STUDIES ON β-LACTAM ANTIBIOTICS. II

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF α-HYDROXYIMINOARYLACETYL CEPHALOSPORINS

TAKAO TAKAYA*, HISASHI TAKASUGI, TAKASHI MASUGI
HIROMU KOCHI and HIROSHI NAKANO

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

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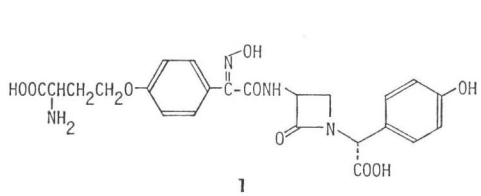
The synthesis and antibacterial activity *in vitro* of the 2-aryl-2-hydroxyiminoacetyl cephalosporins (**2**) are described. Within this cephalosporin series, analogs (**9f**~**13f**) with 2-hydroxyimino-2-(3-hydroxyphenyl)acetyl group at the 7-position of a cephem nucleus were found to have the highest antibacterial activity against a wide-range of microorganisms, including β-lactamase-producing bacteria. Structure-activity relationships of **2** are discussed.

Nocardicin A, found by IMANAKA[1] from the fermentation broth of *Nocardia uniformis* subsp. *tsuyamanensis* ATCC 21806, is a new single β-lactam antibiotic with a unique acyl side chain (Fig. 1). It is active against Gram-negative bacteria, especially against *Pseudomonas aeruginosa*, and highly stable to β-lactamases derived from various species of bacteria[2]. Thus this finding gave us an important clue to develop a new family of cephalosporins (**2**)[3,4] bearing the 2-aryl-2-hydroxyiminoacetyl** side chain at the 7-position of a cephem nucleus.

Recently, the Glaxo group has reported the synthesis and assignment of *syn*- and *anti*-isomers of 7β-(2-hydroxyiminophenylacetamido)cephalosporanic acid, obtained by condensation of 7-aminocephalosporanic acid (7-ACA) with α-dichloroacetoxyiminophenylacetic acid.[5,6] Development of this work to thienyl and furyl analogs led to the discovery of cefuroxime.

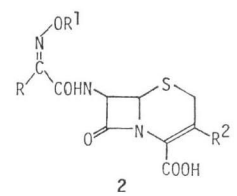
In this paper, we report the chemistry and *in vitro* antimicrobial profile of these cephalosporins (**2**) as well as some *in vitro* structure-activity correlation studies. Similar to the *syn-anti* structure-activity

Fig. 1.



1

Structure of nocardicin A



2

General structure of oxyiminoarylacetic cephalosporins

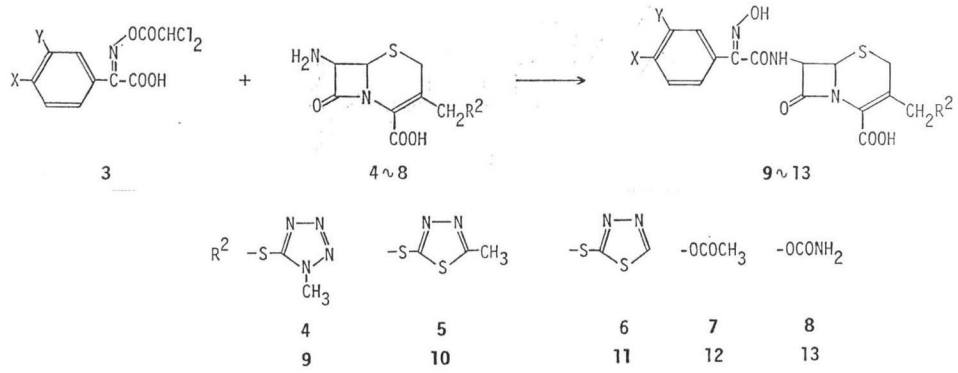
** There exist two possible configurations with respect to the hydroxyimino group, in terms of *syn* and *anti*, or *Z* and *E*.

