SEMISYNTHETIC β-LACTAM ANTIBIOTICS. 8

STRUCTURE-ACTIVITY RELATIONSHIPS OF α-SULFOCEPHALOSPORINS

HIROAKI NOMURA, ISAO MINAMI, TAKENORI HITAKA and TAKESHI FUGONO

Central Research Division, Takeda Chemical Industries, Ltd., Juso, Osaka 532, Japan

(Received for publication May 26, 1976)

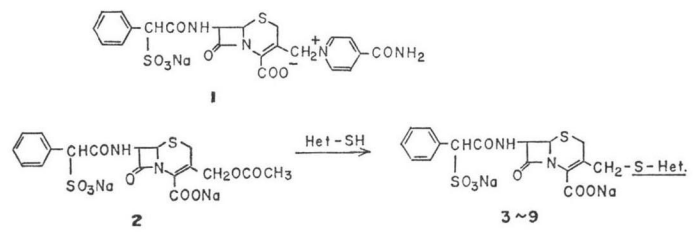
Synthesis and *in vitro* activity of a number of cephalosporins having α-sulfoacyl- or other acyl groups, *e. g.*, α-carboxyacyl- and α-sulfoaminoacyl- at the 7-position and bearing a variety of heterocyclic thioether or pyridinium moieties at the 3-position are described.

Despite the recent massive efforts in searching for broad spectrum semisynthetic cephalosporins, the overwhelming majority of compounds reported in the literature showed essentially no activity against *Pseudomonas aeruginosa*. A striking exception to this is sulfocephalosporins.[1] 7-(D-α-Sulfophenylacetamido)-3-(4-carbamoylpyridinium)methyl-3-cephem-4-carboxylic acid **(1)**, a representative compound in this series, showed a potent antipseudomonal activity almost comparable to that of gentamicin, *i.e.*, the *in vitro* activity was over ten times stronger than those of carbenicillin and sulbenicillin[1,2] and the protective activity in mice from infection with *P. aeruginosa* was 4~40 times more potent than that of carbenicillin.[2] A strong bactericidal activity with the MBC values essentially equal to the MICs[2] is another outstanding characteristic of **1**. The spectrum of antibacterial activity was unique and noteworthy in that, despite the surprisingly potent inhibitory effect on *P. aeruginosa*, the activity against other gram-negative bacteria, *e.g.*, *Escherichia coli* and *Proteus* species were merely modest.

As an extension of our studies to synthesize a new cephalosporin which is highly inhibitory to *P. aeruginosa* as well as a wide range of other gram-negative bacteria, we tried to make more modifications of sulfocephalosporins by introducing a variety of substituents at the 3-position or by replacing an R group in the 7-acyl side chain.

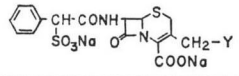
Reaction of 7-(α-sulfophenylacetamido)cephalosporanic acid, **2**, with selected five-membered heterocyclic thiol gave the corresponding 3-heterocyclic thioethers (**3**~**9**) as outlined in Scheme 1. Table 1 summarizes the antibacterial activity of these 7-(α-sulfophenylacetamido)-3-heterocyclic thio-

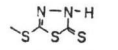
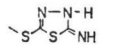
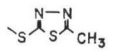
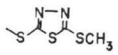
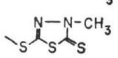
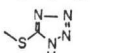
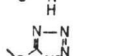
Scheme 1



Het = Heterocyclic group

Table 1. Antibacterial activities



No.	Y	MIC μg/ml				
		P. aeruginosa NCTC 10490	*E. coli* NIHJ	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209P	*S. aureus* Pc-R*
3		12.5	6.25	3.13	1.56	12.5
4		25	25	6.25	6.25	12.5
5		100	25	6.25	6.25	12.5
6		> 100	25	6.25	3.13	12.5
7		> 100	50	50	0.78	3.12
8		50	50	6.25	50	50
9		100	25	6.25	3.12	12.5

* Clinical isolates, No. 87

methyl-3-cephem acids. Compounds having an N–H group in a heterocyclic ring attached to the thiomethyl side chain at the 3-position had a relatively broad spectrum including a significant activity against *P. aeruginosa*. In comparison to **1**, cephalosporins in this class generally displayed improved activity against most gram-negative bacteria and much less activity against *P. aeruginosa*. In the case of compound **3**, the improvement in activity against *E. coli* was 4-fold while the antipseudomonal activity was 30 times less than that of **1**.

