NEW CEPHALOSPORINS WITH 7-ACYL GROUPS DERIVED FROM β-KETOACIDS

II. FURTHER MODIFICATIONS OF 7-(3-OXOBUTYRYLAMINO)-CEPHALOSPORINS

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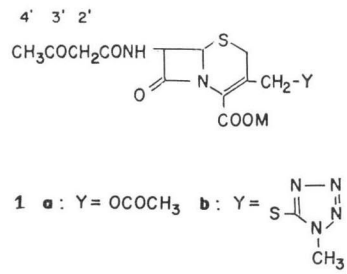
New cephalosporins modified in the acyl part of 7-(3′-oxobutyrylamino)cephalosporins (**1**), which have been described in the preceding paper, were synthesized by thiolation at the 2′- or the 4′-position, or by transforming the 3′-oxo group into a 3′-imino group. The most active compound *in vitro* was 3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-7-(4-methylthio-3-oxobutyrylamino)ceph-3-em-4-carboxylic acid (**7c**), which showed superior *in vitro* activity against Gram-positive and Gram-negative bacteria compared to the parent cephalosporin (**1b**) with the same 3-substituent. The ED_{50} value for **7c**, however, was essentially equal to that of **1b** in mice infected with *Escherichia coli* O-111.

In the preceding paper[1] we described the synthesis and the antimicrobial properties of 7-(β-keto-acylamino)cephalosporins in which the acyl groups are relatively simple, *e.g.* 3-oxobutyryl, 3-phenyl- or 3-heterocyclic-3-oxobutyryls. Among these, the *in vitro* activity of 3-[[(1-methyl-1H-tetrazol-5-yl)-thio]methyl]-7-(3-oxobutyrylamino)ceph-3-em-4-carboxylic acid (**1b**) was fairly good and almost as potent as cephalothin (CET). Its *in vivo* activity was 6.5 times more potent than CET in mice infected with *Escherichia coli*.

These results encouraged us to pursue our initial working hypothesis that the introduction of β-ketoacyl moieties might give the cephalosporins better antimicrobial properties.

This paper deals with the modifications of the 3′-oxobutyrylamino side chain of **1b** by substituting various thiols for the hydrogen at the 2′-methylene or at the 4′-methyl position, or by substituting imino groups for the oxygen of the 3′-oxo group.

Fig. 1.



Chemistry

1. Modification at the 2′-Methylene Position

Treatment of 7-(3-oxobutyrylamino)cephalosporanic acid (**1a**) with N-bromosuccinimide (NBS) or N-chlorosuccinimide (NCS) resulted in the 2′-halogenated compounds (**2**), which, being too unstable to be isolated, were reacted *in situ* with thiols to afford 2′-thiolated compounds (**3a**～**e**).

The NMR spectra (D_2O) of **3a**～**e**, except for **3d**, exhibited absorption for the 4′-methyl group as a

Scheme 1.

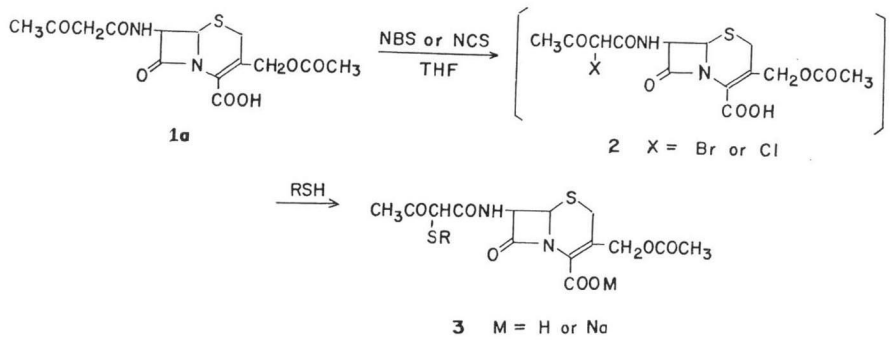


Table 1. 7-(2-Substituted-thio-3-oxobutyrylamino)cephalosporanic acids (**3**)

Compound	R	M	Yield %	Formula[a]	IR (β-lactam) KBr, cm^{-1}
3a	CH_3	Na	68	$C_{15}H_{17}N_2O_7S_2Na$[b]	1755
b	$HOCH_2CH_2$	Na	72	$C_{16}H_{19}N_2O_8S_2Na \cdot 0.5H_2O$	1755
c	$NaOCOCH_2$	Na	77	$C_{16}H_{16}N_2O_9S_2Na_2 \cdot 1.5H_2O$[c]	1760
d		H	46	$C_{19}H_{19}N_3O_7S_2 \cdot H_2O$	1770
e		H	64	$C_{19}H_{19}N_3O_7S_2 \cdot 0.5H_2O$[d]	1770

[a]: Unless otherwise indicated, analytical results for C, H and N for all compounds were within 0.4% of the theoretical value.
[b]: N, calcd., 6.60; found, 6.17.
[c]: N, calcd., 5.41; found, 4.87.
[d]: N, calcd., 8.86; found, 8.27.