R^1–C–CO–
||
N–OH
syn (*Z*)

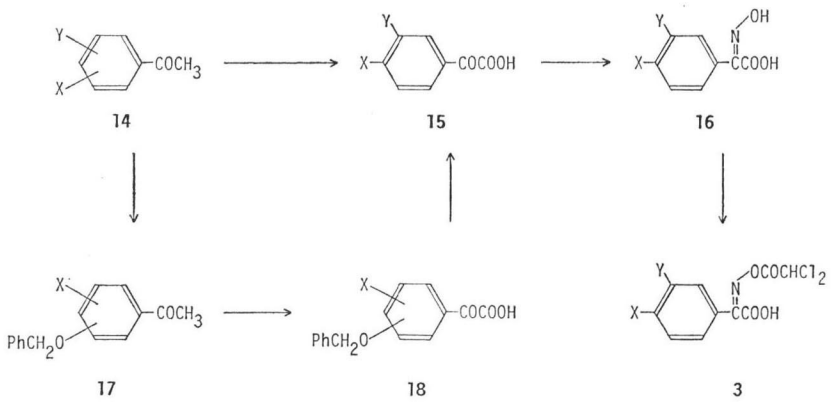
R^1–C–CO–
||
HO–N
anti (*E*)

The assignment of *syn*-configuration is based on analogies with nocardicins[9] and other oxyiminoacetyl derivatives[2,3] by reference to the NMR spectra of those oximes.

Scheme 1.



Scheme 2.



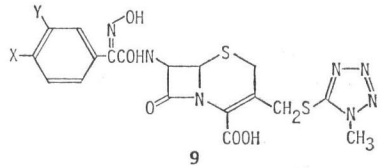
relationship for nocardicins,[7,8] a *syn*-isomer of (oxyiminoacetyl)cephems always had greater antibacterial activity than an *anti*-isomer.[3~6] Therefore we here discuss the *in vitro* activity of the cephalosporins (**2**) with *syn*-configuration.

Chemistry

As outlined in Scheme 1, the novel hydroxyiminoacetyl cephalosporins (**9**~**13**) were prepared by acylation of 7-aminocephalosporanic acid and its analogs with different 3-substituents with 2-aryl-2-dichloroacetoxyiminoacetic acids (**3**). The acids (**3**) were converted to the corresponding acid chlorides with PCl_5 or activated with VILSMEIER reagent for the above coupling reaction. Activation with VILSMEIER reagent, prepared from dimethylformamide (DMF) and phosphoryl chloride ($POCl_3$) or thionyl chloride ($SOCl_2$) was the most satisfactory method used for coupling the 7-aminocephems (**4**~**8**) with the oxyiminoacetic acids (**3**) bearing a phenolic hydroxyl function. In order to facilitate acylation of the 7-aminocephems (**4**~**8**) under non aqueous conditions, **4**~**8** were solubilized in an organic solvent by trimethylsilylation using *O*,*N*-bis(trimethylsilyl)acetamide (BSA) or *N*-(trimethylsilyl)acetamide (MSA).

Scheme 2 summarizes the synthesis of various side-chain acids (**16**). Substituted arylglyoxylic acids (**15**) were prepared by oxidation of acetophenones (**14**) with selenium dioxide (SeO_2)-pyridine.[10,11] In oxidation of the acetophenones (**14**, Y=OH) bearing a phenolic hydroxyl function, however, protection

Table 1. Effect on antibacterial activity of substitution in the phenyl ring.



9

Compounds			MIC (mcg/ml)						
			S. aureus		*E. coli*		*K. pneumoniae*	*P. mirabilis*	*P. vulgaris*
	X	Y	206	278	324	352[a]	417	501	601
9a	H	H	0.2	0.39	0.2	3.13	0.78	3.13	6.25
9b	H	Cl	0.1	0.2	1.56	12.5	3.13	12.5	6.25
9c	Cl	H	0.2	0.2	6.25	50	12.5	100	50
9d	H	F	0.2	0.39	0.39	6.25	1.56	12.5	6.25
9e	F	H	0.39	0.39	3.13	25	3.13	12.5	12.5
9f	H	OH	0.39	0.78	0.1	1.56	0.39	3.13	12.5
9g	OH	H	0.39	0.78	0.2	6.25	0.39	0.78	6.25
9h	H	OCH_3	0.2	0.39	1.56	25	3.13	12.5	25
9i	OCH_3	H	0.78	0.78	6.25	50	12.5	50	50
9j	H	CH_3	0.2	0.39	0.78	12.5	3.13	25	12.5
9k	CH_3	H	0.39	0.39	3.13	25	6.25	25	25
9l	H	$NHSO_2CH_3$	0.78	1.56	0.78	25	1.56	6.25	12.5
9m	$NHSO_2CH_3$	H	0.78	1.56	0.39	50	1.56	6.25	6.25
9n	H	CF_3	0.2	0.2	12.5	100	12.5	50	25
9o	H	SO_2NH_2	0.78	0.78	0.2	12.5	0.78	3.13	6.25
9p	SCH_3	H	0.2	0.39	6.25	25	12.5	50	50
9q	OH	OCH_3	0.39	0.78	0.78	12.5	0.39	6.25	6.25
9r	OH	Cl	0.78	0.78	0.2	3.13	0.39	0.78	6.25
9s	OH	NO_2	0.78	0.78	0.39	3.13	0.39	0.2	12.5
9t	OH	$NHSO_2CH_3$	1.56	1.56	0.39	12.5	0.39	3.13	12.5

