SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF TRIAZOLE AND ISOXAZOLE DERIVATIVES OF AMPICILLIN

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In order to improve the antibacterial activity of ampicillin, new penicillin derivatives having a 1-aryltriazole-4-carboxamide group or a 5-arylisoxazole-3-carboxamide group at the α-position of benzylpenicillin or *p*-hydroxybenzylpenicillin were synthesized. Some compounds in these series were found to possess high activity against *Pseudomonas* and other Gram-negative bacteria. In addition, structure-activity relationships, especially the effect of the hydrophobic character of the compounds on activity, were investigated.

Recently the opportunistic infectious diseases have become the most serious problem in chemotherapy. They have been mainly caused by troublesome Gram-negative bacteria, especially *Pseudomonas aeruginosa*. Furthermore, the infectious diseases, caused by other glucose non-fermenting Gram-negative rods and anaerobic bacteria, have gradually increased.

These situations have stimulated the search for newer antibiotics and it is known that modifications of the amino group of ampicillin or amoxicillin have led to compounds with improved antibacterial activity, particularly against *P. aeruginosa*. Typical compounds are piperacillin,[1] apalcillin,[2] azlocillin,[3] TEI-2012,[4] CI-867,[5] *etc.*

In our laboratory two series of penicillins having a 1-aryltriazole-4-carboxamide group or a 5-arylisoxazole-3-carboxamide group at the α-position of benzylpenicillin or *p*-hydroxybenzylpenicillin have been prepared. These penicillins have similar structures and have shown similar antibacterial activity.

Some of these compounds have shown good activity against Gram-negative bacteria, including antipseudomonal activity. Furthermore, these compounds are of interest because of their favorable potencies against various glucose non-fermenting Gram-negative rods and anaerobic bacteria.

We report here the synthesis and *in vitro* microbiological evaluation of these compounds. In addition, structure-activity studies, especially the effect of the hydrophobic character of the compounds on microbiological potency, are presented.