It is tempting to speculate from these results that the selective antipseudomonal activity of **1** is presumably due to the coexistence of a negative charge in the sulfo group and a positive charge in the 3-pyridiniummethyl side chain. In order to ascertain this assumption, we synthesized a couple of cephalosporins having both an α-acidic group and a 3-pyridiniummethyl side chain, 7-(α-sulfoaminophenylacetamido)- and 7-(α-carboxyphenylacetamido)-3-(4-carbamoylpyridinium)methyl-3-cephem-4-carboxylate (**10** and **11** in Scheme 2). Antibacterial spectra of these compounds are shown in Table 2. They showed a definite inhibitory effect on *P. aeruginosa*. The antibacterial spectra of

Scheme 2

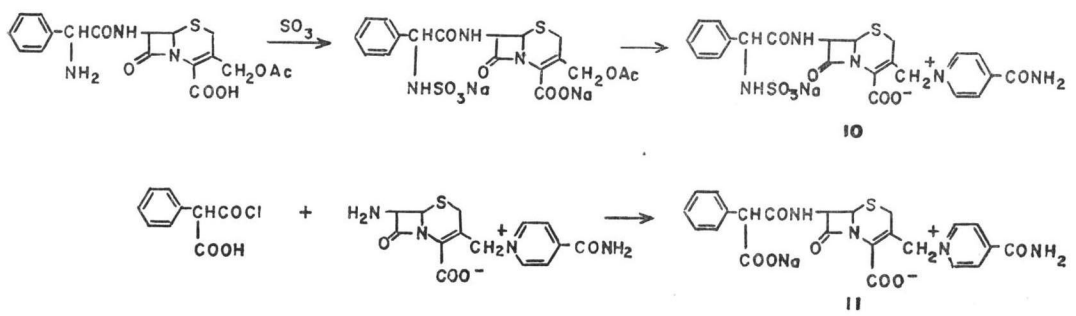
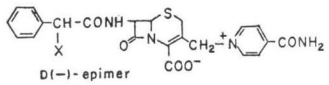


Table 2. Antibacterial activities



No.	X	MIC μg/ml					
		P. aeruginosa IFO 3080	*P. aeruginosa* NCTC 10490	*E. coli* NIHJ	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209P	*S. aureus* Pc-R**
1	$-SO_3Na$	1.56	0.39	25	50	3.12	3.12
10	$-NHSO_3Na$	12.5	50	50	50	3.12	3.12
11	-COONa*	50	50	50	100	1.25	6.25

* DL-epimer ** Clinical isolates, No. 87

Scheme 3

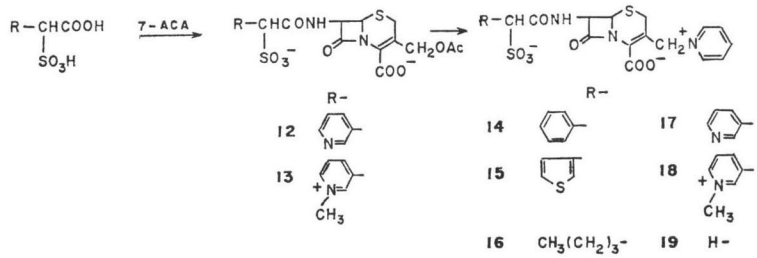
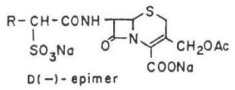


Table 3. Antibacterial activities

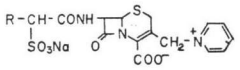


No.	R	MIC μg/ml				
		P. aeruginosa NCTC 10490	*E. coli* NIHJ	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209P	*S. aureus* Pc-R*
2		6.24	25	12.5	1.56	3.12
12		50	>100	50	12.5	25
13		>100	>100	>100	50	50

* Clinical isolates, No. 87.

these compounds suggest that acidic groups, such as $-SO_3H$,- $NHSO_3H$ and -COOH, when they coexist with a positive charge attached to the 3-methylene side chain, contribute to the improved antipseudomonal activity and the decreased activity against other gram-negative bacteria. The difference in the level of antipseudomonal activity among these compounds might be due to the difference in acidity and spatial size of the groups, which affects the penetrating ability of the molecule through the bacterial cell wall or the interaction with the enzyme concerned.

