Semisynthetic β-Lactam Antibiotics. 6.[1] Sulfocephalosporins and Their Antipseudomonal Activities

Hiroaki Nomura,* Takeshi Fugono, Takenori Hitaka, Isao Minami, Toshiyuki Azuma, Shiro Morimoto, and Toru Masuda

Central Research Division, Takeda Chemical Industries, Ltd., Juso, Osaka 532, Japan. *Received May 14, 1974*

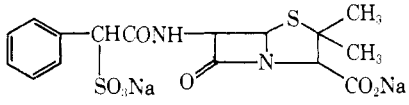
A number of derivatives of 7-(α-sulfophenylacetamido)cephalosporanic acid were synthesized and their antibacterial activities against several microorganisms have been determined *in vitro*. Among these compounds, 7-(D-α-sulfophenylacetamido)ceph-3-em-3-pyridiniummethyl-4-carboxylate (D-5a) and its 3-(4′-carbamoylpyridinium)methyl analog (D-5b) showed antipseudomonal activities approximately ten times more potent than that of D-α-sulfobenzylpenicillin (SBPC). Structure–activity relationships of these new cephalosporins are described.

An increase in infection caused by *Pseudomonas aeruginosa* throughout the world[2] makes urgent the discovery of a highly effective antipseudomonal medicament without toxicity. Gentamicin and polymixin have high activities against *Ps. aeruginosa* but are not free from toxic side effects.[3] Carbenicillin and sulbenicillin (I) have virtually no toxicity[4,5] but have relatively large minimal inhibitory concentrations (MIC) values for *Ps. aeruginosa*.[6-8]

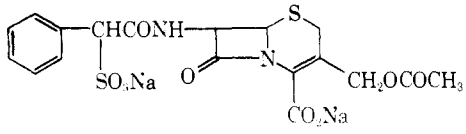
In previous articles,[7,9-11] we reported a new series of semisynthetic penicillins, a typical compound being I, which has a broad antibacterial spectrum including *Ps. aeruginosa* and has been in clinical use. Followed by extensive studies in the penicillin field, we directed our efforts to the synthesis of new cephalosporins bearing a 7-sulfoacyl side chain.

Our initial attempt at synthesis was focused on 7-(α-sulfophenylacetamido)cephalosporanic acid (4), a key compound, from which a number of cephalosporins having a modified side chain at the C_3 position could be derived by nucleophilic substitution.

Treatment of 7-aminocephalosporanic acid (3) with α-sulfophenylacetyl chloride (2) gave 4 as needles. By using optically active acid chlorides, D- and L-2, the corresponding diastereoisomers, D- and L-4, were individually prepared as in the case of 1.[7]

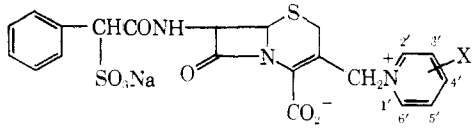


1



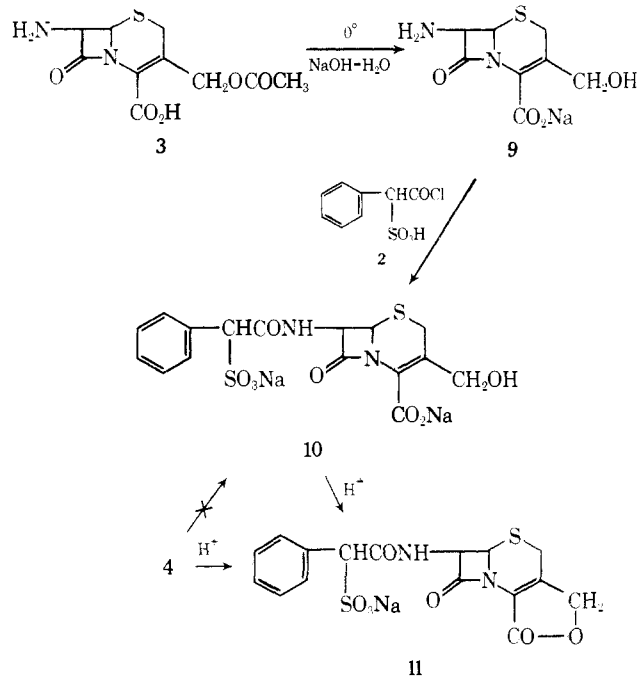
4

The first modified compound synthesized was the desacetyl derivative 10 which was obtained as a by-product in the acylation of 3. 7-Aminocephalosporadesic acid (9) was presumably an intermediate. In fact, treatment of 3 with



5a, X = -H
b, X = -$CONH_2$ (4′)
c, X = -CH=NOH (4′)
d, X = -$NHCOCH_3$ (4′)
e, X = -Br (3′)
f, X = -$(CH)_3$ (2′,4′,6′)

Scheme I



2 molar equiv of NaOH for 0.5 hr at 0° and successive chromatography gave 9 in high yields. On acylation, 9 yielded 10 quantitatively. Acid treatment of 4 led to lactonization to yield 11.