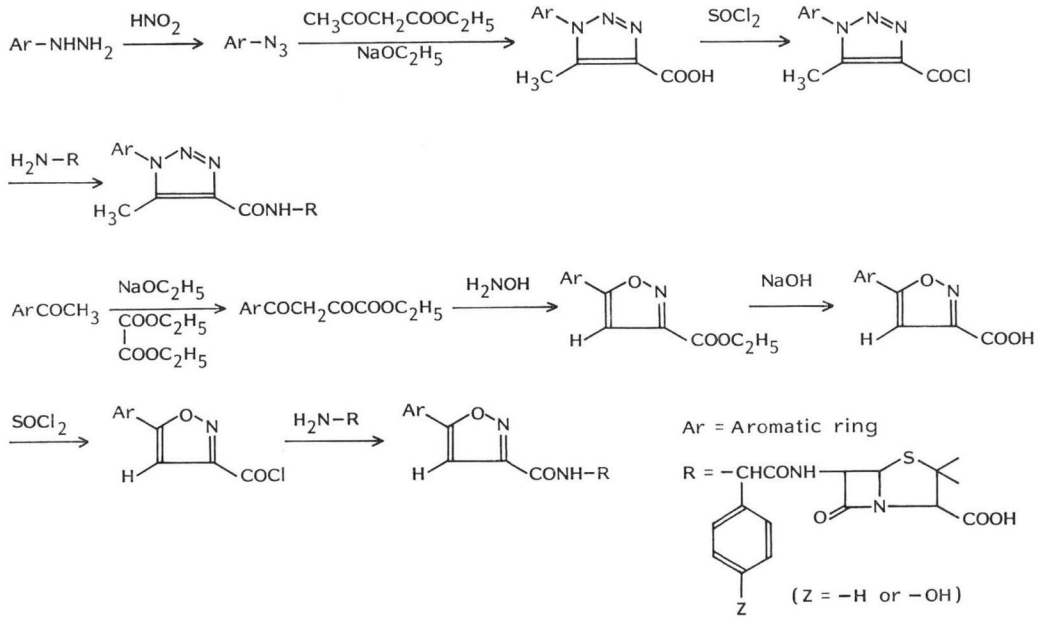
Chemistry

The synthesis of these series of penicillins was carried out as outlined in Chart 1. The side chains, 1-aryltriazole-4-carboxylic acids[6] and 5-arylisoxazole-3-carboxylic acids[7] were prepared according to the previously reported procedure. The condensation of the side chain to the ampicillin or amoxicillin nucleus was accomplished by the acid chloride method. The activation of the carboxyl group to the carbonyl chloride was carried out with thionyl chloride.

The penicillins having the amino-substituted phenyltriazole group were prepared by reduction of the corresponding nitro-substituted compounds.

The final products were isolated as water soluble sodium salts by treatment with sodium 2-ethyl-

Chart 1.



hexanoate. The structures and properties of these series of penicillins are given in Tables 1 and 2.

Antimicrobial Activity

The minimum inhibitory concentrations (MICs) of these series of penicillins against clinically important bacteria are shown in Table 3. MIC was assayed by the agar dilution method and inoculum size was one loopful of 10^6 cfu/ml.

From Table 3 it can be seen that most of these compounds are distinctly more active than carbenicillin against *P. aeruginosa*. The compounds **7** and **19** were selected for the evaluation of the *in vitro* activity against glucose nonfermenting Gram-negative rods and anaerobic bacteria. They were found to have good activity against these bacteria, as presented in Table 4.

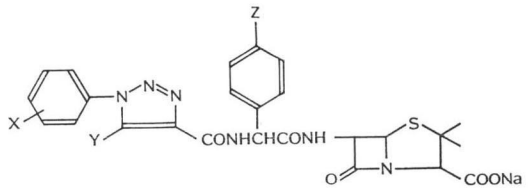
The most active anti-pseudomonal penicillins so far prepared by derivatization of the amino group of ampicillin or amoxicillin characteristically have a hydroxyl group or a carbonyl group at the β-position of the carbonyl group of the amide or ureido linkage formed with ampicillin or amoxicillin.[1~5] A similar conclusion was reached with regard to apalcillin and its related compounds by KOMATSU *et al.*[8] In contrast, the penicillins reported in this paper have high activity, despite the absence of either the hydroxyl group or the carbonyl group.

Table 3 indicates the following relationships between structure and activity.

(1) Comparison of the activity of **17** with that of **1** and **16** shows that a bulky phenyl group as substituent in the triazole ring reduces the activity markedly.

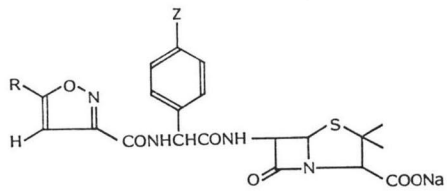
(2) Comparison of the activity between **4** and **5**, **7** and **8**, and between **19** and **20** shows that the amoxicillin derivative is slightly less active than the ampicillin derivative having the same side chain. This fact might be due to the decrease of hydrophobic character as discussed below.

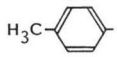
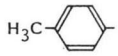
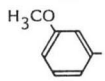
Table 1. Penicillins having a triazole ring.