Table 4. Antibacterial activities



No.	R	MIC μg/ml				
		P. aeruginosa NCTC 10490	*E. coli* NIHJ	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209P	*S. aureus** Pc-R
14	 **	0.39	100	50	3.12	3.12
15		0.78	>100	100	1.56	3.12
16	$CH_3(CH_2)_3$-	3.12	>100	100	0.78	1.56
17		12.5	>100	>100	3.12	12.5
18		50	>100	>100	25.0	25.0
19	 H-	>100	>100	>100	3.12	12.5

* Clinical isolates, No. 87. **Reference 1).

By using modified sulfoacetic acids with various R groups, we synthesized a number of new sulfocephalosporins, **12**~**19**, as shown in Scheme 3. Tables 3 and 4 show the structure-activity relationship of these compounds.

Among the sulfocephalosporins with aromatic or heteroaromatic ring systems as R, phenyl analogs were generally more inhibitory to gram-positive and negative bacteria compared to the corresponding pyridyl and N-methylpyridinium analogs. Accordingly, existence of a basic nitrogen (**12, 17**) and a positive charge in R (**13, 18**) appear to be responsible for the decreased antibacterial activity.

Interestingly, a positive charge existing in a substituent attached to the 3-methylene group contributes to the potent activity against *P. aeruginosa* while that existing in the R group in the 7-acyl side chain appears to weaken the activity against both gram-positive and negative bacteria (Tables 3 and 4). Existence of a basic nitrogen or a positive charge in itself does not appear to cause a marked decrease in the activity of cephalosporins because cephapirin* and related compounds** which possess a pyridyl or N-methyl pyridinium moiety showed potent inhibitory effect on a wide range of gram-positive and negative bacteria.[4~6] Presumably, electrostatic interaction between the negatively charged sulfogroup and the basic center of the pyridyl group in **12, 13, 17** and **18** restricts the free rotation of each substituent to fix the conformation which might be unfavorable for the activity. A sharp decrease in the antibacterial activity can be seen with cephalosporins which have a sterically hindered acyl side chain.[7]

As shown in Table 4, very little change of the 7-(α-sulfoacyl)-moiety in **14** was permissible for retention of the level of antipseudomonal activity and the best results were obtained with compounds having a phenyl (**14**) or thienyl group (**15**). Antipseudomonal activity of the *n*-butyl analogue (**16**) was 4~8 times less while its activity against *Staphylococcus aureus* was 2~4 times stronger than those

* 7-(α-(4-Pyridylthio)acetamido)cephalosporanate.[4,5]

** 7-(α-(3-Pyridylthio)acetamido)cephalosporanate, BL-P 1343.[4] 7-(α(1-Methyl-4-pyridylthio)acetamido) cephalosporanic acid betaine, BL-S 217.[4,5] 7-(α-(1-Methyl-3-pyridylthio)acetamido)cephalosporanic acid betaine, BL-S 226.[4] 7-(α-(3-Pyridyl) acetamido)cephalosporanate.[6]

of **14** and **15**, respectively. The sulfoacetyl analogue (**19**), although showing significant antistaphylococcal activity, had a disappointing inhibitory effect on gram-negative bacteria. The results summarized in Table 4 suggest that a definite similarity can be seen in structure-antipseudomonal activity relationship between sulfocephalosporins and sulfopenicillins.[8]

Experimental Section

Infrared spectra were obtained in KBr disc using a Perkin-Elmer Infracord. NMR spectra were determined in D_2O, $CDCl_3$ and DMSO-d_6 on a Varian T-60 spectrometer. The IR and NMR spectra of all cephalosporins were consistent with structure. Elemental analyses are indicated by symbols of the elements and the results were within $\pm 0.4\%$ of theoretical values.

Minimum inhibitory concentrations

The MIC's of the cephalosporins were determined in twofold dilution (μg/ml) by the agar dilution method. Nutrient agar (pH 7.0) was used as assay medium. The test organism was grown for 18~24 hours on nutrient agar and one loopful of a suspension containing about 1 mg per ml of test organism was used as inoculum. MIC was determined after incubation at 37°C for 18 hours.