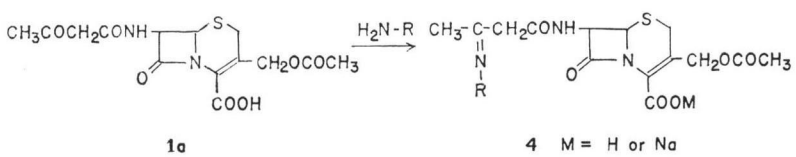
symmetrical doublet indicating that they were 1:1 mixtures of diastereoisomers which differ in configuration at the 2′-position. However, **3d** exhibited the absorption as a singlet, thus the diastereomeric constitution of **3d** remains uncertain.

2. Modification at the 3′-Oxo Group

The reaction of **1a** with amines such as hydroxylamine, alkoxyamine, semicarbazide or hydrazines under mild conditions afforded the 3′-imino compounds (**4a**~**f**) without affecting the β-lactam ring, which has been reported to undergo ring fissions with these reagents.[2]

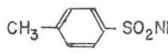
The NMR spectra (D_2O) of **4a**~**e**, except for **4d** and **4f**, showed the absorption for the 4′-methyl group as a symmetrical doublet indicating that the 1:1 mixtures of *syn*- and *anti*-geometric isomers at

Scheme 2.



the imino group had been isolated. However, **4d** and **4f** showed the absorption as a singlet, thus their isomeric constitution remains uncertain.

Table 2. 7-(3-Substituted-iminobutyrylamino)cephalosporanic acids (**4**)

Compound	R	M	Yield %	Formula[a]	IR (β-lactam) KBr, cm^{-1}
4a	HO	H	78	$C_{14}H_{17}N_3O_7S$	1770
b	CH_3O	H	60	$C_{15}H_{19}N_3O_7S$[b]	1770
c	$CH_2=CHCH_2O$	H	87	$C_{17}H_{21}N_3O_7S$[c]	1765
d	H_2NCONH	H	87	$C_{15}H_{19}N_5O_7S$	1763
e		Na	84	$C_{18}H_{19}N_6O_6SNa \cdot 2H_2O$[d]	1755
f		H	61	$C_{21}H_{24}N_4O_5S_2$	1770

[a]: See, footnote a of Table 1.
[b]: N, calcd., 10.91; found, 10.44.
[c]: H, calcd., 5.15; found, 4.62.
[d]: N, calcd., 16.59; found, 16.07.

3. Modification at the 4′-Methyl Position

Acylation of 7-aminocephalosporanic acid (7-ACA) with 4-halogeno-3-oxobutyryl halogenides[3,4,5] gave 7-(4-halogeno-3-oxobutyrylamino)cephalosporanic acid (**5**). Treatment of **5** with thiols in the presence of sodium hydrogen carbonate in aqueous tetrahydrofuran (THF) at room temperature gave 4′-thiolated compounds (**6a**～**l**). They were also obtained by one-pot reaction from 7-ACA without isolation of **5**.

6a and **6b** were subjected to further modification at the 3-acetoxymethyl side chain, which was effected by the nucleophilic displacement of the acetoxy group with heterocyclic thiols by the conventional method[6] to afford **7a**～**f**.

The cephalosporins thus prepared were characterized and tested as free acids or as their sodium salts (Tables 1～4).

Scheme 3.

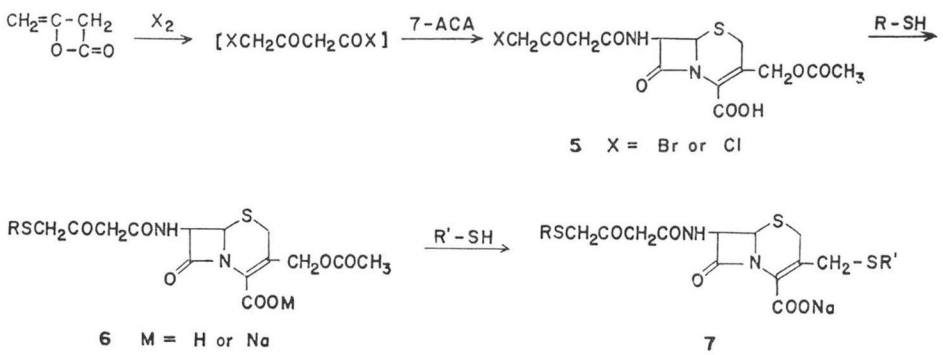
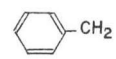
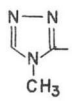
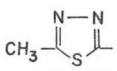


Table 3. 7-(4-Substituted-thio-3-oxobutyrylamino)cephalosporanic acids (6)