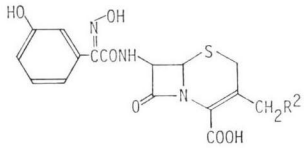
[a] Cephalosporinase producer

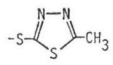
of the hydroxyl group with benzylhalide was utilized. Oxidation of the *O*-benzyl derivatives (**17**) with SeO_2-pyridine gave the corresponding arylglyoxylic acids (**18**), which were debenzylated with HCl-acetic acid to provide the hydroxyarylglyoxylic acids (**15f, g, q, r, s, t**). *Syn*-2-aryl-2-hydroxyiminoacetic acids (**16**) were predominantly prepared from the α-ketoacids (**15**) and hydroxylamine by the modified methods of AHMAD[12] and the Glaxo group.[5,6] The dichloroacetoxyiminoacetic acids (**3**) were prepared by acylation on **16** with dichloroacetyl chloride,[5,6] in order to protect the hydroxyimino group prior to activation of a carboxyl group in **3** using VILSMEIER reagent or PCl_5.

Biological Results and Discussion

The *in vitro* activity of the cephalosporins (**9a**~**9t**) against selected Gram-positive and Gram-negative organisms is shown in Table 1. Structure-activity relationships associated with 7-acyl side chain variation are conveniently dealt with keeping 3-substituent (R^2) constant as the *N*-methyltetrazolylthiomethyl group which is known to give most favorable antimicrobial activity against Gram-negative bacteria. The following structure-activity relationships are derived from the Table 1. i) The 7β-[2-hydroxyimino-2-(substituted phenyl)acetamido]cephalosporins have much more potent activity against Gram-

Table 2. Effect of substituents (R^2) at the 3-position on antibacterial activity.



Compounds R^2		MIC (mcg/ml)						
		S. aureus 206	*S. aureus* 278	*E. coli* 324	*E. coli* 352[a)]	*K. pneumoniae* 417	*P. mirabilis* 501	*P. vulgaris* 601
9f		0.39	0.78	0.1	1.56	0.39	3.13	12.5
10f		0.39	0.78	0.1	1.56	0.2	3.13	3.13
11f		0.2	0.39	0.1	1.56	0.2	1.56	3.13
12f	$-OCOCH_3$	0.78	0.78	0.2	3.13	0.1	0.78	12.5
13f	$-OCONH_2$	0.78	0.78	0.2	1.56	0.2	0.39	6.25

a) Cephalosporinase producer

positive bacteria than against Gram-negative bacteria. ii) The *meta* substituted phenyl derivatives are more active than the corresponding *para* derivatives against Gram-negative bacteria.* However, there is little difference in activity against Gram-positive between the *meta* and *para* substituted derivatives. iii) In comparison with the unsubstituted phenyl derivative, introduction of a hydroxyl or sulfonamide function into the phenyl ring enhances the activity against Gram-negative, and in contrast the introduction of a chlorine atom increases the activity against Gram-positive. However, other substituents tend to reduce the antibacterial activity. Substitution of both *meta* and *para* positions in the phenyl ring reduces slightly the activity against both Gram-positive and Gram-negative bacteria.