7-(2-Sulfoamino-2-phenylacetamido)cephalosporanic acid

To a stirred solution of cephaloglycin (anhydrous, 1.25 g, 3.1 mmol) and triethylamine (0.51 ml, 3.7 mmol) in CH_2Cl_2 (50 ml), was added dropwise SO_3-triethylamine (0.67 g, 3.7 mmol as SO_3) in CH_2Cl_2 (2 ml). The mixture was allowed to react for 2 hours at 20°C. After evaporation of the solvent, the residual solid was dissolved in a small amount of water and passed through a column of Amberlite IR-120 (Na-form, 110 ml) to remove triethylamine. The effluent and washings were concentrated and chromatographed on a column of Amberlite XAD-2 (100~400 mesh, 3.5×100 cm) with water as eluant, under the examination by UV absorption at 254.5 nm. The eluate containing the cephalosporanic acid was collected and freeze-dried to give a colorless powder of the product, 0.69 g. IR (KBr) 1760 (β-lactam), 1680 (-CONH-), 1610 ($-COO^-$), 1230 ($-SO_2-$), 1048 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 2.06 (3H, s, $-OCOCH_3$), 3.28, 3.51 (2H, dd, C_2-H), 4.76 (2H, d, C_3-CH_2-), 4.98 (1H, s, Ph-CH-), 5.02 (1H, d, C_6-H), 5.64 (1H, d, C_7-H), 7.44 (5H, s, Ph-H). *Anal.* ($C_{15}H_{17}N_3S_2O_9Na_2 \cdot 2.5\ H_2O$) C, H, N.

Potassium 7-(2-sulfoamino-2-phenylacetamido)-3-(4-carbamoylpyridinium)-methyl-3-cephem-4-carboxylate (**10**)

The cephalosporanic acid (0.53 g, 1 mmol) described in the above experiment was added to a solution of isonicotinamide (0.244 g, 2 mmol) and KI (8.3 g) in water (5.6 ml). After the mixed solution had been heated for 2 hours at 70°C, the reaction mixture was chromatographed on Amberlite XAD-2 (2.5×90 cm) with elution by water. The cephalosporin fraction was collected and concentrated *in vacuo*, followed by lyophilization to give 10 as a colorless powder, 0.31 g. IR (KBr disc) 1765 (β-lactam), 1690 (-CONH-), 1645 (-CONH-), 1615 ($-COO^-$), 1225 ($-SO_2-$), 1040 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.09, 3.47 (2H, dd, C_2-H), 5.01 (1H, s, Ph-CH-), 5.14 (1H, d, C_6-H), 5.47 (1H, d, C_7-H), 7.38 (5H, s, Ph-H), 8.28 and 9.05 (4H, dd, pyridinium-H). *Anal.* ($C_{22}H_{20}N_5S_2O_8K \cdot 3H_2O$) C, H, N.

Sodium 7-(2-sulfo-2-phenylacetamido)-3-(heterocyclic thio)-methyl-3-cephem-4-carboxylate (**3~9**)

General procedure: The stirred aqueous solution of 7-(α-sulfo-α-phenylacetamido)cephalosporanic acid[1] (disodium salt, 257 mg, 0.5 mmol), an appropriate heterocyclic thiol (0.75 mmol) and $NaHCO_3$ (50 mg, 0.6 mmol) in water (0.5 ml) was kept at 40°C for 8 hours. The reaction mixture was chromatographed on a column of Amberlite XAD-2 (100~400 mesh, 3×80 cm) with water as eluant. The desired cephalosporin fraction was collected and lyophilized to yield a slightly yellowish powder (**3~9**). The spectral data of each compound are as follows.

Disodium 7-(2-sulfo-2-phenylacetamido)-3-[2-(5-mercapto-1,3,4-thiadiazolyl)-thio]methyl-3-cephem-4-carboxylate (**3**)

IR (KBr disc) 1750 (β-lactam), 1670 (-CONH-), 1600 ($-COO^-$), 1530 (-CSNH-), 1285 (-CSNH-), 1215 ($-SO_2-$), 1045 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.23 and 3.62 (2H, dd, 18 cps, C_2-H), 3.81 and 4.29 (2H, dd, 13 cps, C_3-CH_2-), 5.02 (1H, d, 4.5 cps, C_6-H), 5.69 (1H, d, 4.5 cps, C_7-H), 5.14 (1H, s, Ph-

CHCO–).