Compound	X	Y	Z	Formula	Calcd.			Found			Rf
					C	H	N	C	H	N	
1	H	CH_3–	H	$C_{26}H_{25}N_6O_5SNa \cdot 1.5H_2O$	53.50	4.85	14.40	53.77	4.54	14.57	0.32
2	*o*-CH_3–	CH_3–	H	$C_{27}H_{27}N_6O_5SNa \cdot 1.6H_2O$	54.09	5.09	14.02	54.51	4.95	13.56	0.29
3	*m*-CH_3–	CH_3–	H	$C_{27}H_{27}N_6O_5SNa \cdot 2H_2O$	53.45	5.16	13.86	53.53	4.77	13.63	0.24
4	*p*-CH_3–	CH_3–	H	$C_{27}H_{27}N_6O_5SNa \cdot 2.3H_2O$	52.98	5.21	13.73	52.60	4.73	13.41	0.22
5	*p*-CH_3–	CH_3–	HO–	$C_{27}H_{27}N_6O_6SNa \cdot 1.4H_2O$	53.00	4.92	13.74	53.78	5.12	12.86	0.41
6	*o*-Cl–	CH_3–	H	$C_{26}H_{24}N_6O_5SCl \cdot Na \cdot 1H_2O$	51.27	4.31	13.80	51.21	4.41	13.24	0.27
7	*m*-Cl–	CH_3–	H	$C_{26}H_{24}N_6O_5SCl \cdot Na \cdot 1.5H_2O$	50.45	4.56	13.58	50.74	4.25	13.38	0.20
8	*m*-Cl–	CH_3–	HO–	$C_{26}H_{24}N_6O_6SCl \cdot Na \cdot 1.5H_2O$	47.88	4.49	12.89	47.94	4.21	12.68	0.31
9	*p*-Cl–	CH_3–	H	$C_{26}H_{24}N_6O_5SCl \cdot Na \cdot 0.5H_2O$	51.96	4.36	13.98	52.20	4.60	13.27	0.21
10	*p*-NO_2–	CH_3–	H	$C_{26}H_{24}N_7O_7SNa \cdot 2H_2O$	48.97	4.39	15.38	49.58	4.24	15.04	0.29
11	*p*-NH_2–	CH_3–	H	$C_{26}H_{26}N_7O_5SNa \cdot 1.5H_2O$	52.17	4.85	16.39	52.83	4.89	15.84	0.42
12	*p*-CH_3O–	CH_3–	H	$C_{27}H_{27}N_6O_6SNa \cdot 0.5H_2O$	54.16	5.23	14.04	54.51	4.99	13.46	0.29
13	*p*-C_2H_5O–	CH_3–	H	$C_{28}H_{29}N_6O_6SNa \cdot 0.5H_2O$	55.15	4.97	13.79	55.57	5.09	13.68	0.20
14	*p*-$PhCH_2O$–	CH_3–	H	$C_{33}H_{31}N_6O_6SNa \cdot 1.5H_2O$	57.47	4.93	12.19	57.42	4.70	12.25	0.07
15	*p*-HO–	CH_3–	H	$C_{26}H_{25}N_6O_6SNa \cdot 0.5H_2O$	53.70	4.48	14.46	53.28	4.42	14.86	0.38
16	*p*-CH_3–	H	H	$C_{26}H_{25}N_6O_5SNa \cdot 4H_2O$	49.68	5.29	13.37	49.76	4.94	14.16	0.27
17	H	Ph–	H	$C_{31}H_{27}N_6O_5SNa \cdot 1.7H_2O$	57.34	4.73	12.95	57.82	4.63	12.59	0.18

Table 2. Penicillins having a isoxazole ring.



Compound	R	Z	Formula	Calcd.			Found			Rf
				C	H	N	C	H	N	
18		H	$C_{26}H_{23}N_4O_6SNa \cdot 1.3H_2O$	55.17	4.57	9.90	55.50	4.73	9.56	0.22
19		H	$C_{27}H_{25}N_4O_6SNa \cdot 1.2H_2O$	56.08	4.79	9.69	56.10	4.81	9.45	0.08
20		HO–	$C_{27}H_{25}N_4O_7SNa \cdot H_2O$	53.28	4.81	9.21	53.17	4.78	8.82	0.23
21		H	$C_{26}H_{22}N_5O_8SNa \cdot 1.4H_2O$	50.96	4.09	11.43	50.93	4.10	11.08	0.17
22		H	$C_{27}H_{25}N_4O_7SNa \cdot 1.2H_2O$	54.57	4.66	9.43	54.34	4.71	9.33	0.18
23		H	$C_{25}H_{22}N_5O_6SNa \cdot 1.1H_2O$	53.29	4.34	12.43	52.86	4.44	12.03	0.41
24		H	$C_{24}H_{21}N_4O_7SNa \cdot 1.3H_2O$	51.84	4.29	10.07	52.54	4.83	9.00	0.26
25		H	$C_{24}H_{21}N_4O_6SNa \cdot 1H_2O$	50.87	4.10	9.89	50.81	4.36	9.48	0.24
26	CH_3–	H	$C_{21}H_{21}N_4O_6SNa \cdot 0.9H_2O$	50.77	4.64	11.28	51.34	5.02	10.96	0.57