Compound	R	M	Yield[a] %	Formula[c]	IR (β-lactam) KBr, cm^{-1}
6a	CH_3	Na	10[b]	$C_{15}H_{17}N_2O_7S_2Na \cdot H_2O$	1765
b	C_2H_5	H	60	$C_{16}H_{20}N_2O_7S_2$	1781
c	$Me_2NCH_2CH_2$	Na	45	$C_{18}H_{24}N_3O_7S_2Na$[d]	1774
d	$HOCH_2CH_2$	Na	55	$C_{16}H_{19}N_2O_8S_2Na \cdot H_2O$	1768
e	$HSCH_2CH_2$	H	49	$C_{16}H_{20}N_2O_7S_3 \cdot 0.5AcOEt$[e]	1785
f	H_2NCOCH_2	H	22	$C_{16}H_{19}N_3O_8S_2$[f]	1780
g	$NaOCOCH_2$	Na	47	$C_{16}H_{18}N_2O_9S_2Na_2 \cdot 2.5H_2O$	1766
h	$MeOCOCH_2$	Na	74	$C_{17}H_{19}N_2O_9S_2Na \cdot 0.5H_2O$	1773
i		Na	21	$C_{18}H_{23}N_4O_7S_2Na \cdot H_2O$	1774
j		Na	36	$C_{21}H_{21}N_2O_7S_2Na \cdot 1.5H_2O$	1764
k		Na	56	$C_{17}H_{18}N_5O_7S_2Na \cdot 1.5H_2O$	1765
l		Na	10[b]	$C_{17}H_{17}N_4O_7S_3Na \cdot 2.5H_2O$	1767

[a]: Yields are calculated from **5**.
[b]: Compound was prepared from 7-ACA by one-pot reaction.
[c]: See, footnote a of Table 1.
[d]: H, calcd., 5.02; found, 5.66.
[e]: H, calcd., 4.91; found, 4.40.
[f]: N, calcd., 9.43; found, 8.67.

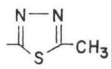
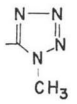
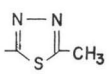
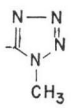
Antimicrobial Activity

The *in vitro* antimicrobial activities of the cephalosporanic acid obtained by modifications at the 2′-methylene or the 3′-oxo group of 3′-oxobutyryl side chain of **1a** are shown in Table 5. Data for the cephalosporanic acids modified at the 4′-methyl group and for compounds with the 3-heterocyclicthiomethyl groups are listed in Table 6.

It can be seen that most of the modifications at the 2′-(**3a**～**e**) and 3′-position (**4a**～**f**) of the acyl part of **1a** resulted in a decrease of either or both of the activities against Gram-positive and Gram-negative bacteria, with the exception of **4d** which exhibited a slight increase in the activity against Gram-positive bacteria.

The modifications at the 4′-methyl group produced various effects on the activity. Incorporation of carbamoylmethylthio- (**6f**), carboxymethylthio- (**6g**) or methoxycarbonylmethylthio- (**6h**) groups produced enhancement of the activity against Gram-negative bacteria, but the latter two modifications brought about reduction of the activity against Gram-positive bacteria. Introduction of a thio-group bearing a bulky (**6i, k, l**) or a hydrophobic (**6e, j**) group resulted in reduction of the activity against Gram-negative bacteria. Introduction of a methylthio- (**6a**) or an ethylthio- (**6b**) group enhanced the activities against both Gram-positive and Gram-negative bacteria.

Table 4. Sodium 3-[(heterocyclic-thio)methyl]-7-(4-substituted-thio-3-oxobutyrylamino)ceph-3-em-4-carboxylates (**7**)

Compound	R	R′	Yield[a] %	Formula[b]	IR (β-lactam) KBr, cm^{-1}
7a	CH_3		20	$C_{15}H_{15}N_4O_5S_4Na \cdot 2H_2O$	1763
b	CH_3		13	$C_{16}H_{17}N_4O_5S_4Na \cdot 1.5H_2O$	1767
c	CH_3		10	$C_{15}H_{17}N_6O_5S_3Na \cdot 2H_2O$[c]	1766
d	CH_3CH_2		15	$C_{16}H_{17}N_4O_5S_4Na \cdot 1.5H_2O$	1762
e	CH_3CH_2		8	$C_{17}H_{19}N_4O_5S_4Na \cdot 2H_2O$[d]	1766
f	CH_3CH_2		11	$C_{16}H_{19}N_6O_5S_3Na \cdot 1.5H_2O$	1763

[a]: Yields are calculated from **6**.
[b]: See, footnote a of Table 1.
[c]: N, calcd., 16.27; found, 15.25.
[d]: H, calcd., 4.24; found, 3.63.

Table 5. *In vitro* activity of 7-(2-substituted-thio-3-oxobutyrylamino)cephalosporanic acids (**3**) and 7-(3-substituted-iminobutyrylamino)cephalosporanic acids (**4**)

Compound	MIC (mcg/ml)[a]				
	S. aureus 209P	*S. aureus* 1840	*E. coli* NIHJ JC-2	*K. pneumoniae* DT	*P. vulgaris* IFO-3988
3a	<0.78	6.25	100	25	50
b	1.56	1.56	100	50	>100
c	3.13	3.13	100	100	100
d	3.13	12.5	100	50	>100
e	3.13	12.5	>100	50	>100
4a	3.13	3.13	50	25	>100
b	<0.78	1.56	100	50	>100
c	<0.78	<0.78	>100	>100	>100
d	1.56	1.56	50	25	>100
e	3.13	6.25	100	50	>100
f	3.13	3.13	50	50	100
1a	3.13	3.13	50	25	>100

a: The MIC's were determined by the two-fold serial dilution method on Tripticase soy agar (BBL).

