each other.

b) It was difficult to read the δ value because the signals overlapped with those of water.

2.35 (q, 2H, C–CH_2), 3.22 (s, 3H, N–CH_3), 3.48 (s, 3H, N–CH_3), 5.55 (s, 1H, olefinic proton) and 8.22 (s, 1H, olefinic proton).

Anal. Calcd. for $C_{10}H_{13}NO_3$: C 61.53, H 6.71, N 7.18.
Found: C 61.23, H 6.85, N 6.99.

6-Ethyl-4-(1*H*)-pyridone-3-carboxylic Acid (**1j**)

To a solution of 3-(dimethylaminomethylene)-4-oxo-6-methyl-2-pyrone (6.00 g, 0.0307 mole) in 44 ml of water was added 19 g of ammonia water (28%), causing rapid crystallization. After stirring at room temperature for 30 minutes dimethylamine (50% aqueous; 2.77 g, 0.0307 mole) was added to give a clear solution which was heated at 50°C for 30 minutes and then cooled to 20°C. Acidification to pH 2 by the addition of 50% aqueous sulfuric acid and stirring for 1 hour in an ice-water bath gave a precipitate, which was collected, washed with cold water, and dried *in vacuo*: yield 3.29 g (64.1%). An analytical sample was recrystallized from EtOH - H_2O.

mp 262~265°C (dec.); IR (Nujol) 1657, 1590 cm^{-1}; NMR (CF_3COOH) δ 1.48 (t, 3H, CH_3), 3.11 (q, 2H, CH_2), 7.31 (s, 1H, pyridine 5-H), 9.03 (b, 1H, pyridine 2-H).

Anal. Calcd. for $C_8H_9NO_3$: C 57.45, H 5.42, N 8.42.
Found: C 57.31, H 5.53, N 8.20.

5-Bromo-6-methyl-4-(1*H*)-pyridone-3-carboxylic Acid (**1k**)

To a solution of 6-methyl-4-(1*H*)-pyridone-3-carboxylic acid, **1i** (15.3 g, 0.1 mole) in 300 ml of acetic acid was added dropwise a solution of bromine (17.6 g) in 50 ml of acetic acid at 90~100°C for 2 hours. The reaction mixture was further stirred at 90~95°C for 1 hour and allowed to cool to room temperature. The precipitate was collected, washed with 30 ml of acetic acid, then with 50 ml of ethanol and dried *in vacuo*: yield 12.5 g (53.9%). An analytical sample was recrystallized from water.

mp 283~285°C (dec.); IR (KBr) 1700, 1630, 1485 cm^{-1}; NMR (CF_3COOD) δ 2.98 (s, 3H, CH_3), 9.08 (s, 1H, pyridine 2-H).

Anal. Calcd. for $C_7H_6BrNO_3$: C 36.23, H 2.61, Br 34.44, N 6.04.
Found: C 36.47, H 2.35, Br 34.16, N 6.11.

6-Methyl-5-nitro-4-(1*H*)-pyridone-3-carboxylic Acid (**1l**)

To an ice-cold solution of 6-methyl-4-(1*H*)-pyridone-3-carboxylic acid, **1i** (76.5 g, 0.5 mole) in 190 ml of concentrated sulfuric acid was added fuming nitric acid (37.8 g). The reaction mixture was heated to 70~75°C and maintained at that temperature for 7 hours. The resulting mixture was poured into 1 kg of ice-water. The precipitate was collected, washed with water, then with acetone, and dried *in vacuo* at 60°C: yield 65.1 g (65.7%).

mp 242~244°C (dec.); IR (KBr) 1700, 1650, 1620, 1545, 1500 cm^{-1}; NMR (CF_3COOH) δ 2.87 (3H, s, CH_3), 9.13 (1H, s, pyridine 2-H).

Anal. Calcd. for $C_7H_6N_2O_5$: C 42.43, H 3.05, N 14.14.
Found: C 42.44, H 2.96, N 14.28.