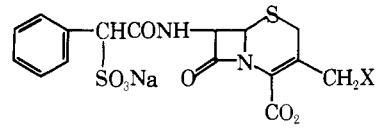
In contrast to 3, alkaline treatment of 4 resulted in the hydrolytic cleavage of the β-lactam ring before deacetylation. Scheme I summarizes these reactions.

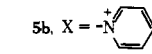
Antibacterial testing demonstrated that D-4 was moderately active against gram-positive and gram-negative organisms including *Ps. aeruginosa*. The desacetyl cephalosporin 10 gave only meager results, while the lactone 11 showed activity against *Staphylococcus aureus*. MIC data of these compounds are listed in Tables I and II.

Further modification of 4 was performed as follows. Treatment with pyridine in an aqueous solution gave a 3-pyridiniummethyl compound 5. Addition of a large quantity of inorganic neutral salts to the reaction mixture caused a high conversion of 4 to 5, as observed in the cephaloridine synthesis.[12]

α-Sulfocephalosporins in this series were obtained as a mixture of two diastereoisomers. Chromatography on a column of Amberlite XAD-2 was effective for separation of the diastereoisomers into the respective pure isomers. The L isomer of 4, 5a, and 5b had a shorter retention time than those of the corresponding D isomers as in the case of 1.[7] For resolving the diastereoisomeric mixture, fractional crystallization was also useful. In all cases listed in Table I, the needles which were crystallized from EtOH-H_2O

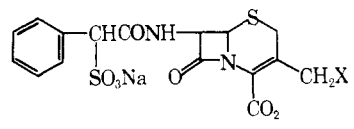
Table I. Antibacterial Activities of D- and L-Cephalosporins

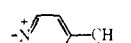
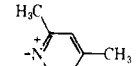
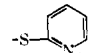
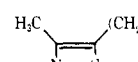
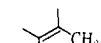


Organism	4, X = -OAc		5a, X = 		5b, X = 		SBPC, D-1
	D	L	D	L	D	L	
S. aureus 209P	3.12[a]	50	1.56	3.12	3.12	12.5	0.78
S. aureus Pc-R[b]	3.12	100	3.12	12.5	3.12	25	12.5
B. subtilis PCI 219	6.25	50	6.25	50	6.25	50	0.10
Sa. lutea PCI 1001	12.5	>100	6.25	100	12.5	>100	0.20
E. coli IFO 3044	25	>100	100	>100	25	>100	12.5
P. vulgaris IFO 3045	12.5	>100	50	>100	50	>100	1.56
P. morganii IFO 3168	100	>100	>100	>100	100	>100	3.13
P. mirabilis IFO 12255	50	>100	50	>100	50	>100	1.56
Ps. aeruginosa IFO 3448	25	>100	1.56	25	1.56	25	12.5
Ps. aeruginosa NCTC 10490	3.12	100	0.39	12.5	0.39	25	3.12

[a]Minimal inhibitory concentration (MIC) in μg/ml as determined by the agar dilution method. [b]Penicillin G resistant strain.

Table II. Antibacterial Activities of 7-(D-α-Sulfophenylacetamido)cephalosporanic Acid and Its Derivatives



Organism	5c, X = 	5d, X =	5e, X = 	5f, X = 	7a, X = 	7b, X =	8, X = 	10, X = -OH	11, X = 	SBPC, 1
Ps. aeruginosa IFO 3080	6.25[a]	25	100	100	100	>100	6.25	>100	>100	12.5
Ps. aeruginosa 10490	1.56	6.25	25	25	25	50	1.56	50	>100	3.12
S. aureus 209P	3.12	3.12	3.12	12.5	3.12	1.56	1.56	25	3.12	0.39
S. aureus No. 87[b]	6.25	6.25	12.5	12.5	6.25	1.56	3.12	50	3.12	6.25

[a]Minimal inhibitory concentration (MIC) in μg/ml as determined by the agar dilution method. [b]Penicillin G resistant strain.

were the D isomer and the mother liquor contained the L isomer as a main component.