Relationship between Hydrophobic Character and Activity

It has already been reported that there is a close relationship between hydrophobic character and antibacterial activity of β-lactam antibiotics against Gram-negative bacteria.[9] For example, BIAGI *et al.* reported that the activity of penicillins against *Escherichia coli* increases linearly with the hydrophilic character of the molecules.[9] It was also assumed that the high lipid content of the cell wall of Gram-negative bacteria such as *E. coli* could retain highly hydrophobic compounds, thus allowing a more hydrophilic β-lactam antibiotic to pass more easily through the outermembrane.

The penicillins described in this paper have a fairly high hydrophobic character and therefore it was particularly interesting to examine the relationship between this property and activity.

The hydrophobic character of 1-aryltriazole analogs and 5-arylisoxazole analogs was estimated by means of reversed-phase thin-layer chromatography and is expressed as a chromatographic Rm value,[9] related to the partition coefficient and calculated from the formula:

$$Rm = \log (1/Rf - 1)$$

Rf value was measured by a modification of SAWAI's procedure[10] to determine slight differences in the hydrophobic character of these analogs. The Rf values obtained are shown in Tables 1 and 2.

Antibacterial activity is expressed as log 1/MIC. In the case of *E. coli* and *P. aeruginosa* the average of MIC values against two strains given in Table 3 was used. Compounds **16** and **17** were excluded from

Table 3. Comparative *in vitro* activity (MIC, μg/ml) of the penicillins in Tables 1 and 2.

Compounds	*E.c.*	*E.c.**	*K.p.*	*P.v.*	*S.m.*	*P.a.*	*P.a.**
1	12.5	6.25	1.56	0.20	6.25	6.25	12.5
2	12.5	6.25	0.78	0.20	3.13	6.25	6.25
3	6.25	3.13	0.39	≧0.10	1.56	3.13	6.25
4	6.25	3.13	0.20	≧0.10	1.56	6.25	6.25
5	12.5	3.13	1.56	≧0.10	3.13	6.25	6.25
6	12.5	6.25	0.78	0.20	1.56	6.25	6.25
7	6.25	3.13	0.39	≧0.10	3.13	6.25	6.25
8	12.5	6.25	1.56	0.20	3.13	6.25	6.25
9	6.25	3.13	0.39	0.20	3.13	12.5	12.5
10	12.5	3.13	0.78	0.20	12.5	12.5	12.5
11	6.25	3.13	1.56	≧0.10	6.25	6.25	12.5
12	6.25	3.13	0.39	≧0.10	3.13	6.25	12.5
13	6.25	3.13	0.39	0.20	1.56	6.25	12.5
14	6.25	3.13	≧0.10	0.20	1.56	6.25	6.25
15	12.5	3.13	1.56	0.20	6.25	12.5	12.5
16	6.25	3.13	0.39	≧0.10	6.25	6.25	6.25
17	50.0	25.0	6.25	6.25	50.0	25.0	25.0
18	12.5	6.25	0.39	0.20	6.25	6.25	12.5
19	6.25	3.13	0.39	0.39	6.25	6.25	6.25
20	12.5	6.25	1.56	0.39	25.0	12.5	12.5
21	25.0	6.25	0.78	0.20	12.5	12.5	12.5
22	12.5	6.25	0.78	0.20	6.25	12.5	12.5
23	25.0	12.5	3.13	≧0.10	12.5	6.25	6.25
24	25.0	12.5	3.13	0.39	50.0	12.5	12.5
25	25.0	6.25	1.56	0.39	12.5	12.5	12.5
26	50.0	25.0	12.5	0.78	100.0	25.0	50.0
Ampicillin	6.25	3.13	6.25	0.78	25.0	>100	>100
Carbenicillin	6.25	3.13	50.0	0.78	1.56	25.0	50.0