Because of the activities of **6a** and **6b**, they were selected for further modification at the 3-substituent. Displacement of the 3-acetoxy group of **6a** and **6b** with heterocyclic thiols such as 1, 3, 4-thiadiazolethiol (**7a** and **7d**), 5-methyl-1, 3, 4-thiadiazolethiol (**7b** and **7e**) or 1-methyltetrazolethiol (**7c** and **7f**) enhanced the activities against both Gram-positive and Gram-negative bacteria. In this series, **7c** was the most

Table 6. *In vitro* activity of 7-(4-substituted-thio-3-oxobutyrylamino)cephalosporins (**6** and **7**)

Compound	MIC (mcg/ml)*				
	S. aureus 209P	*S. aureus* 1840	*E. coli* NIHJ JC-2	*K. pneumoniae* DT	*P. vulgaris* IFO-3988
6a	<0.78	1.56	25	6.25	100
b	<0.78	<0.78	25	12.5	100
c	6.25	25	>100	100	>100
d	3.13	6.25	50	25	100
e	3.13	12.5	>100	100	100
f	1.56	3.13	25	12.5	100
g	25	25	50	12.5	100
h	1.56	6.25	50	25	50
i	3.13	12.5	>100	100	>100
j	<0.78	<0.78	>100	>100	>100
k	6.25	25	100	50	>100
l	3.13	12.5	100	50	>100
7a	<0.78	<0.78	12.5	6.25	25
b	<0.78	<0.78	6.25	6.25	25
c	<0.78	1.56	1.56	1.56	50
d	<0.78	1.56	3.13	3.13	25
e	<0.78	1.56	25	25	50
f	<0.78	1.56	6.25	6.25	25
1b	<0.78	<0.78	6.25	3.13	100

* See, footnote a of Table 5.

Table 7. *In vivo* activity of sodium 3-[(heterocyclic-thio)methyl]-7-(4-substituted-thio-3-oxobutyrylamino)-ceph-3-em-4-carboxylates (**7a, c, e**) and 3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-7-(3-oxobutyryl-amino)ceph-3-em-4-carboxylic acid (**1b**)

Organism	Route	ED_{50}[a] (mg/kg)			
		7a	**7c**	**7e**	**1b**
E. coli O–111	sc	>20	6.21	>20	5.97

a: The ED_{50} values are expressed as the dose of compound which afforded protection to 50% of the mice (male mice; Slc-ICR strain) challenged intraperitoneally with 10^5 CFU/animal of test organism. A single dose (5 mice per one dose) was administered subcutaneously immediately after challenge.

active compound and showed activity against Gram-negative bacteria superior to the parent 7-(3-oxobutyrylamino) analogue (**1b**).

The protective effects (ED_{50} values) of some of the cephalosporins in mice infected with *E. coli* O-111 are shown in Table 7. It is noteworthy that no better protective effect was found with **7c** as compared with **1b**, although **7c** was superior to **1b** in *in vitro* activity against the Gram-negative bacteria tested. The other compounds (**7a** and **7c**) did not show useful protective effects.

Experimental

Infrared (IR) spectra were measured in a KBr disk using a Hitachi EPI-S_2 infrared spectrophotometer. NMR spectra were determined on a Varian HA-100 spectrometer, using tetramethylsilane as a standard. All melting points are uncorrected.

7-(2-Substituted-3-oxobutyrylamino)cephalosporanic acids (3)

To a stirred solution of **1a** (0.5 mmol) in THF (5 ml) was added NCS (0.072 g, 0.54 mmol) or NBS (0.096 g, 0.54 mmol) and the mixture was stirred for 1 hour at room temperature. To the mixture was added an appropriate thiol (0.5 mmol) and $NaHCO_3$ (0.084 g, 1.0 mmol). After 0.5-hour stirring, the reaction mixture was washed with AcOEt. The aqueous layer was acidified to pH 2 with H_3PO_4 and extracted twice with AcOEt. The combined extracts were washed with water, dried and evaporated *in vacuo*. The residue was triturated with *n*-hexane to yield a free acid of **3** (M=H) as powder. The sodium salt **3** (M=Na) was obtained by adding a solution of 2 N sodium 2-ethylhexanoate in isopropyl alcohol to a free acid of **3** (M=H) dissolved in a small portion of AcOEt until no further precipitate was formed. Filtration of the precipitate gave a sodium salt of **3** (M=Na). NMR data of each compound are as follows:

3a: (D_2O) δ 2.19 (s, $OCOCH_3$), 2.25 & 2.27 (each s, CH_3CO), 2.48 & 2.50 (each s, CH_3S), 3.47 & 3.77 (ABq, J=18 Hz, C_2–H_2), 4.78 & 5.00 (ABq, J=13 Hz, C_3–CH_2), 5.24 (d, J=5 Hz, C_6–H), 5.75 (d, J=5 Hz, C_7–H).