On nmr spectra, the difference in chemical shifts between each pair of the isomers was observed to be analogous to the relationship between D- and L-**1**.[7] Signals due to protons bound to the cephem ring system in D-**4**, **-5a**, and **-5b** were observed in a slightly lower field than those of the respective L isomers. The optical purity of a partially epimerized sample of **4** or **5** was estimated by measuring the ratio of signal intensities of the corresponding protons of the isomers.

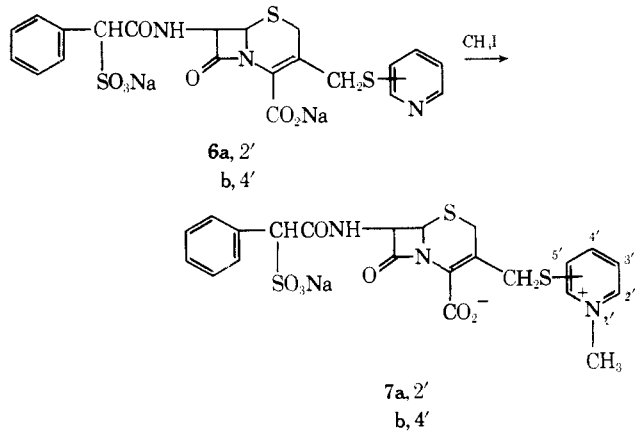
The *in vitro* antibacterial activities of these cephalosporins are shown in Table I. The MIC values of β-lactam antibiotics are affected by the stereochemistry of the side-chain acyl moiety.[13,14] In fact, activities of the D isomers were much more potent than those of the corresponding L isomers. The most striking characteristic was the fact that some of the α-sulfocephalosporins synthesized showed remarkable activity against *Ps. aeruginosa* and far less activities against other gram-negative bacteria, exemplified by **5a-d**. The antipseudomonal activity of D-**5a** was about ten times more active than that of D-**1**.

An extended test against 89 clinical isolates of this species showed that D-**5a** inhibited more than 93.3% of the strains at a concentration of 50 μg/ml. Based on these findings, D-**5a** or the analog, D-**5b**, appears to be one of the most potent antipseudomonal compounds ever known among the β-lactam antibiotics.

Aiming to improve the inhibitory spectrum against gram-negative bacteria by introduction of a variety of substituents onto the pyridinium ring of **5a**, we synthesized a number of substituted pyridinium compounds, **5b-f**, and a thiazolium derivative, **8** (Table II). Introduction of a substituent at the ortho and meta positions of the pyridine ring of **5a** resulted in decreased activity. However, introduction at the para position did not significantly alter the inhibitory potency.

Another modification of **4** was made by replacing the acetoxyl group with *o*- and *p*-mercaptopyridine, followed by N-methylation with methyl iodide (Scheme II). The

Scheme II



resulting pyridiniumthio derivatives **7a** and **7b**, however, showed much less antipseudomonal activities compared to **5a**, suggesting that the potency decreased with the increase in interatomic distance between the 3-methylene carbon atom and the positive charge of the pyridinium ring in the 3 side chain.

In the present series, the potent antipseudomonal activity appears to depend on the existence of both an α-sulfo group in the 7-phenylacetyl side chain and a positive charge of the nitrogen directly attached to the 3-methylene carbon.

Experimental Section

7-(DL-α-Sulfophenylacetamido)cephalosporanic Acid (DL-4). DL-α-Sulfophenylacetyl chloride (monoetherate, 710 mg, 2.3 mmol) in ether was added dropwise with stirring at 0–5° to a solution of 7-aminocephalosporanic acid (7-ACA, 545 mg, 2.0 mmol), NaOH (80 mg, 2.0 mmol), and $NaHCO_3$ (395 mg, 4.7 mmol) in water (12 ml). The mixture was allowed to react at 0–5° for 20 min. After removal of the organic layer, the aqueous solution was adjusted to pH 6.0 and then lyophilized. Crystallization from water–pyridine–Me_2CO gave DL-4 as colorless needles: 720 mg; mp 165° dec; $[\alpha]^{23}D$ + 78° (*c* 1.01, H_2O); ir (KBr) 1755 (β-lactam), 1680 (-CONH-), 1610 ($-CO_2^-$), 1220 ($-SO_2-$), 1039 cm^{-1} ($-SO_3^-$); uv max (H_2O) 261 mμ (ε 8340). *Anal.* ($C_{18}H_{16}O_9N_2S_2Na_2 \cdot 2H_2O$) C, H, N, S.