Organisms selected for inclusion in this table are:
E.c., *E. coli* NIHJ JC-2; *E.c.**, *E. coli* K12 C600; *K.p.*, *K. pneumoniae* PCI-602; *P.v.*, *P. vulgaris* OX19; *S.m.*, *S. marcescens* IAM1184; *P.a.*, *P. aeruginosa* IFO3445; *P.a.**, *P. aeruginosa* ATCC 10145.

Table 4. Comparative *in vitro* activity (MIC, μg/ml) of compounds **7** and **19** against glucose non-fermenting Gram-negative rods and anaerobic bacteria.

Compound	**7**	**19**	Carbenicillin
Pseudomonas cepacia 9*	1.56	3.13	>100
Pseudomonas maltophilia 17*	1.56	1.56	25
Alcaligenes faecalis 16*	3.13	6.25	>100
Achromobacter xylosoxidans 9*	0.78	1.56	>100
Bacteroides fragilis ATCC 25285	0.78	3.13	6.25
Bacteroides fragilis GM7000	0.78	1.56	6.25
Clostridium perfringens ATCC 13123	≧0.39	≧0.39	0.78

* Clinical isolate.

the investigation because they have a different substituent Y.

Plots of log 1/MIC vs. Rm (Figs. 1 and 2) show that the antibacterial activity of isoxazole analogs against *E. coli* and *Klebsiella pneumoniae* is correlated with the hydrophobic character of the compounds. In the case of the triazole analogs, the same was found for the activity against *K. pneumoniae*. In the case of the other bacteria tested, a clear relationship was not noticed.

These results do not agree with those of BIAGI. However a similar trend to that found in the present series of penicillins has been reported for the activity of piperacillin and its analogs against *K. pneumoniae* and *Serratia marcescens*.[1] These findings may suggest different penetration mechanisms for the hydro-

Fig. 1. The relationship between Rm value of isoxazole analogs and their activity against *K. pneumoniae* (○) and *E. coli* (△).

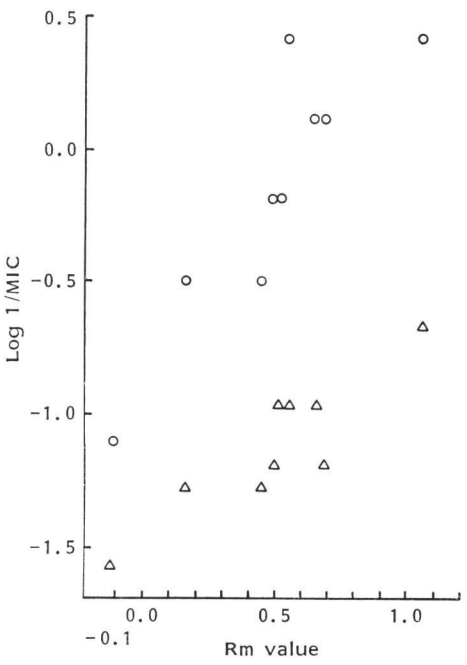
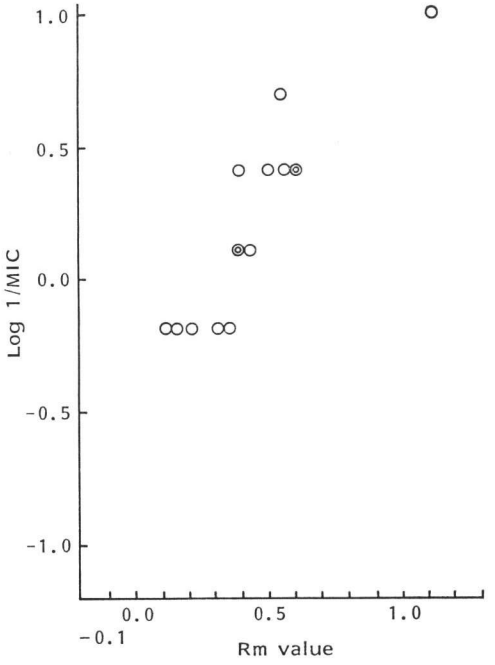