3b: (D_2O) δ 2.19 (s, $OCOCH_3$), 2.50 & 2.52 (each s, CH_3CO), 2.92 & 2.93 (each t, J=6 Hz, CH_2S), 3.47 & 3.78 (ABq, J=18 Hz, C_2–H_2), 3.85 (t, J=6 Hz, $HOCH_2$), 4.79 & 4.99 (ABq, J=13 Hz, C_3–CH_2), 5.24 (d, J=5 Hz, C_6–H), 5.75 (d, J=5 Hz, C_7–H).

3c: (D_2O) δ 2.20 (s, $OCOCH_3$), 2.40 & 2.51 (each s, CH_3CO), 3.49 & 3.79 (ABq, J=18 Hz, C_2–H_2), 3.50 & 3.64 (each s, $OCOCH_2S$), 5.24 (d, J=5 Hz, C_6–H), 5.74 (d, J=5 Hz, C_7–H).

3d: (DMSO-d_6-D_2O-$NaHCO_3$) δ 2.19 (s, $OCOCH_3$), 2.22 (s, CH_3CO), 3.45 & 3.75 (ABq, J=18Hz, C_2–H_2), 4.82 & 5.02 (ABq, J=13 Hz, C_3–CH_2), 5.16 (d, J=5 Hz, C_6–H), 5.86 (d, J=5 Hz, C_7–H), 7.25 & 8.30 (ABq, J=6 Hz, pyridine-H).

3e: (D_2O-$NaHCO_3$) δ 2.21 (s, $OCOCH_3$), 2.30 & 2.40 (each s, CH_3CO), 3.47 & 3.77 (ABq, J=18 Hz, C_2–H_2), 5.20 & 5.22 (each d, J=5 Hz, C_6–H), 5.76 & 5.88 (each d, J=5 Hz, C_7–H), 7.5 & 8.3 (m, pyridine-H).

7-(3-Substituted-iminobutyrylamino)cephalosporanic acids (4)

The preparation of 7-(3-hydroxyiminobutyrylamino)cephalosporanic acid (**4a**) is described as a representative example. To a stirred solution of **1a** (0.356 g) and $NaHCO_3$ (0.168 g) in water (10 ml), $NH_2OH \cdot HCl$ (0.076 g) was added. After stirring for 0.5 hour, the reaction mixture was layered with AcOEt and acidified to pH 2 with 2 N HCl. After vigorous shaking, the organic layer was separated, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was triturated with Et_2O to give **4a** (0.29 g) as a powder. **4b**~**f** were prepared by the same method. **4e** (M=Na) was obtained with the subsequent transformation of the acid into the sodium salt and chromatographic separation on an Amberlite XAD-2 column eluting with water. NMR data of each compound are as follows:

4a: (DMSO-d_6) δ 1.75 & 1.80 (each s, CH_3), 2.00 (s, $OCOCH_3$), 3.06 & 3.28 (each s, CH_2CO), 3.39 & 3.63 (ABq, J=18 Hz, C_2–H_2), 4.67 & 4.98 (ABq, J=13 Hz, C_3–CH_2), 5.04 (d, J=5 Hz, C_6–H), 5.64 (dd, J=5 & 8 Hz, C_7–H), 8.84 & 8.96 (each d, J=8 Hz, CONH).

4b: (DMSO-d_6) δ 1.76 & 1.80 (each s, CH_3), 2.00 (s, $OCOCH_3$), 3.08 & 3.24 (each s, CH_2CO), 3.39 & 3.63 (ABq, J=18 Hz, C_2–H_2), 3.68 & 3.70 (each s, CH_3O), 4.65 & 4.97 (ABq, J=13 Hz, C_3–CH_2), 5.04 (d, J=5 Hz, C_6–H), 5.62 (dd, J=5 & 8 Hz, C_7–H), 8.92 & 9.00 (each d, J=8 Hz, CONH).

4c: (DMSO-d_6) δ 1.81 & 1.82 (each s, CH_3), 2.00 (s, $OCOCH_3$), 3.10 & 3.30 (each s, CH_2CO), 3.40 & 3.64 (ABq, J=18 Hz, C_2–H_2), 4.45 (m, CH_2O), 4.67 & 4.99 (ABq, J=13 Hz, C_3–CH_2), 5.06 (d, J=5 Hz, C_6–H), 5.21 & 5.23 (each d, J=12 Hz, CH_2=), 5.63 (dd, J=5 & 8 Hz, C_7–H), 5.8 (m, =CH), 8.94 & 9.02 (each d, J=8 Hz, CONH).

4d: (DMSO-d_6) δ 1.80 (s, CH_3), 2.00 (s, $OCOCH_3$), 3.15 (s, CH_2CO), 3.41 & 3.64 (ABq, J=18 Hz, C_2–H_2), 4.67 & 4.99 (ABq, J=12 Hz, C_3–CH_2), 5.06 (d, J=5 Hz, C_6–H), 5.64 (dd, J=5 & 8 Hz, C_7–H), 6.16 (s, $CONH_2$), 8.94 (d, J=8 Hz, CONH), 9.01 (s, NHN=).