In this series, the influence of various 3-substituents of the cephem nucleus on antibacterial activity was also examined by keeping the 7-acyl side chain constant as the 2-(3-hydroxyphenyl)-2-hydroxyimino-acetyl radical. Their *in vitro* activity can be seen in Table 2. The cephem (**11f**) with 1,3,4-thiadiazolyl group shows the best activity against *Staphylococcus aureus*. The analog (**13f**) with a carbamoyloxy group exhibits the best activity against *Proteus* species. However, all of the cephems in Table 2 tend to show essentially little difference in activity against *Klebsiella pneumoniae* and *Escherichia coli*. It is noteworthy that all of these compounds exhibit good activity against Gram-negative bacteria such as the β-lactamase producing *Escherichia coli* 352.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at

* The *ortho* derivatives with the *syn* configuration were difficult to isolate in pure form because of their labile isomerization to the corresponding *anti*-isomer.

100 MHz on a JEOL-MH 100 NMR spectrometer using Me_4Si as an internal standard. IR spectra were taken on a Hitachi 260-10 spectrophotometer or Shimadzu IR-420 spectrophotometer.

Antibiotic Susceptibility

All the *in vitro* antibacterial activity 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 10^6 C.F.U./ml. *E. coli* 352 is cephalosporin-resistant strain.

Preparation of 4-Benzyloxy-3-methanesulfonamidoacetophenone (**17**, $X=NHSO_2CH_3$)

To a solution of 4-benzyloxy-3-nitroacetophenone (**17**, $X=NO_2$) (40 g, 0.147 mole) in ethanol (800 ml) and H_2O (300 ml) $Na_2S\cdot 9H_2O$ (80 g) was added portionwise at 90°C, and the mixture was refluxed for 3 hours under stirring. The reaction mixture was concentrated to about 300 ml and the precipitates were filtered, washed with H_2O, and dried (P_2O_5) to give 25.5 g (71.8%) of 3-amino-4-benzyloxyacetophenone (**17**, $X=NH_2$); mp 113~114°C (EtOH); IR (nujol) 3475, 3375, 1660 cm^{-1}; NMR ($CDCl_3$) δ 2.45 (3H, s), 4.00 (2H, br. s), 5.10 (2H, s), 6.84 (1H, d, J=8Hz), 7.28 (1H, dd, J=2, 8Hz), 7.29 (6H, m).

A solution of methanesulfonyl chloride (6.3 g, 0.064 mole) in methylene chloride (20 ml) was added dropwise to a mixture of **17** ($X=NH_2$) (12 g, 0.05 mole) and pyridine (8 g, 0.1 mole) in methylene chloride (100 ml) under ice-cooling for 30 minutes, and the reaction mixture was stirred at room temperature for 4 hours. After removing the solvent, H_2O was added to the residue and the resulting solution was adjusted to pH 1.0 with conc. HCl. The precipitates were filtered, washed with H_2O, and dried (P_2O_5) to give 15.4 g (96.6%) of **17**; mp 124~126°C; IR (nujol) 3300, 1670, 1600, 1330, 1150 cm^{-1}; NMR (DMSO-d_6) δ 2.43 (3H, s), 2.90 (3H, s), 5.20 (2H, s), 7.10 (1H, d, J=8Hz), 7.30 (5H, br. s), 7.50 (1H, dd, J=2, 8Hz), 7.8 (1H, d, J=2Hz), 9.10 (1H, s).

General Preparation of Substituted Arylglyoxylic Acids (**15**)

Method A: To a solution of a substituted acetophenone (**14**) (0.1 mole) in pyridine (100 ml) SeO_2 (0.15 mole) was added portionwise at 60~70°C, and the mixture was stirred at similar temperature for 5~8 hours. After filtration, the filtrate was evaporated *in vacuo* to afford the residue, which was dissolved in a mixture of H_2O and ethyl acetate (AcOEt) with stirring. The separated aqueous layer was adjusted to pH 2.0 with 40% aqueous H_3PO_4, and extracted with AcOEt. The AcOEt layer was washed with brine, dried ($MgSO_4$), and evaporated to dryness. The residue was triturated with benzene or petroleum ether to afford a substituted arylglyoxylic acid (**15**).