Disodium 7-(2-sulfo-2-phenylacetamido)-3-[2-(5-amino-1,3,4-thiadiazolyl)-thio]methyl-3-cephem-4-carboxylate (**4**)

IR (KBr disc) 1760 (β-lactam), 1680 (–CONH–), 1605 ($-COO^-$), 1210 ($-SO_2-$), 1040 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.23 and 3.67 (2H, dd, 18 cps, C_2–H), 3.72 and 4.36 (2H, dd, 14 cps, C_3–CH_2–), 5.05 (1H, d, 4.5 cps, C_6–H), 5.67 (1H, 4.5 cps, C_7–H), 5.13 (1H, s, Ph–CHCO–).

Disodium 7-(2-sulfo-2-phenylacetamido)-3-[2-(5-methyl-1,3,4-thiadiazolyl)-thio]methyl-3-cephem-4-carboxylate (**5**)

IR (KBr) 1760 (β-lactam), 1680 (–CONH–), 1605 ($-COO^-$), 1200 ($-SO_2-$), 1040 (SO_3^-) cm^{-1}; NMR (D_2O) 2.70 (3H, s, $-CH_3$), 3.24 and 3.55 (2H, dd, 18 cps, C_2–H), 3.93 and 4.36 (2H, dd, 14 cps, C_3–CH_2–), 5.01 (1H, d, 4.5 cps, C_6–H), 5.68 (1H, d, 4.5 cps, C_7–H), 5.12 (1H, s, Ph–CHCO–).

Disodium 7-(2-sulfo-2-phenylacetamido)-3-[2-(5-methylthio-1,3,4-thiadiazolyl)thio]methyl-3-cephem-4-carboxylate (**6**)

IR (KBr disc) 1755 (β-lactam), 1675 (–CONH–), 1600 ($-COO^-$), 1035 cm^{-1}; NMR (D_2O) 2.72 (3H, s, $-SCH_3$), 3.27 and 3.62 (2H, dd, 18 cps, C_2–H), 3.99 and 4.37 (2H, dd, 13.8 cps, C_3–CH_2–), 5.05 (1H, d, 4.5 cps, C_6–H), 5.71 (1H, d, 4.5 cps, C_7–H), 5.15 (1H, s, Ph–CHCO–), 7.5 (5H, Ph–H).

Disodium 7-(2-sulfo-2-phenylacetamido)-3-[2-(4-methyl-5-mercapto-1,3,4-thiadiazolyl)thio]methyl-3-cephem-4-carboxylate (**7**)

IR (KBr disc) 1760 (β-lactam), 1677 (–CONH–), 1604 ($-COO^-$), 1037 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.23 and 3.48 (2H, dd, 18 cps, C_2–H), 3.70 (3H, s, N–CH_3), 3.95 and 4.33 (2H, dd, 13.5 cps, C_3–CH_2–), 5.00 (1H, d, 4.5 cps, C_6–H), 5.76 (1H, d, 4.5 cps, C_7–H), 5.19 (1H, s, Ph–CHCO–), 7.4～7.6 (5H, Ph–H).

Trisodium 7-(2-sulfo-2-phenylacetamido)-3-[5-(tetrazolyl)thio]methyl-3-cephem-4-carboxylate (**8**)

IR (KBr disc) 1760 (β-lactam), 1220 ($-SO_2-$), 1050 ($-SO_3^-$) cm^{-1}; NMR (d_6-DMSO+D_2O) 3.42 and 3.70 (2H, dd, 18 cps, C_2–H), 4.22 (C_3–CH_2–), 4.87 and 4.99 (1H, ss, Ph–CH–CO–), 5.08 and 5.15 (1H, dd, 5 cps, C_6–H), 5.74 and 5.80 (1H, dd, 5 cps, C_7–H), 7.4～7.8 (5H, m, Ph–H). *Anal.* ($C_{17}H_{13}N_6O_7S_3Na_3 \cdot 4H_2O$) C, H, N.

Disodium 7-(2-sulfo-2-phenylacetamido)-3-[5-(1-methyltetrazolyl)thio]-methyl-3-cephem-4-carboxylate (**9**)

IR (KBr disc) 1760 (β-lactam), 1675 (–CONH–), 1210 ($-SO_2-$), 1038 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.34 and 3.60 (2H, dd, 18 cps, C_2–H), 3.99 (3H, s, N–CH_3), 4.01 and 4.25 (2H, dd, 14 cps, C_3–CH_2–), 5.10 (1H, s, Ph–CHCO–), 5.25 (1H, d, 4.5 cps, C_6–H), 5.65 (1H, d, 4.5 cps, C_7–H), 7.5 (5H, Ph–H).