7-(D-α-Sulfophenylacetamido)cephalosporanic Acid (D-4). 7-ACA (1.36 g, 5.0 mmol) was acylated with D-(−)-α-sulfophenylacetyl chloride (1.54 g, 5.0 mmol) under the same conditions described above. The aqueous layer of the reaction mixture was adjusted to pH 6 and chromatographed on a column of Amberite XAD-2 (100–200 mesh, 5.0 × 90 cm) using water as eluent. Under monitoring by uv absorption at 254.5 mμ, 10-ml fractions were collected. Fractions containing α-sulfophenylacetic acid, 10 and 4, were eluted successively. A combination of the desired D-4 fractions was evaporated to 0.1 vol *in vacuo*. Lyophilization of the concentrate and a following crystallization from EtOH–H_2O gave D-4 as needles: 350 mg; mp >200°; $[\alpha]D$ 97.7° (*c* 1.0, H_2O); nmr (D_2O) 3.26, 3.56 (each 1 H, two sets of doublets, J = 18 Hz, C_2-H), 4.79, 4.82 (each 1 H, C_3-H), 5.08 (1 H, d, J = 4.5 Hz, C_6-H), 5.72 (1 H, d, J = 4.5 Hz, C_7-H), 5.10 (1 H, s, PhCH–), 7.51 (5 H, m, PhH). *Anal.* ($C_{18}H_{16}O_9N_2S_2Na_2 \cdot 2H_2O$) C, H, N, S.

7-(L-α-Sulfophenylacetamido)cephalosporanic Acid (L-4). The L isomer was synthesized by treating 3 with L-α-sulfophenylacetyl chloride and working up as described above. Crystallization from H_2O–pyridine–Me_2CO gave L-4 as needles: mp >200°; $[\alpha]^{23}D$ +67.1° (*c* 1.01, H_2O); nmr (D_2O) 3.44, 3.84 (each 1 H, two sets of doublets, J = 18 Hz, C_2-H), 4.80, 4.86 (each 1 H, C_3-H), 5.12 (1 H, d, J = 5.0 Hz, C_6-H), 5.68 (1 H, d, J = 5.0 Hz, C_7-H), 2.13 (3 H, s, $-COCH_3$), 5.11 (1 H, s, PhCH–), 7.50 (5 H, m, PhH). *Anal.* ($C_{18}H_{16}O_9N_2S_2Na_2 \cdot 2H_2O$) C, H, N, S.

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-pyridiniummethyl-4-carboxylate (D-5a). A mixture of D-4 (257 mg, 0.5 mmol), KSCN (1.2 g, 12.9 mmol), and pyridine (60 mg, 0.76 mmol) in 0.3 ml of H_2O was kept at 50° for 25 hr. The reaction mixture was chromatographed on Amberite XAD-2 (100–200 mesh, 3 × 80 cm) with water as eluent. Three peaks appeared on the elution curve recorded by uv absorption at 254.5 mμ. The fraction corresponding to the first peak contained KSCN, the second fraction contained impurities derived from cephalosporin compound, and the third, the main peak, contained the desired cephalosporin. The third fraction was evaporated *in vacuo* and lyophilized to give a slightly yellowish powder: yield 0.17 g. Crystallization from MeOH gave D-5a as colorless needles: mp >200°; $[\alpha]^{23}D$ +44.1° (*c* 1.00, H_2O); uv max (H_2O) 257 mμ (ε 12,040); ir (KBr) 1760 (β-lactam), 1665 (-CONH-), 1610 ($-CO_2^-$), 1205 ($-SO_2-$), 1030 cm^{-1} ($-SO_3^-$); nmr (D_2O) 2.97, 3.35 (each 1 H, two sets of doublets, J = 18 Hz, C_2-H), 5.27, 5.40 (each 1 H, C_3-H), 5.07 (1 H, d, J = 5.2 Hz, C_6-H), 5.71 (1 H, d, J = 5.2 Hz, C_7-H), 5.10 (1 H, s, PhCH–), 7.47 (5 H, m, PhH), 8.04, 8.55, 8.90 (5 H, pyridinium). *Anal.* ($C_{21}H_{18}O_7N_3S_2Na \cdot 2.5H_2O$) C, H, N, S.