4e: (D_2O) δ 2.17 & 2.22 (each s, CH_3), 2.22 (s, $OCOCH_3$), 3.43 & 3.75 (ABq, J=18 Hz, C_2–H_2), 4.78 & 4.98 (ABq, J=12 Hz, C_3–CH_2), 5.21 (d, J=5 Hz, C_6–H), 5.76 (d, J=5 Hz, C_7–H), 7.22 (t, J=5 Hz, pyrimidine-H), 8.69 (d, J=5 Hz, pyrimidine-H).

4f: (DMSO-d_6) δ 1.79 (s, CH_3), 1.98 (s, $OCOCH_3$), 2.33 (s, CH_3–Ph), 3.07 (s, CH_2CO), 3.37 & 3.61 (ABq, J=17 Hz, C_2–H_2), 4.64 & 4.95 (ABq, J=12 Hz, C_3–CH_2), 5.00 (d, J=5 Hz, C_6–H), 5.56 (dd,

J=5 & 8 Hz, C_7–H), 7.29 & 7.67 (ABq, J=8 Hz, phenyl-H), 8.92 (d, J=8 Hz, CONH), 10.14 & 10.30 (each s, NHN=).

7-(4-Halogeno-3-oxobutyrylamino)cephalosporanic acids (**5**)

To a stirred solution of diketene (3.4 g, 40 mmol) in CH_2Cl_2 (10 ml) at −30°C, a solution of Br_2 (6.4 g, 40 mmol) in CH_2Cl_2 (10 ml) was added dropwise and stirring was continued for an additional 10 minutes. The solution of 3-oxobutyryl bromide thus formed was added to a stirred cold (−30°C) solution of 7-ACA (10.9 g) and Et_3N (8.1 g) in CH_2Cl_2 (100 ml). The mixture was allowed to warm to room temperature over a period of 1 hour and evaporated *in vacuo*. The residue was shaken vigorously with AcOEt (100 ml) and 10% H_3PO_4 (100 ml). The aqueous layer was saturated with NaCl and extracted twice with AcOEt. The combined extracts were washed with saturated NaCl and dried over $MgSO_4$ and evaporated *in vacuo*. The residue was layered with Et_2O and allowed to stand overnight. The crystals formed were collected to afford 7-(4-bromo-3-oxobutyrylamino)cephalosporanic acid (8.0 g, 46%). *Anal.* Calcd. for $C_{14}H_{15}BrN_2O_7S \cdot 0.25Et_2O$: C, 39.40; H, 3.75; N, 6.13. Found: C, 39.20; H, 3.63; N, 6.09. IR 1780 (β-lactam), 1735, 1650 cm^{-1}; NMR (DMSO-d_6) δ 2.01 (s, $OCOCH_3$), 3.54 (m, C_2–H_2), 3.62 (s, $COCH_2CO$), 4.37 (s, $BrCH_2$), 4.67 & 5.01 (ABq, J=14 Hz, C_3–CH_2), 5.08 (d, J=4 Hz, C_6–H), 5.66 (dd, J=4 & 8 Hz, C_7–H), 9.04 (d, J=8, CONH).

When a solution of Cl_2 in CCl_4 was used instead of the solution of Br_2 in CH_2Cl_2 in the above procedure, the crystals of 7-(4-chloro-3-oxobutyrylamino)cephalosporanic acid were obtained in a yield of 40%. mp 135~140°C (dec.). *Anal.* Calcd. for $C_{14}H_{15}ClN_2O_7S$: C, 43.03; H, 3.87: N, 7.14. Found: C, 43.01; H, 3.89; N, 7.18. IR 1790 (β-lactam) cm^{-1}; NMR (DMSO-d_6) δ 2.00 (s, $OCOCH_3$), 3.41 & 3.64 (ABq, J=18 Hz, C_2–H_2), 3.56 (s, $COCH_2CO$), 4.50 (s, $ClCH_2$), 4.67 & 5.00 (ABq, J=13 Hz, C_3–CH_2), 5.07 (d, J=4.5 Hz, C_6–H), 5.66 (dd, J=4.5 & 8 Hz, C_7–H), 9.04 (d, J=8 Hz, CONH).

7-(4-Substituted-thio-3-oxobutyrylamino)cephalosporanic acid (**6**)

(A) From 7-ACA without isolation of the intermediate (**5**)

4-Bromo-3-oxobutyryl bromide (4 mmol) was reacted with 7-ACA as mentioned above and to the reaction mixture pyridine (10 mmol) and methanethiol (10 mmol, 5 N THF solution) were added at 0°C. After stirring for 25 minutes at 0°C and for 40 minutes at room temperature, the reaction mixture was evaporated *in vacuo*. The residue was dissolved in 10% $NaHCO_3$ and shaken with AcOEt. The aqueous layer was acidified to pH 2 with H_3PO_4 and extracted twice with AcOEt. The combined extracts were washed with saturated NaCl, dried and evaporated *in vacuo*. To a solution of the residue dissolved in a small portion of AcOEt was added 1 ml of 2 N sodium 2-ethylhexanoate in isopropyl alcohol. The precipitate was rapidly collected and dissolved in water, which on purification by column chromatography on Amberlite XAD-2 and lyophilization of the fraction containing the desired product gave sodium 7-(4-methylthio-3-oxobutyrylamino)cephalosporanate (**6a**) (0.17 g). **6l** was prepared by the same method.