7-(DL-2-Carboxy-2-phenylacetamido)-3-(4-carbamoylpyridinium)-methyl-3-cephem-4-carboxylic acid monosodium salt (**11**)

To an ice-cooled, stirred solution of 7-amino-3-(4-carbamoylpyridinium)methyl-3-cephem-4-carboxylic acid betaine (556 mg, 1.5 mmol) and $NaHCO_3$ (315 mg, 3.76 mmol) in water (15 ml), was added α-carboxyphenylacetyl chloride[8] (420 mg, 1.88 mmol) in AcOEt (1 ml). The mixture was allowed to react for 15 minutes at 0～5°C. After AcOEt had been removed, the reaction mixture was passed through a column of Amberlite CG-50 then chromatographed on Amberlite XAD-2 with water as eluant. Fractions containing the product were lyophilized to give 50 mg of the desired cephalosporin as a colorless powder. IR (KBr) 1772 (β-lactam), 1675 (–CONH–), 1610 ($-COO^-$), 1515, 1390, 1350 cm^{-1}; NMR (D_2O) 3.1, 3.53 and 3.18, 3.60 (2H, four d, C_2–H), 4.52 and 4.59 (1H, ss, –CH(COO^-)CO–), 5.13 and 5.16 (1H, dd, C_6–H), 5.39 and 5.49 (2H, dd, C_3–CH_2–), 5.66 (1H, d, C_7–H), 7.29 (5H, s, Ph–H), 8.33 and 9.08 (4H, dd, pyridinium-H). *Anal.* ($C_{23}H_{19}N_4O_7SNa \cdot 3H_2O$) N.

2-Mercapto-2-(3-thienyl)acetic acid

2-Bromo-2-(3-thienyl)acetic acid (4.4 g, 20 mmol), prepared according to the method described by GRONOWITZ[9] was allowed to react with thiourea (1.7 g, 21.4 mmol) in EtOH (50 ml) for 3 hours at 60°C. Evaporation and crystallization from benzene afforded isothiouronium salt as reddish needles. IR (KBr disc) 1757 (–COOEt), 1645 (C=N), 710 (thiophene ring). Hydrolysis in 10% NaOH (20 ml) for 2.5 hours at 100°C followed by the usual working up yielded 2.15 g of the desired acetic acid as color-

less needles (from *n*-hexane-benzene). IR (KBr disc) 2550 (–SH), 1693 (–COOH), 1414, 692 (thiophene ring) cm^{-1}; NMR (D_2O) 2.63 (1H, d, 8.5 cps, –SH), 4.77 (1H, d, 8.5 cps, –CH–), 7.12~7.37 (3H, m, thiophene ring-H), 11.16 (1H, s, –COOH). *Anal.* ($C_6H_6O_2S_2$) C, H.

2-Sulfo-2-(3-thienyl)acetic acid

To a mixed solution of 2-mercapto-2-(3-thienyl)acetic acid (0.7 g, 4 mmol) and KOH (6 mmol) in water (25 ml), was added dropwise a 6% aqueous $KMnO_4$ solution (10.5 ml) over a period of 1.5 hours at room temperature. Addition of MeOH (2 ml) and filtration gave a colorless solution. After being passed through a column of Amberlite IR-120 (H-form), the mixture was chromatographed on an Amberlite XAD-2 column with water as eluant. Working up in the usual way gave 130 mg (30%) of the desired acid as colorless needles. IR (KBr disc) 1727 (–COOH), 1257, 1190 ($-SO_2-$), 1030 ($-SO_3^-$), 668 (thiophene ring); NMR (D_2O) 5.29 (1H, s, methylene-H), 7.28~7.66 (3H, m, thiophene ring-H).

2-Sulfo-2-(3-thienyl)acetyl chloride

This compound was prepared from 2-sulfo-2-(3-thienyl)acetic acid by a procedure similar to that described in our previous paper.[10] NMR ($CDCl_3$) 5.73 (1H, s, –CHCOCl), 7.25~7.55 (3H, m, thiophene ring-H).