The dicyclohexylamine salt was crystallized from MeOH–dioxane as colorless needles: mp 163° (turn brown), 182° dec. *Anal.* ($C_{33}H_{41}O_7N_4S_2 \cdot 1.5H_2O$) C, H, N, S.

7-(L-α-Sulfophenylacetamido)ceph-3-em-3-pyridiniummethyl-4-carboxylate (L-5a). By using L-4 in place of D-4 in the above-mentioned reaction, L-5a was obtained. No racemization at the asymmetric carbon of the 7-acyl side chain occurred during the reaction. Chromatography on an XAD-2 column and the subsequent lyophilization yielded pure L epimer: $[\alpha]^{23}D$ +9.8° (*c* 1.00, H_2O); nmr (D_2O) 3.21, 3.51 (each 1 H, two sets of doublets, J = 18 Hz, C_2-H), 5.38, 5.47 (each 1 H, C_3-H), 5.20 (1 H, d, J = 4.8 Hz, C_6-H), 5.72 (1 H, d, J = 4.8 Hz, C_7-H), 5.14 (1 H, s, PhCH–), 7.47 (5 H, m, PhH), 8.09, 8.54, 8.91 (5 H, pyridinium). *Anal.* ($C_{21}H_{18}O_7N_3S_2Na \cdot 3H_2O$) C, H, N.

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-(4′-carbamoylpyridinium)methyl-4-carboxylate (D-5b). The reaction of D-4 (550 mg, 1.0 mmol) with isonicotinamide (180 mg, 1.5 mmol) in water (1 ml) containing excess KSCN was carried out as described above. Chromatographic isolation and recrystallization from EtOH–H_2O gave D-5b as colorless needles: $[\alpha]^{23}D$ +16.5° (*c* 1.08, H_2O); mp 175° dec; uv max (H_2O) 263 mμ (ε 14,600); ir (KBr) 1765, 1692 (-CONH-), 1645 (-CONH-), 1615 ($-CO_2^-$), 1029 cm^{-1} ($-SO_3^-$); nmr (D_2O) 2.99, 3.56 (each 1 H, two sets of doublet, J = 18 Hz, C_2-H), 5.40, 5.51 (each 1 H, C_3-H), 5.13 (1 H, d, J = 4.8 Hz, C_6-H), 5.73 (1 H, d, J = 4.8 Hz, C_7-H), 5.10 (1 H, s, PhCH–), 7.40 (5 H, m, PhH), 8.31, 9.07 (each 2 H, two sets of doublets, J = 6.6 Hz, pyridinium H). *Anal.* ($C_{22}H_{19}O_8N_4S_2Na \cdot 2H_2O$) C, H, N, S.

7-(L-α-Sulfophenylacetamido)ceph-3-em-3-(4′-carbamoylpyridinium)methyl-4-carboxylate (L-5b). L-5b was synthesized as a colorless amorphous powder in a similar way as described above by using L-4 as the starting compound: $[\alpha]^{23}D$ −16.8° (*c* 1.01, H_2O); nmr (D_2O) 3.15, 3.68 (each 1 H, two sets of doublets, J = 18 Hz, C_2-H), 5.45, 5.56 (each 1 H, C_3-H), 5.22 (1 H, d, J = 4.8 Hz, C_6-H), 5.74 (1 H, d, J = 4.8 Hz, C_7-H), 5.08 (1 H, s, PhCH–), 7.47 (5 H, m, PhH), 8.32, 9.11 (each 2 H, two sets of doublets, J = 6.5 Hz, pyridinium H). *Anal.* ($C_{22}H_{19}O_8N_4S_2Na \cdot 2.5H_2O$) C, H, N.

7-(α-Sulfophenylacetamido)ceph-3-em-3-substituted-pyridiniummethyl-4-carboxylate (5c–f). A solution containing 1.0 mmol of D-4, 25 mmol of KSCN, and 1.5 mmol of a substituted pyridine as listed in Table II in 0.7 ml of water was kept at 50° for 18 hr. The reaction mixture was purified by chromatography in a manner similar to that described in the previous section. Structure and purity of the products were confirmed by spectral data.