Fig. 2. The relationship between Rm value of triazole analogs and their activity against *K. pneumoniae*.



phobic penicillins prepared by modification of the amino group of ampicillin or amoxicillin. Such a possibility has recently been mentioned by SAWAI *et al.*[11,12]

Experimental

Infrared spectra were measured on a Shimadzu IR-430 spectrophotometer. NMR spectra were measured on a Varian EM-390 (90MHz) or T-60 (60MHz) spectrometer using TMS as an internal standard. All compounds were analyzed for C, H, N and the analytical results of penicillins are given in Tables 1 and 2.

Side Chain Synthesis

Side chain acids, triazole-4-carboxylic acids and isoxazole-3-carboxylic acids were prepared similarly to the reported methods.[6,7] Examples illustrating the preparation of typical compounds will hereinafter be described.

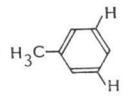
1-(3-Chlorophenyl)-5-methyl-1,2,3-triazole-4-carboxylic Acid

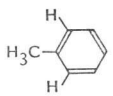
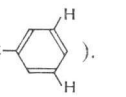
To a mixture of 3-chlorophenylhydrazine sulfate (19.15 g, 50 mmol) and 12 N HCl (7 ml) in water (100 ml), covered with ether (50 ml), a solution of sodium nitrite (9 g, 130 mmol) in water (25 ml) was added dropwise with stirring at $-3\sim-5$°C. After stirring for 2 hours, the ether layer was separated, dried and evaporated *in vacuo* under 25°C to give 12.0 g of 3-chlorophenylazide as a residue. This residue and ethyl acetoacetate (11.2 g, 86 mmol) were dissolved in ethanol (10 ml) and added to a sodium ethoxide solution (sodium 2.2 g, ethanol 30 ml) with stirring at 5°C. The mixture was refluxed for 1 hour. After cooling, water (50 ml) was added and the mixture was concentrated. The pH of the residual solution was brought to 2 with 12 N HCl and a precipitate was collected by filtration. The solid thus obtained was dissolved in 1.3% NaOH (450 ml) and precipitated by adding 12 N HCl. The reprecipitate was collected and dried under vacuum to give 10.5 g (44.2%) of the product; mp $192\sim194$°C;

NMR (DMSO-d_6) δ 2.53 (3H, s, CH_3–), 7.45~7.87 (4H, m, Ph-H).

Anal. Calcd. for $C_{10}H_8N_2O_2Cl$: C 50.53, H 3.40, N 17.68.
Found: C 50.89, H 3.18, N 17.83.