7-[2-Sulfo-2-(3-thienyl)acetamido]cephalosporanic acid

An AcOEt solution (10 ml) of 2-sulfo-2-(3-thienyl)acetyl chloride prepared from 1.5 g (6.8 mmol) of 2-sulfo-2-(3-thienyl)acetic acid was added dropwise to an ice-cooled, stirred solution of 7-ACA (1.84 g, 6.8 mmol) and NaOH (13.6 mmol) in water (25 ml). The mixture was allowed to react at 0~6°C for 30 minutes. The aqueous layer was collected, adjusted to pH 6.5 and concentrated *in vacuo*. Chromatography on a column of Amberlite XAD-2 with water as eluant, followed by the usual work-up yielded 700 mg of the cephalosporin as an almost colorless powder. IR (KBr disc) 1750 (β-lactam, –OAc), 1677 (–CONH–), 1605 ($-COO^-$), 1225 ($-SO_2-$), 1043 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 2.13 (3H, s, $-COOCH_3$), 3.54 (2H, dd, C_2–H), 5.10 (1H, d, 4.7 cps, C_6–H), 5.24 (1H, s, –CHCON–), 5.70 (1H, d, 4.7 cps, C_7–H), 7.38~7.67 (3H, m, thiophene ring-H).

Potassium 7-[2-sulfo-2-(3-thienyl)acetamido]-3-pyridiniummethyl-3-cephem-4-carboxylate (**15**)

A mixture of the preceding cephalosporin (260 mg, 0.5 mmol), KSCN (97 mg, 1 mmol) and pyridine (118 mg, 1.5 mmol) in water (2.5 ml) was kept at 40°C for 24 hours. The reaction mixture was chromatographed on a column of Amberlite XAD-2 with water as eluant. By the usual work-up procedure,[1] we obtained 50 mg of the product as an almost colorless crystalline powder. IR (KBr disc) 1764 (β-lactam), 1675 (–CONH–), 1613 ($-COO^-$), 1040 ($-SO_3^-$); NMR (D_2O) 3.09 and 3.50 (2H, dd, C_2–H), 5.14 (1H, d, 4.7 cps, C_6–H), 5.22 (1H, s, –CHCON–), 5.35 and 5.47 (2H, dd, C_3–CH_2–), 5.71 (1H, d, 4.7 cps, C_6–H), 7.35~7.58 (3H, m, thiophene ring-H), 8.08 (2H, m, pyridinium-H(m)), 8.54 (1H, m, pyridinium-H(p)), 8.91 (2H, m, pyridinium-H(o)).

7-(α-Sulfo-*n*-caproamido)cephalosporanic acid sodium salt

To a cooled solution of 7-ACA (2.56 g, 9.4 mmol), triethylamine (2.85 g, 28.2 mmol) in $CHCl_3$ (50 ml), was added dropwise a solution of α-sulfo-*n*-caproyl chloride, obtained from α-sulfo-*n*-caproic acid (2.5 g, 12.8 mmol), in $CHCl_3$ (5 ml) under stirring. The mixture was allowed to react for 60 minutes at 10°C. After evaporation, the residual solid was dissolved in a small amount of water and passed through a cation-exchanger. Chromatography and the successive work up in the usual way gave the desired product as an almost colorless powder, 200 mg. IR (KBr disc) 1760 (β-lactam), 1670 (–CONH–), 1610 ($-COO^-$), 1380 and 1230 ($-SO_2-$), 1045 ($-SO_3^-$).

Potassium 7-(α-sulfo-*n*-caproamido)-3-pyridiniummethyl-3-cephem-4-carboxylate (**16**)

This compound was synthesized by the reaction of disodium 7-(α-sulfo-*n*-caproamido)cephalosporanate (450 mg, 1 mmol) with pyridine (395 mg, 5 mmol) in an aqueous solution (1 ml) containing KSCN (485 mg, 5 mmol) under conditions similar to those described for compound 3. IR (KBr disc) 1763 (β-lactam), 1676 (–CONH–), 1613 ($-COO^-$), 1038 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 0.93 (3H, t, CH_3–), 1.37 (4H, m, $-CH_2CH_2-$), 1.97 (2H, m, $-CH_2CH-$), 3.54 (2H, dd, C_2–H), 3.83 (1H, t, –CHCON–), 5.21 (1H, C_6–H), 5.50 (2H, C_3-CH_2–), 5.75 (1H, d, C_7–H), 8.17 (2H, q, 1.5, 6.5 cps, pyridinium-H(m)), 8.56 (1H, pyridinium-H(p)), 8.97 (2H, q, 1.5, 6.5 cps, pyridinium-H(o)).