Method B (in case of the acetophenone with a phenolic hydroxyl function): A mixture of a substituted acetophenone (**14**, Y=OH) (0.1 mole), benzyl chloride (0.105 mole), and K_2CO_3 (0.12 mole) in DMF (100 ml) was stirred at 100°C for an hour. The reaction mixture was poured into H_2O, and the aqueous solution was extracted with AcOEt. The separated AcOEt layer was washed with brine, dried ($MgSO_4$), and evaporated to dryness. The residue was recrystallized from an appropriate solvent to afford a benzyloxyacetophenone derivative (**17**) as crystal.

To solution of **17** (0.1 mole) in pyridine (100 ml) SeO_2 (0.15 mole) was added portionwise at 100°C with vigorous stirring. The reaction mixture was stirred at 100~110°C for 3~5 hours, and worked up in a similar manner to the Method A to give **18**.

A mixture of **18** (0.1 mole) and conc. HCl (1.0~1.2 mole) in acetic acid (120 ml) was refluxed for 3 hours. The reaction mixture was concentrated *in vacuo* to afford the residue, which was dissolved in AcOEt. The AcOEt solution was washed with brine, dried ($MgSO_4$), and evaporated. The residue was triturated with hexane or benzene to afford a substituted arylglyoxylic acid (**15**).

General Preparation of 2-Hydroxyimino-2-arylacetic Acid (**16**)

Method A: To a solution of **15** (0.01 mole) in aqueous $NaHCO_3$ solution (0.01 mole) was added an aqueous NH_2OH solution [prepared from $NH_2OH\cdot HCl$ (0.011 mole) and $NaHCO_3$ (0.011 mole) in H_2O], and the mixture was stirred at room temperature for 18~25 hours. The reaction mixture was washed with diethyl ether. The separated aqueous layer was acidified to pH 1.0 with 10% HCl, and the acidified solution was extracted with AcOEt. The AcOEt layer was washed with brine and dried

Table 3. Physical data and yields of **3** and **16**.

	Method	**16**				**3**		
		Yield (%)	mp °C(dec.)	IR ν_{max}^{Nujol} (cm^{-1})		Yield (%)	IR ν_{max}^{Nujol} (cm^{-1})	
b	A	70.7	166	3170	1700	87.9	1755	
d	A	84.1	159	3275	1705	92.0	1754	
e	B	84.0	144	3250	1698	82.4	1770	
f	A	80.1	122	3200	1700	94.8	1760	1730
g	A	92.0	156	3250	1690	91.4	1770	1740
h	B	56.9	150	3300	1715	87.5	1750	
i	B	63.0	137	3250	1730	93.0	1780	1725
j	A	93.8	150	3275	1715	94.0	1785	1750
k	A	94.4	151	3175	1695	84.1	1750	
l	B	75.6	141	3250	1702	81.2	1772	1735
m	A	62.0	156	3250	1730	91.0	1780	1730
n	B	77.2	164	3210	1700	77.0	1790	1720
o	A	45.0	162	3250	1705	84.5	1785	1740
p	A	60.0	138	3250	1705	90.0	1770	1750
q	A	72.1	156	3250	1710	87.3	1795	1705
r	A	84.9	162	3350	1730	85.0	1730	1700
s	A	81.3	95	3300	1725	*		
t	A	93.3	172	3300	1710	*		

* This compound was not isolated.

($MgSO_4$). The solvent was evaporated and the residue was crystallized or triturated with an appropriate solvent to afford 2-hydroxyimino-2-arylacetic acid (**16**) as crystal or powder.

Method B: To a 1 N methanolic solution of NH_2OH (0.011 mole) **15** (0.011 mole) was added under ice-cooling, and the mixture was refluxed for 10~30 minutes. After removing the solvent *in vacuo*, the residue was dissolved in 1 N NaOH solution, and worked up in a similar manner to the Method A to give **16**.

General Preparation of 2-Dichloroacetoxyimino-2-arylacetic Acid (**3**)

To a solution of dichloroacetyl chloride (0.04 mole) in methylene chloride (20~30 ml) **16** (0.01 mole) was added under ice-cooling, and the mixture was stirred at room temperature for 20~60 minutes. Hexane was added to the reaction mixture under ice-cooling to form precipitates, which were collected by filtration. The dichloroacetoxyiminoacetyl derivatives (**3**) obtained above were used for the coupling reaction without further purification.