5c [3-(4′-hydroxyiminomethylpyridinium)-]: ir (KBr) 1764 (β-lactam), 1670 (-CONH-), 1613 ($-CO_2^-$), 1280, 1210 ($-SO_2-$), 1036 cm^{-1} ($-SO_3^-$); nmr (D_2O) 3.2, 3.5 (each 1 H, two sets of doublets, C_2-H), 5.06 (1 H, C_6-H), 5.08 (1 H, PhCH–), 5.35, 5.46 (each 1 H, $-CH_2-$), 5.68 (1 H, C_7-H), 7.45 (5 H, PhH), 8.10 (2 H, H_3·,† H_5·), 8.30 (1 H, -CH=NOH), 8.42 (2 H, H_2·, H_6·).

5d [3-(4′-acetaminopyridinium)-]: ir (KBr) 1762 (β-lactam), 1722 ($-NHCOCH_3$), 1673 (-CONH-), 1610 ($-CO_2^-$), 1513, 1227, 1200 ($-SO_2-$), 1035 cm^{-1} ($-SO_3^-$); nmr (D_2O) 2.30 (3 H, s, $-NHCOCH_3$), 3.1, 3.4 (each 1 H, two sets of doublet, C_2-H), 5.07 (1 H, C_6-H), 5.10 (1 H, PhCH–), 5.25 (2 H, $-CH_2-$), 5.4, 5.5 (1 H, C_7-H), 7.45 (5 H, PhH), 8.05 (2 H, H_3·, H_5·), 8.60 (2 H, H_2·, H_6·).

5e [3-(3′-bromopyridinium)-]: ir (KBr) 1778 sh, 1765 (β-lactam), 1725 sh, 1670 (-CONH-), 1615 ($-CO_2^-$), 1210 ($-SO_2-$), 1038 cm^{-1} ($-SO_3^-$); nmr (D_2O) 3.1, 3.4 (each 1 H, two sets of doublet, C_2-H), 5.17 (1 H, C_6-H), 5.26 (1 H, PhCH–), 5.4, 5.5 (each 1 H, $-CH_2-$), 5.72 (1 H, C_7-H), 7.6 (5 H, PhH), 8.0 (1 H, H_5·), 8.7–9.1 (2 H, H_4·, H_6·), 9.33 (1 H, H_2·).

5f [3-(2′,4′,6′-trimethylpyridinium)-]: ir (KBr) 1754 (β-lactam), 1670 (-CONH-), 1600 ($-CO_2^-$), 1210 ($-SO_2-$), 1037 cm^{-1}

†In all spectra H_2·, H_3·, . . . , and H_6· refer to the protons attached to the 2′, 3′, . . . , and 6′ positions of the pyridinium ring, respectively.

($-SO_3^-$); nmr (D_2O) 2.57 (3 H, s, 4′-CH_3-), 2.80 (6 H, s, 2′,6′-CH_3-), 3.2, 3.5 (each 1 H, two sets of doublets, C_2-H), 5.08 (1 H, C_6-H), 5.09 (1 H, PhCH-), 5.35, 5.4 (each 1 H, $-CH_2$-), 5.64 (1 H, C_7-H), 7.5 (5 H, PhH and 2 H, $H_{3'}$, $H_{5'}$).

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-(2′-pyridylthio)methyl-4-carboxylate (6a). A solution containing 514 mg (1.0 mmol) of D-4, 100 mg of $NaHCO_3$, and 133 mg (1.2 mmol) of 2-mercaptopyridine in water (1.1 ml) was kept at 60° for 4 hr. The reaction mixture was purified in a similar manner described above to give **6a**: 250 mg; ir (KBr) 1758 (β-lactam), 1680 (-CONH-), 1605 ($-CO_2^-$), 1210 ($-SO_3^-$), 1040 cm^{-1} ($-SO_3^-$); nmr (D_2O) 3.10, 3.55 (2 H, two sets of doublets, C_2-H), 3.78, 4.38 (2 H, two sets of doublets, $-CH_2$-), 4.91 (1 H, C_6-H), 5.08 (1 H, PhCH-), 5.62 (1 H, C_7-H), 7.1–7.7 (8 H), 8.35 (1 H).

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-(4′-pyridylthio)methyl-4-carboxylate (6b). **6b** was prepared from D-4 and 4-mercaptopyridine in 46% yield in the same way: ir (KBr) 1760 (β-lactam), 1677 (-CONH-), 1610 ($-CO_2^-$), 1210 ($-SO_3^-$), 1040 cm^{-1} ($-SO_3^-$); nmr (D_2O) 3.01, 3.45 (each 1 H, two sets of doublets, C_2-H), 3.70, 4.25 (each 1 H, two sets of doublets, $-CH_2$-), 4.88 (1 H, C_6-H), 5.04 (1 H, PhCH-), 5.60 (1 H, C_7-H), 7.3 (7 H), 8.28 (2 H).