5-(4-Methylphenyl)isoxazole-3-carboxylic Acid

Sodium (3.8 g, 165 mmol) was dissolved in 100 ml of anhydrous ethanol. To this ice-cooled solution, a mixture of 4-methylacetophenone (20.1 g, 150 mmol) and diethyl oxalate (21.9 g, 150 mmol) was slowly added with stirring. The mixture was stirred at 0°C for 1 hour and at room temperature for 6 hours. After standing overnight, the mixture was poured into ice-cold water and the pH of the water phase was brought to 4 with 6 N H_2SO_4. The separated oil was extracted with benzene. The organic layer was dried and evaporated *in vacuo* to give 10.0 g of ethyl (4-methylbenzoyl)pyruvate as a residual oil. Without further purification, this compound was dissolved in ethanol (140 ml) and hydroxylamine hydrochloride (2.78 g, 40 mmol) was added. The mixture was refluxed for 1.5 hours and evaporated *in vacuo* to give a residual solid. That was recrystallized from ethanol - water to give 5.4 g of ethyl 5-(4-methylphenyl)isoxazole-3-carboxylate; mp 53~54°C; NMR (DMSO-d_6) δ 1.36 (3H, t, $-CH_2CH_3$), 2.38 (3H, s, CH_3–Ph), 4.40 (2H, q, $-CH_2CH_3$), 7.37 (3H, m, isoxazole C_4-H+ ), 7.82 (2H, d, ).

This product was dissolved in ethanol (50 ml) and 13.3% NaOH (18 ml) and the mixture was refluxed for 1.5 hours. After cooling, the mixture was poured into ice-cold water and covered with ether. The pH of the water phase was brought to 1.0 with 12 N HCl. The organic layer was separated and the water layer was extracted by ether. The combined organic layers were dried and evaporated *in vacuo* to give a residual solid, which was crystallized from benzene to give 4.0 g (13.1%) of the product; mp 193~194°C (dec.); NMR (DMSO-d_6) δ 2.36 (3H, s, CH_3–), 7.30 (1H, s, isoxazole C_4-H), 7.36 (2H, d, ), 7.82 (2H, d, ).

Anal. Calcd. for $C_{11}H_9NO_3$: C 65.02, H 4.46, N 6.89.
Found: C 65.18, H 4.22, N 6.86.

(2*S*,5*R*,6*R*)-6-[(*R*)-2-[1-(3-Chlorophenyl)-5-methyl-1,2,3-triazole-4-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic Acid Sodium Salt (7)

A mixture of 1-(3-chlorophenyl)-5-methyl-1,2,3-triazole-4-carboxylic acid (1.19 g, 5 mmol) and thionyl chloride (10 ml) was stirred at 80°C for 4 hours. After the mixture was evaporated, dry benzene (10 ml) was added and the mixture was concentrated *in vacuo* again to give the corresponding acid chloride as a residue. It was dissolved in dry acetonitrile (15 ml).

Ampicillin trihydrate (1.81 g, 4.5 mmol) was suspended in a mixture of water (25 ml) and acetonitrile (10 ml), and the pH of the suspension was adjusted carefully to 8.5 with 2 N NaOH under ice-cooling. To this solution, the acid chloride solution previously prepared was added dropwise with stirring and ice-cooling. Subsequently, the solution was stirred for 1 hour under ice-cooling and for 1 hour at room temperature. During the reaction, the pH of the mixture was kept at 7.5~8.0 with 2 N NaOH and 2 N HCl. Water (15 ml) was added and the mixture was evaporated *in vacuo* under 30°C to remove acetonitrile. The water solution was covered with ethyl acetate (100 ml) and acidified to pH 1.5 with 2 N HCl. The organic layer was separated and the water layer was extracted by ethyl acetate (100 ml). The combined organic layers were dried and evaporated to a syrup *in vacuo*, which was triturated with a mixture of ether - petroleum ether (1: 1). The triturated material was collected by filtration and dried under vacuum. To a suspension of the solid thus obtained in 10 ml of methanol, 2.14 ml of 33% butanol solution of sodium 2-ethylhexanoate was added with stirring under ice-cooling. After stirring

for 15 minutes, the insoluble material was removed and 200 ml of ether was added with stirring. The precipitate was collected by filtration and dried under vacuum to give 2.10 g (75.5%) of **7**; IR (Nujol) 1760 cm^{-1} (β-lactam); NMR (DMSO-d_6) δ 1.45 (3H, s, $-CH_3$), 1.55 (3H, s, $-CH_3$), 2.55 (3H, s, triazole-CH_3), 3.90 (1H, s, C_2-H), 5.40 (2H, m, C_5-H+C_6-H), 5.95 (1H, d, Ph-C*H*-CO), 7.30~7.70 (9H, m, Ph-H), 8.50 (1H, d, -CONH-), 9.20 (1H, d, -CONH-).