General Procedure for Acylation of **4**~**8**

Acid Chloride Method: To a suspension of **3** (0.01 mole) in methylene chloride (20~30 ml) was added PCl_5 (0.01 mole) under ice-cooling, and the reaction mixture was stirred at similar temperature for 30~60 minutes. The resultant solution was evaporated *in vacuo*. The residue was dissolved in methylene chloride to produce an acid chloride solution of **3**. To a solution of **4** (0.01 mole) and BSA (0.03~0.04 mole) or MSA (0.06~0.08 mole) in methylene chloride (30~40 ml) was added the above acid chloride solution at −20°C, and the reaction mixture was stirred at −20~0°C for an hour. After removing the solvent, the residue was dissolved in a mixture of H_2O and AcOEt. To the separated AcOEt layer was added H_2O, and the mixture was adjusted to pH 7.0 with a saturated $NaHCO_3$ solution. The aqueous layer was acidified to pH 2.0 with 10% HCl, and extracted with AcOEt. The AcOEt solution was washed with brine, and dried ($MgSO_4$). The solvent was evaporated and the residue was triturated with Et_2O to afford **9**.

VILSMEIER Reagent Method: A mixture of DMF (0.011 mole) and $POCl_3$ (0.011 mole) was heated at 40~50°C for 30 minutes to prepare VILSMEIER reagent. To a suspension of the above VILSMEIER

Table 4. NMR and IR spectral data of **9a**~**t**.

Compounds			NMR (DMSO-d_6, δ)								IR ν_{max}^{Nujol} (cm^{-1})			
9	X	Y	=N-OH 1H,s	CONH 1H,d J=9Hz	C_7-H 1H,dd J=5,9Hz	C_6-H 1H,d J=5Hz	C_3-CH_2 2H,dd J=13Hz	C_2-H_2 2H,dd J=18Hz	N-CH_3 3H,s	Ring protons	=N-OH	β-Lactam	COOH	CONH
a	H	H	11.65	9.68	5.79	5.20	4.31	3.72	3.90	7.3~7.8 5H, m	3200	1770	1710	1665
b	H	Cl	11.92	9.72	5.86	5.20	4.34	3.75	3.95	7.4~7.70 4H,m	3250	1770	1710	1660
c	Cl	H	11.80	9.65	5.80	5.15	4.30	3.73	3.95	7.5 4H,m	3230	1775	1710	1660
d	H	F	11.94	9.78	5.90	5.23	4.37	3.76	3.95	7.1~7.8 4H,m	3275	1775	1715	1670
e	F	H	11.63	9.65	5.82	5.15	4.30	3.80	3.91	7.5~7.80 4H,m	3200	1770	1710	1665
f	H	OH	11.60	9.60	5.85	5.17	4.36	3.77	3.96	6.7~7.5 4H,m	3250	1770	1710	1670
g	OH	H	11.25	9.57	5.85	5.17	4.32	3.73	3.95	6.80 2H,d, J=9Hz 7.43 2H,d, J=9Hz	3250	1770	1710	1660
h	H	OCH_3	11.63	9.65	5.82	5.13	4.30	3.70	3.92	7.10 4H, m 3.72 3H, s	3250	1770	1710	1660
i	OCH_3	H	11.40	9.65	5.87	5.20	4.35	3.75	3.93	7.55 2H,d,J=12Hz 7.00 2H,d,J=12Hz 3.80 3H, s	3250	1775	1705	1660
j	H	CH_3	11.62	9.66	5.88	5.20	4.33	3.74	3.93	7.1~7.6 4H, m 2.32 3H, s	3250	1775	1710	1665
k	CH_3	H	11.48	9.58	5.82	5.16	4.30	3.70	3.92	7.10 2H,d,J=7Hz 7.43 2H,d,J=7Hz 2.31 3H, s	3260	1770	1710	1665
l	H	$NHSO_2CH_3$	12.11	9.55	5.82	5.13	4.28	3.67	3.86	9.81 1H, s 6.95~7.70 4H,m 2.95 3H, s	3250	1775	1710	1665
m	$NHSO_2CH_3$	H	11.55	9.65	5.95	5.18	4.31	3.72	3.90	7.21 2H,d,J=9Hz 7.50 2H,d,J=9Hz 3.02 3H, s	3250	1770	1705	1660