(B) From isolated **5**

A solution of **5** (1 mmol) in THF (10 ml) was added dropwise to a stirred solution of $NaHCO_3$ (2 mmol) and an appropriate thiol (1 mmol) in water (10 ml). After stirring for 1 hour, THF was evaporated *in vacuo*. The aqueous mixture was washed with AcOEt, acidified to pH 2 and then extracted twice with AcOEt. The combined organic layers were washed with saturated NaCl, dried and evaporated *in vacuo*. The residue was triturated with *n*-hexane. By this procedure a free acid of **6** (M=H) was obtained as a powder. When the requirement of further purification was indicated by TLC, IR or NMR, **6** was changed to sodium salt by using sodium 2-ethylhexanoate as described in Method A, and purified by column chromatography on Amberlite XAD-2. NMR data of each compound are as follows.

6a: (D_2O) δ 2.14 (s, CH_3S), 2.18 (s, $OCOCH_3$), 3.46 & 3.76 (ABq, J=18 Hz, C_2–H_2), 3.60 (s, SCH_2CO), 4.80 & 5.00 (ABq, J=12 Hz, C_3–CH_2), 5.23 (d, J=5 Hz, C_6–H), 5.77 (d, J=5 Hz, C_7–H).

6b: (DMSO-d_6) δ 1.15 (t, J=7 Hz, $C\underline{H_3}CH_2$), 2.01 (s, $OCOCH_3$), 2.46 (q, J=7 Hz, $CH_3C\underline{H_2}$), 3.43 & 3.65 (ABq, J=18 Hz, C_2–H_2), 3.44 (s, $COCH_2CO$), 3.57 (s, SCH_2CO), 4.67 & 4.99 (ABq, J=13 Hz, C_3–CH_2), 5.08 (d, J=4 Hz, C_6–H), 5.67 (dd, J=4 & 8 Hz, C_7–H), 9.01 (d, J=8 Hz, CONH).

6c: (D_2O) δ 2.15 (s, $OCOCH_3$), 2.94 (t, J=7 Hz, $CH_2C\underline{H_2}S$), 2.95 (s, $2\times CH_3$), 3.38 (t, J=7 Hz, $NC\underline{H_2}CH_2$), 3.41 & 3.73 (ABq, J=18 Hz, C_2–H_2), 4.75 & 4.95 (ABq, J=13 Hz, C_3–CH_2), 5.19 (d, J=

5 Hz, C_6–H), 5.72 (d, J=5 Hz, C_7–H).

6d: (D_2O) δ 2.18 (s, $OCOCH_3$), 2.77 (t, J=7 Hz, $CH_2\underline{CH_2}S$), 3.46 & 3.77 (ABq, J=18 Hz, C_2–H_2), 3.80 (t, J=7 Hz, $O\underline{CH_2}CH_2$), 4.79 & 4.99 (ABq, J=13 Hz, C_3–CH_2), 5.23 (d, J=5 Hz, C_6–H), 5.76 (d, J=5 Hz, C_7–H).

6e: (DMSO-d_6) δ 2.00 (s, $OCOCH_3$), 2.7~3.0 (m, CH_2CH_2), 2.8~3.2 (m, SCH_2CO & $COCH_2CO$), 3.40 & 3.63 (ABq, J=18 Hz, C_2–H_2), 4.66 & 4.98 (ABq, J=13 Hz, C_3–CH_2), 5.06 (d, J=4 Hz, C_6–H), 5.69 (dd, J=4 & 8 Hz, C_7–H), 8.87 (d, J=8 Hz, CONH).

6f: (DMSO-d_2) δ 2.00 (s, $OCOCH_3$), 3.06 (s, $COCH_2CO$), 3.41 & 3.64 (ABq, J=18 Hz, C_2–H_2), 3.56 (s, $COCH_2S$ & SCH_2CO), 4.68 & 4.99 (ABq, J=12 Hz, C_3–CH_2), 5.06 (d, J=5 Hz, C_6–H), 5.66 (dd, J=5 & 8 Hz, C_7–H), 6.93 & 7.36 (br. s, NH_2), 8.98 (d, J=8 Hz, CONH).

6g: (D_2O) δ 2.17 (s, $OCOCH_3$), 3.26 (s, $OCOCH_2S$), 3.45 & 3.75 (ABq, J=18 Hz, C_2–H_2), 4.76 & 4.97 (ABq, J=13 Hz, C_3–CH_2), 5.21 (d, J=5 Hz, C_6–H), 5.75 (d, J=5 Hz, C_7–H).

6h: (D_2O) δ 2.16 (s, $OCOCH_3$), 3.44 (s, $OCOCH_2S$), 3.45 & 3.75 (ABq, J=18 Hz, C_2–H_2), 3.77 (s, CH_3), 4.79 & 4.99 (ABq, J=13 Hz, C_3–CH_2), 5.20 (d, J=5 Hz, C_6–H), 5.74 (d, J=5 Hz, C_7–H).