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-(1′-methylpyridinium-2′-thio)methyl-4-carboxylate (7a). The mixture of 565 mg (1.0 mmol) of **6a** and 3.0 ml of CH_3I was allowed to stir about 60 hr at room temperature. After CH_3I was evaporated, the residue was purified in a manner similar to that described above to give **7a**: 200 mg; ir (KBr) 1761 (β-lactam), 1672 (-CONH-), 1611 ($-CO_2^-$), 1206 ($-SO_3^-$), 1034 cm^{-1} ($-SO_3^-$); nmr (D_2O) 3.24, 3.63 (each 1 H, two sets of doublets, C_2-H), 4.13 (3 H, NCH_3), 4.32 (2 H, $-CH_2$-), 5.04 (1 H, C_6-H), 5.10 (1 H, PhCH-), 5.65 (1 H, C_7-H), 7.5 (5 H, PhH), 7.5–8.7 (4 H).

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-(1′-methylpyridinium-4′-thio)methyl-4-carboxylate (7b). 7b was prepared from **6b** in 45% yield in the same way: ir (KBr) 1760 (β-lactam), 1677 (-CONH-), 1632 (C=N), 1607 ($-CO_2^-$), 1210 ($-SO_3^-$), 1036 cm^{-1} ($-SO_3^-$); nmr (D_2O) 3.21, 3.61 (each 1 H, two sets of doublets, C_2-H), 4.16 (5 H, NCH_3 and $-CH_2$-), 5.03 (1 H, C_6-H), 5.18 (1 H, PhCH-), 5.65 (1 H, C_7-H), 7.4 (5 H, PhH), 7.72 (2 H).

7-(D-α-Sulfophenylacetamido)ceph-3-em-3-[4′-methyl-5′-(β-hydroxyethyl)thiazolium]methyl-4-carboxylate (8). A solution of 500 mg (0.97 mmol) of D-4, 4 g of KSCN, and 415 mg (2.9 mmol) of 4-methyl-5-(β-hydroxyethyl)thiazole in water (2.5 ml) was allowed to react at 50° for 10 hr. The reaction mixture was worked up in the similar manner to afford 8 in 42.8% yield: ir (KBr) 1770 (β-lactam), 1680 (-CONH-), 1615 ($-CO_2^-$), 1210 ($-SO_2$-), 1040 cm^{-1} ($-SO_3^-$); nmr (D_2O) 2.43 (3 H, s, $-CH_3$), 3.1, 3.16 (each 1 H, $-CH_2$- at C_2), 3.1, 3.23 (2 H, two sets of doublets, $-CH_2CH_2OH$), 3.78, 3.87 (2 H, t, $-CH_2OH$), 5.08 (1 H, s, $-CHSO_3^-$), 5.15 (1 H, C_6-H), 5.20 (2 H, d, $-CH_2$- at C_3), 5.72 (1 H, d, J = 5.0 Hz, C_7-H), 7.43 (5 H, s, PhH), 9.12 (1 H, s, thiazolium ring $H_{2'}$).

7-Aminocephalosporadesic Acid (9). 7-ACA (0.6 g, 2.2 mmol) was dissolved in an aqueous solution (12 ml) containing 0.176 g of NaOH (4.4 mmol) at 0°. The solution was left standing for 30 min under ice cooling and then chromatographed on a column of XAD-2 (29 × 90 cm) using water as eluent. The fractions containing desacetyl compound were collected and lyophilized to yield a colorless powder: 0.4 g; nmr (D_2O) 3.55, 3.63 (each 1 H, two sets of doublets, J = 18 Hz), 4.30 (2 H, s, $-CH_2$- at C_3), 4.80 (1 H, d, J = 3.5 Hz, C_7-H), 5.10 (1 H, d, J = 3.5 Hz, C_6-H); ir (KBr) 3400 (OH), 2950 (CH), 1750 (β-lactam), 1605 cm^{-1} ($-CO_2^-$). *Anal.* ($C_8H_9O_4N_2SNa\cdot H_2O$) C, H, N.