Compounds **1**~**10** and **12**~**17** were similarly prepared.

(2*S*,5*R*,6*R*)-6-[(*R*)-2-[1-(4-Aminophenyl)-5-methyl-1,2,3-triazole-4-carboxamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic Acid Sodium Salt (**11**)

A suspension of 30% Pd-$BaCO_3$ (7.2 g) in water (90 ml) was placed in a steel bomb and activated for 1 hour at 30 atm of hydrogen. Then the solution of the corresponding nitro analog (**10**) (3 g, 4.71 mmol) in water (75 ml) was added. The reduction was carried out at room temperature for 1 hour at 30 atm of hydrogen. After the removal of the catalyst, the solution was covered with ethyl acetate (300 ml) and the pH of the water phase was brought to 1.5 with 2 N HCl. The organic layer was separated and the water layer was extracted by ethyl acetate (300 ml). The combined organic layers were dried and evaporated *in vacuo* under 30°C. The residue was triturated with ether and the triturated material was collected by filtration and dried under vacuum. The solid thus obtained was converted to monosodium salt by the method described above to give 2.0 g (71%) of **11**; IR (Nujol) 1760 cm^{-1} (β-lactam); NMR (DMSO-d_6) δ 1.43 (3H, s, $-CH_3$), 1.55 (3H, s, $-CH_3$), 2.43 (3H, s, triazole-CH_3), 4.20 (1H, s, C_2-H), 5.37~5.67 (2H, m, C_5-H+C_6-H), 5.94 (1H, d, Ph-C*H*-CO-), 6.67~7.67 (9H, m, Ph-H).

(2*S*, 5*R*, 6*R*)-3,3-Dimethyl-6-[(*R*)-2-[5-(4-methylphenyl)isoxazole-3-carboxamido]-2-phenylacetamido]-7-oxo-4-thia-1-azabicyclo[3,2,0]heptane-2-carboxylic Acid Sodium Salt (**19**)

By a similar procedure to that used for compound (**7**), ampicillin trihydrate (1.81 g, 4.5 mmol) was allowed to react with 5-(4-methylphenyl)isoxazole-3-carboxylic acid (0.97 g, 5 mmol) to give 1.48 g (57%) of **19**; IR (Nujol) 1765 cm^{-1} (β-lactam); NMR (DMSO-d_6) δ 1.42 (3H, s, $-CH_3$), 1.52 (3H, s, $-CH_3$), 2.38 (3H, s, CH_3-Ph), 3.92 (1H, s, C_2-H), 5.30~5.58 (2H, m, C_5-H+C_6-H), 5.93 (1H, d, Ph-C*H*-CO-), 7.10~7.60 (8H, m, Ph-H+isoxazole C_4-H+ ), 7.79 (2H, d, ), 8.87 (1H, d, -CONH-), 9.04 (1H, d, -CONH-).

Compounds **18** and **20**~**26** were similarly prepared.

Reversed-phase Thin-layer Chromatography

The polar mobile phase was a 2: 1 mixture of sodium acetate-Veronal buffer (pH 7.0) and acetone. The nonpolar stationary phase was a silanized silica gel plate (Merck silica gel 60 F_{254} silanized Art. 5747). Before use, the silica gel plate was activated by heating for 30 minutes at 120°C. Penicillins were dissolved in distilled water to give about 10 mg/ml and about 0.5~2.0 μl of solution was spotted on the thin-layer plate. The development was carried out for about 1 hour at room temperature. After the developed plate was dried, the penicillin was detected by UV (253.5 nm) and iodine vapor.

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