1-Ethyl-1,4-dihydro-4-oxo-1,5-naphthyridine-3-carboxylic Acid (**1p**)

To a solution of 1,4-dihydro-4-oxo-1,5-naphthyridine-3-carboxylic acid, **1m** (9.50 g, 0.05 mole) and potassium hydroxide (85%, 9.88 g, 0.15 mole) in a mixture of 70 ml of water and 60 ml of ethanol was added ethyl iodide (35.1 g, 0.225 mole). The mixture was refluxed with stirring for 3.5 hours and then cooled to 40°C. The reaction mixture was acidified to pH 2 by the addition of 6 N HCl and then heated to 90°C to remove excess ethyl iodide. The resulting mixture was cooled to room temperature and filtered to remove unreacted starting material (1.76 g). Concentration of the filtrate to half of its original volume *in vacuo* gave 4.4 g (49.5% yield) of the product which was purified by recrystallization from ethanol.

mp 251~253°C (dec.); IR (KBr) 1730, 1620, 1595 cm^{-1}; NMR (CF_3COOD) δ 1.78 (t, 3H, CH_3), 4.89 (q, 2H, –CH_2–), 8.68 (dd, 1H, naphthyridine 7-H), 9.33~9.43 (m, 3H, naphthyridine 2-H, 5-H, and 8-H).

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C 60.54, H 4.62, N 12.84.
Found: C 60.60, H 4.42, N 12.90.

Active Esters (**2, 3**)

The following *N*-hydroxysuccinimide esters and *p*-nitrophenyl esters were prepared by methods previously described[4,5].

3a: mp 167~168.5°C; NMR (CF_3COOH) δ 7.58 (d, 2H, J=9.6 Hz, phenyl protons), 8.42 (d, 2H, phenyl protons), 8.30~8.50 (m, 1H, pyridine 5-H), 9.23 (d, 1H, J=6 Hz, pyridine 4-H), 9.47 (d, 1H, J=8 Hz, pyridine 6-H), 9.72 (broad s, 1H, pyridine 2-H).

2e: mp 235~242°C (dec.); NMR (DMSO-d_6) δ 2.83 (s, 4H, succinimide protons), 6.37 (t, 1H, pyridine 5-H), 7.83 (dd, 1H, pyridine 4-H), 8.31 (dd, 1H, pyridine 6-H).

2g: mp 224~225°C (dec.); NMR (CF_3COOD) δ 3.10 (s, 4H, succinimide protons), 6.60 (s, 1H, pyridine 5-H), 8.87 (s, 1H, pyridine 2-H).

2i: mp 220~234°C (dec.); NMR (CF_3COOH) δ 2.88 (s, 3H, CH_3), 3.17 (s, 4H, succinimide protons), 7.47 (s, 1H, pyridine 5-H), 9.28 (s, 1H, pyridine 2-H).

2j: mp 214~215.5°C; NMR (DMSO-d_6) δ 1.17 (t, 3H, CH_3), 2.51 (q, 2H, CH_2), 2.84 (s, 4H, succinimide protons), 6.18 (s, 1H, pyridine 5-H), 8.41 (s, 1H, pyridine 2-H).

2k: mp 247~252°C (dec.); NMR (DMSO-d_6) δ 2.46 (s, 3H, CH_3), 2.86 (s, 4H, succinimide protons), 8.46 (s, 1H, pyridine 2-H).

2l: mp 183~185°C (dec.); NMR (CF_3COOH) δ 2.80 (s, 3H, CH_3), 3.15 (s, 4H, succinimide protons), 9.13 (s, 1H, pyridine 2-H).

2n: mp 250~257°C (dec.); NMR (CF_3COOD) δ 3.17 (s, 4H, succinimide protons), 3.23 (s, 3H, CH_3), 8.37 (d, 1H, J=9 Hz, naphthyridine 7-H), 9.10 (d, 1H, J=9 Hz, naphthyridine 8-H), 9.33 (s, 1H, naphthyridine 2-H).

2p: mp 215~225°C (dec.); NMR (CF_3COOH) δ 1.75 (t, 3H, CH_3), 3.15 (s, 4H, succinimide protons), 4.78 (q, 2H, $-CH_2$), 8.63 (dd, 1H, naphthyridine 7-H), 9.23~9.35 (m, 3H, naphthyridine 2-H, 6-H, and 8-H).

3q: mp 177~179°C; NMR (CF_3COOH) δ 1.78 (t, 3H, J=7 Hz, CH_3), 4.70 (q, 2H, J=7 Hz, $-CH_2$), 7.55 (d, 2H, J=9 Hz, phenyl protons), 7.67 (d, 1H, J=8 Hz, pyridone 5-H), 8.47 (d, 2H, J=9 Hz, phenyl protons), 8.75 (dd, 1H, J=8, 2 Hz, pyridone 6-H), 9.47 (d, 1H, J=2 Hz, pyridone 2-H). **3b**[5], **3c**[5], **2f**[4], **2m**[15], and **2o**[4] were prepared as previously reported.