7-(D-α-Sulfophenylacetamido)cephalosporadesic Acid (10). To a mixed solution of **9** (270 mg, 1.0 mmol) and $NaHCO_3$ (170 mg, 2.0 mmol) in water (5 ml), we added dropwise D-α-sulfophenylacetyl chloride (280 mg, 1.0 mmol) in ether at below 5° under stirring. The reaction mixture was applied to a column of XAD-2 (2.5 × 80 cm) and eluted with water. The fractions corresponding to the main peak were collected and lyophilized. Crystallization from EtOH-H_2O gave colorless needles: mp 185° dec; $[\alpha]^{23}D$ +103° (c 1.01, H_2O); ir (KBr) 1760 (β-lactam), 1675 (-CONH-), 1605 ($-CO_2^-$), 1210 ($-SO_2$-), 1042 cm^{-1} ($-SO_3^-$). *Anal.* ($C_{16}H_{14}O_8N_2S_2Na_2\cdot H_2O$) C, H, N.

7-(α-Sulfophenylacetamido)cephalosporadesic Acid Lactone (11). The cephalosporin **4** (500 mg) was dissolved in 6 ml of 1 *N* HCl. The solution was left standing for 24 hr at room temperature. The resulting solution was adjusted to pH 6 and chromatographed on XAD-2 (3 × 80 cm). Elution with water gave an eluate containing the starting compound **4** (20 mg). Changing the eluent to 20% MeOH-H_2O gave a main fraction which, on lyophilization, gave lactone 11 as a colorless powder: 151 mg; ir (KBr) 1785 (β-lactam, γ-lactone), 1675 (-CONH-), 1200 (br, $-SO_2$-), 1036 cm^{-1} ($-SO_3^-$). *Anal.* ($C_{16}H_{13}O_7N_2S_2Na\cdot 2H_2O$) C, H, N, S.

Minimal Inhibitory Concentrations. The MIC's of the cephalosporins were determined by the agar dilution method. Nutrient agar was used as the assay medium. One loopful of a suspension containing about 1 mg/ml of test organism was inoculated on each assay plates. The MIC's were determined after incubation of the plate at 37° for 18 hr.

Acknowledgments. The authors express their gratitude to Dr. S. Tatsuoka, Director of the division, and Drs. E. Ohmura and K. Morita for their kind encouragement.

References

(1) H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Morimoto, and T. Masuda, *Heterocycles*, **2**, 67 (1974).
(2) E. M. Hersh, G. P. Bodey, B. A. Nies, and E. J. Freireich, *J. Amer. Med. Ass.*, **193**, 105 (1965).
(3) R. L. Jao and G. G. Jackson, *J. Amer. Med. Ass.*, **189**, 123 (1964).
(4) P. Acred, D. M. Brown, E. T. Knudsen, G. N. Rolinson, and R. Sutherland, *Nature (London)*, **215**, 25 (1967).
(5) Y. Murata, T. Usui, H. Yamaguchi, T. Kanabayashi, S. Ogiwara, M. Tada, T. Aomori, S. Orita, and H. Yokotani, *Takeda Kenkyusho Ho*, **20**, 262 (1971).
(6) E. T. Knudsen, G. N. Rolinson, and R. Sutherland, *Lancet*, 1312 (1967).
(7) S. Morimoto, H. Nomura, T. Fugono, T. Azuma, I. Minami, M. Hori, and T. Masuda, *J. Med. Chem.*, **15**, 1108 (1972).
(8) K. Tsuchiya, T. Oishi, C. Iwagishi, and T. Iwahi *J. Antibiot.*, 14, 607 (1971).
(9) S. Morimoto, H. Nomura, T. Fugono, T. Ishiguro, and K. Maeda, *J. Med. Chem.*, **15**, 1105 (1972).
(10) S. Morimoto, H. Nomura, T. Fugono, I. Minami, T. Ishiguro, and T. Masuda, *J. Antibiot.*, **26**, 146 (1973).
(11) K. Kamiya, Y. Wada, H. Nomura, M. Nishikawa, and S. Morimoto, *Chem. Pharm. Bull.*, **21**, 1037 (1973).
(12) J. L. Spencer, F. Y. Siu, B. G. Jackson, H. M. Higgins, and E. H. Flynn, *J. Org. Chem.*, **32**, 500 (1967).
(13) A. Gourevitch, S. Wolfe, and J. Lein, *Antibacteriol. Ag. Chemother.*, 576 (1961).
(14) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746 (1966).