6i: (D_2O) δ 2.16 (s, $OCOCH_3$), 3.35 (s, SCH_2CO), 3.38 (s, 3×CH_3), 3.43 & 3.74 (ABq, J=18 Hz, C_2–H_2), 4.76 & 4.96 (ABq, J=12 Hz, C_3–CH_2), 5.18 (d, J=5 Hz, C_6–H), 5.77 & 5.78 (each d, J=5 Hz, C_7–H).

6j: (D_2O) δ 2.14 (s, $OCOCH_3$), 3.39 (s, CH_2S), 3.5 (m, C_2–H_2), 3.65 (s, SCH_2CO), 4.8 (m, C_3–CH_2), 5.13 (d, J=4 Hz, C_6–H), 5.74 (d, J=4 Hz, C_7–H), 7.25 (s, Ph).

6k: (D_2O) δ 2.18 (s, $OCOCH_3$), 3.43 & 3.74 (ABq, J=18 Hz, C_2–H_2), 3.77 (s, triazole-CH_3), 4.77 & 4.98 (ABq, J=13 Hz, C_3–CH_2), 5.18 (d, J=4.5 Hz, C_6–H), 5.72 (d, J=4.5 Hz, C_7–H), 8.54 (s, triazole-H).

6l: (D_2O) δ 2.18 (s, $OCOCH_3$), 2.76 (s, thiadiazole-CH_3), 3.41 & 3.72 (ABq, J=18 Hz, C_2–H_2), 4.78 & 4.98 (ABq, J=12 Hz, C_3–CH_2), 5.18 (d, J=5 Hz, C_6–H), 5.74 (d, J=5 Hz, C_7–H).

Sodium 3-[(heterocyclic-thio)methyl]-7-(4-substituted-thio-3-oxobutyrylamino)ceph-3-em-4-carboxylate (**7**)

General procedure: A solution of **6** (1 mmol), $NaHCO_3$ (2 mmol) and heterocyclic thiol (1.5 mmol) in a phosphate buffer solution (40 ml, pH 6.4, 0.1 M) was stirred at 60~65°C for 7~8 hours. The solution was concentrated to about 20 ml *in vacuo* and chromatographed on Amberlite XAD-2 column (100~200 mesh, 3.4×30 cm) with water as eluent. The desired fraction were collected and lyophilized to yield **7** as powder. NMR data of each compound are as follows.

7a: (D_2O) δ 2.12 (s, CH_3S), 3.50 & 3.86 (ABq, J=18 Hz, C_2–H_2), 3.58 (s, SCH_2CO), 4.14 & 4.59 (ABq, J=13 Hz, C_3–CH_2), 5.16 (d, J=5 Hz, C_6–H), 5.71 (d, J=5 Hz, C_7–H), 9.47 (s, thiadiazole-H).

7b: (D_2O) δ 2.12 (s, CH_3S), 2.79 (s, thiadiazole-CH_3), 3.47 & 3.86 (ABq, J=18 Hz, C_2–H_2), 3.57 (s, SCH_2CO), 4.03 & 4.53 (ABq, J=14 Hz, C_3–CH_2), 5.16 (d, J=5 Hz, C_6–H), 5.70 (d, J=5 Hz, C_7–H).

7c: (D_2O) δ 2.10 (s, CH_3S), 3.51 & 3.85 (ABq, J=18 Hz, C_2–H_2), 3.57 (s, SCH_2CO), 4.08 (s, tetrazole-CH_3), 4.12 & 4.37 (ABq, J=14 Hz, C_3–CH_2), 5.17 (d, J=5 Hz, C_6–H), 5.69 (d, J=5 Hz, C_7–H).

7d: (D_2O) δ 1.26 (t. J=7 Hz, $C\underline{H_3}CH_2$), 2.58 (q, J=7 Hz, $CH_3C\underline{H_2}$), 3.50 & 3.86 (ABq, J=18 Hz, C_2–H_2), 3.64 (s, SCH_2CO), 4.15 & 4.59 (ABq, J=14 Hz, C_3–CH_2), 5.17 (d, J=5 Hz, C_6–H), 5.71 (d, J=5 Hz, C_7–H), 9.47 (s, thiadiazole-H).

7e: (D_2O) δ 1.29 (t, J=7 Hz, $C\underline{H_3}CH_2$), 2.60 (q, J=7 Hz, $CH_3C\underline{H_2}$), 2.82 (s, thiadiazole-CH_3), 3.49 & 3.89 (ABq, J=18 Hz, C_2–H_2), 3.66 (s, SCH_2CO), 4.05 & 4.57 (ABq, J=14 Hz, C_3–CH_2), 5.18 (d, J=5 Hz, C_6–H), 5.72 (d, J=5 Hz, C_7–H).

7f: (D_2O) δ 1.27 (t, J=7 Hz, $C\underline{H_3}CH_2$), 2.59 (q, J=7 Hz, $CH_3C\underline{H_2}$), 3.53 & 3.87 (ABq, J=18 Hz, C_2–H_2), 3.64 (s, SCH_2CO), 4.10 (s, tetrazole-CH_3), 4.12 & 4.38 (ABq, J=13 Hz, C_3–CH_2), 5.16 (d, J=5 Hz, C_6–H), 5.69 (d, J=5 Hz, C_7–H).

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