Table 4. continued

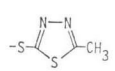
Compounds			NMR (DMSO-d_6, δ)								IR ν_{max}^{Nujol} (cm^{-1})			
9	X	Y	=N–OH 1H,s	CONH 1H,d J=9Hz	C_7-H 1H,dd J=5,9Hz	C_6-H 1H,d J=5Hz	C_3-CH_2 2H,dd J=13Hz	C_2-H_2 2H,dd J=18Hz	N–CH_3 3H,s	Ring protons	=N–OH	β-Lactam	COOH	CONH
n	H	CF_3	12.00	9.76	5.88	5.20	4.33	3.75	4.00	7.55~8.05 4H,m	3200	1770	1710	1665
o	H	SO_2NH_2	11.95	9.73	5.86	5.18	4.32	3.72	3.93	7.58~8.32 4H, m 7.47 2H, s	3250	1765	1705	1675
p	SCH_3	H	11.55	9.65	5.78	5.15	4.32	3.75	3.97	7.45 2H,d,J=8Hz 7.35 2H,d,J=8Hz	3250	1775	1720	1670
q	OH	OCH_3		*	5.80***	5.18	4.15	3.53	3.93	6.6~7.3 3H,m	3200 ~ 3400	1770		1660
r	OH	Cl	11.45	9.65	5.85***	5.20	4.32	3.75	4.00	7.45 1H,d,J=2Hz 7.40 1H,dd,J=2, 8Hz 7.00 1H,d,J=8Hz	3200 ~ 3400	1780	1710	1665
s	OH	NO_2		**	5.79	5.15	4.15	3.60	3.97	6.66 1H,d,J=9Hz 7.50 1H,dd, J=2,9Hz 7.96 1H,d,J=2Hz	3300 ~ 3400	1780	1720	1670
t	OH	$NHSO_2CH_3$		*	5.70***	5.00	4.30	3.54	3.93	6.60 1H,d,J=8Hz 6.84 1H,dd, J=2,8Hz 7.50 1H,d,J=2Hz	3200 ~ 3400	1770		1660

* Na salt. NMR was measured with D_2O.

** NMR was measured with $D_2O+NaHCO_3$.

*** d, J=5Hz

Table 5. NMR and IR spectral data of **10j~13f**.

Compounds	NMR (DMSO-d_6, δ)								IR ν_{max}^{Nujol} (cm^{-1})			
	=N–OH 1H,s	Ring protons 4H,m	CONH 1H,d J=9Hz	C_7-H 1H,dd J=5,9Hz	C_6-H 1H,d J=5Hz	C_3-CH_2 2H,dd J=13Hz	C_2-H_2 2H,dd J=18Hz	R^2	=N–OH	β-Lactam	COOH	CONH
10f 	11.68	6.7~7.5	9.65	5.85	5.23	4.40	3.75	2.75 3H,s	3270	1770	1720	1660
11f 	11.50	6.7~7.5	9.57	5.85	5.16	4.42	3.70	9.5 1H,s	3200	1770	1710	1660
12f $-OCOCH_3$	11.65	6.7~7.5	9.60	5.85	5.18	4.87	3.75	2.0 3H,s	3200	1780	1720	1650
13f $-OCONH_2$*		7.0~7.25		5.85**	5.20	4.70	3.52		3200 ~ 3500	1765		1660

* Na salt. NMR was measured with D_2O.

** d, J=5Hz

reagent in AcOEt (20~30 ml) was added **3** (0.01 mole) at −5°C, and the mixture was stirred at −10~−5°C for 30~60 minutes to produce an activated acid solution of **3**. To a solution of 7β-amino-3-substituted cephalosporanic acid (**4~8**) (0.01 mole) and BSA (0.03~0.04 mole) or MSA (0.06~0.08 mole) in AcOEt (30~40 ml) was added the above activated acid solution at −20°C, and the reaction mixture was stirred at −20~0°C for an hour. The resultant mixture was washed with H_2O. To the separated AcOEt layer was added H_2O, and the mixture was adjusted to pH 7.0 with a saturated $NaHCO_3$ solution. The separated aqueous layer was then acidified to pH 2.0 with 10% HCl, and the acidified solution was extracted with AcOEt. The separated AcOEt layer was washed with brine, and dried ($MgSO_4$). The solvent was evaporated and the residue was triturated with Et_2O to afford **9~13**.

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