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References

1) YAMADA, H.; K. JIMPO, H. TOBIKI, T. KOMATSU, H. NOGUCHI, K. IRIE & T. NAKAGOME: New broad-spectrum cephalosporins with anti-pseudomonal activity. I. Synthesis and antibacterial activity of 7β-[D-2-[(4-hydroxy-1,5-naphthyridine-3-carbonylamino)- and (4-hydroxypyridine-3-carbonylamino)]-2-(4-hydroxyphenyl)acetamido]cephalosporins. J. Antibiotics 36: 522~531, 1983

2) BREUER, H.; U. D. TREUNER, H. J. SCHNEIDER, M. G. YOUNG & H. I. BASCH: Diastereomeric 7-ureidoacetyl cephalosporins. I. Superiority of 7α-H-L-isomers over D-isomers. J. Antibiotics 31: 546~560, 1978

3) SAKAKIBARA, S. & N. INUKAI: The trifluoroacetate method of peptide synthesis. I. The synthesis and use of trifluoroacetate reagents. Bull. Chem. Soc. Jpn. 38: 1979~1984, 1965

4) TOBIKI, H.; H. YAMADA, N. TANNO, K. SHIMAGO, Y. EDA, H. NOGUCHI, T. KOMATSU & T. NAKAGOME: Studies on β-lactam antibiotics. V. Synthesis of 6-[D(−)-α-(acylamino)-4-hydroxyphenylacetamido]-penicillanic acids and antibacterial activity. Yakugaku Zasshi (J. Pharm. Soc. Japan) 100: 133~139, 1980

5) TOBIKI, H.; H. YAMADA, I. NAKATSUKA, K. SHIMAGO, Y. EDA, H. NOGUCHI, T. KOMATSU & T. NAKAGOME: Studies on β-lactam antibiotics. III. Synthesis of 6-[D(−)-α-(acylamino)-phenylacetamido]penicillanic acids and antibacterial activity. Yakugaku Zasshi (J. Pharm. Soc. Japan) 100: 38~48, 1980

6) KILBOURN, E. E. & M. C. SEIDEL: Synthesis of *N*-alkyl-3-carboxy-4-pyridones. J. Org. Chem. 37: 1145~1148, 1972

7) ADAMS, J. T.; C. K. BRADSHER, D. S. BRESLOW, S. T. AMORE & C. R. HAUSER: Synthesis of antimalarials. VI. Synthesis of certain 1,5- and 1,8-naphthyridine derivatives. J. Am. Chem. Soc. 68: 1317~1319, 1946
8) MCOMIE, J. F. W. & I. M. WHITE: Pyrimidines. VII. Some pyrimidine analogues of 4-aminosalicylic acid. J. Chem. Soc. 1953: 4175~4176, 1953
9) KURAISHI, T.: 4,5-Substituted pyridazines. III. Oxidation and solvolysis of 4-methyl-3,6-dichloropyridazine. Chem. Pharm. Bull. 5: 587~589, 1957
10) DORNOW, A.: Über eine direkte Synthese von 3-Cyan-pyridon(2) und seinem 6-Methyl-derivat. Chem. Ber. 73: 153~156, 1940
11) ROSS, W. C. J.: The preparation of some 4-substituted nicotinic acids and nicotinamides. J. Chem. Soc. (C) 1966: 1816~1821, 1966
12) DEN HERTOG, H. J.: On the reactivity of the bromine atoms in brominated pyridines. The preparation of brominated 2- and 4-aminopyridines. Rec. Trav. Chem. 65: 129~140, 1946
13) HEINDL, J.; H. W. KELM, E. DOGS, A. SEEGER & CH. HERRMANN: Untersuchungen über die antibakterielle Aktivität von Chinoloncarbonsäuren. IX(1). Azaanaloga. Di- und trisubstituierte 1,4-Dihydro-4-oxo-1,5-naphthyridine-3-carbonsäuren und 1-Äthyl-4-pyridon-3-carbonsäure. Eur. J. Med. Chem. Chimica Therapeutica 12: 549~555, 1977
14) MORGAN, J. F.: A new synthesis of 4-pyridazones. J. Am. Chem. Soc. 70: 2253~2254, 1948
15) TOBIKI, H.; H. YAMADA, T. MIYAZAKI, N. TANNO, H. SUZUKI, K. SHIMAGO, K. OKAMURA & S. UEDA: Studies on β-lactam antibiotics. IV. Synthesis of apalcillin. Yakugaku Zasshi (J. Pharm. Soc. Japan) 100: 49~55, 1980
16) Japan Society of Chemotherapy: Determination method of MIC. Chemotherapy 23: 1~2, 1975 (in Japanese)