2-Sulfo-2-(3-pyridyl)acetic acid

2-(3-Pyridyl)acetic acid (4.3 g, 31 mmol) was added slowly to liquid SO_3 (14.4 g, 0.18 mol) over a 50 minutes period with mechanical stirring. Stirring was continued for 2.5 hours at 50°C with protection against moisture. The reaction mixture was poured into a large amount of ice water (700 ml), then neutralized with $BaCO_3$ (50 g) and filtered to give a brownish solution. Concentration of this yielded precipitates of the Ba salt (6.7 g). After the Ba ion had been removed with a cation-exchanger, Amberlite IR-120, the acid solution was concentrated to give colorless needles of the desired acid, 2.9 g (42.7%). IR (KBr disc) 1735 (-COOH), 1556, 1280, 1226 ($-SO_2-$), 1189, 1150, 1030 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 5.0 (1H, s, $-\underset{|}{C}\underline{H}CO-$), 7.65~8.80 (4H, m, pyridine-H). *Anal.* ($C_7H_7NO_5S$) C, H, N.

Disodium 7-[2-sulfo-2-(3-pyridyl)acetamido]cephalosporanate (**12**)

To a solution of 2-sulfo-2-(3-pyridyl)acetic acid (0.65 g, 3 mmol) and triethylamine (0.30 g, 3 mmol) in DMF (7.5 ml), was added 0.62 g (3 mmol) of DCC in DMF (0.5 ml) with stirring. After 5 minutes the mixture was added to a cooled $ClCH_2CH_2Cl$ solution (10 ml) of 7-ACA (triethylamine salt, 1.12 g, 3 mmol). After removing the precipitates by filtration, the solution was evaporated to give a yellow powder. Passage of this powder through a column of Amberlite IR-120 with subsequent chromatography on a column of Amberlite XAD-2, followed by work up gave 110 mg of colorless powder. IR (KBr disc) 1762 (β-lactam), 1685 (-CONH-), 1613 ($-COO^-$), 1233 ($-SO_2-$), 1048 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 2.09 (3H, s, $-COCH_3$), 3.31 and 3.55 (2H, dd, 18 cps), 4.80 (2H, dd, C_3-CH_2-), 5.12 (1H, d, 2 cps, C_6-H), 5.20 (1H, s, $-\underset{|}{C}\underline{H}CON-$), 5.70 (1H, d, 2 cps, C_7-H), 7.45~8.79 (4H, m, pyridine ring-H).

Potassium 7-[2-sulfo-2-(3-pyridyl)acetamido]-3-pyridiniummethyl-3-cephem-4-carboxylate (**17**)

This compound was synthesized by the reaction of sodium 7-[2-sulfo-2-(3-pyridyl)acetamido]-cephalosporanate (300 mg, 5.82 mmol) with pyridine (85 mg, 7 mmol) in water (2.7 ml) saturated with KI (4.83 g 29 mmol) at 70°C for 2 hours. Working up in the usual way gave 66 mg of **17** as a colorless powder. IR (KBr disc) 1760 (β-lactam), 1670 (-CONH-), 1610 ($-COO^-$), 1240 ($-SO_2-$), 1045 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.12 and 3.50 (2H, dd, 18 cps, C_2-H), 5.14 (2H, C_3-CH_2-), 5.17 (1H, s, $-\underset{|}{C}\underline{H}CON-$), 5.86 (1H, d, C_6-H), 5.66 (1H, d, 4.5 cps, C_7-H), 7.3~9.3 (9H, m, pyridine-H).

2-Sulfonato-2-(1-methyl-3-pyridino)acetic acid

To a solution of 2-sulfo-2-(3-pyridyl)acetic acid (8.4 g) and Et_3N (0.78 g) in CH_2Cl_2 was added 2.74 g CH_3I. After stirring for 4 hours at room temperature, the product which precipitated was collected by filtration. Treatment with Amberlite IR-120 (H-form) and the following work up gave the desired betaine salt as a colorless crystalline powder. 750 mg. IR (KBr) 1722, 1637, 1220, 1018; NMR (D_2O) 4.53 (3H, s, CH_3-N), 5.45 and 5.65 (1H, ss, =CHCO-), 8.13~9.2 (4H, m, pyridinium-H). *Anal.* ($C_8H_{10}O_5NS \cdot 3.5H_2O$) C, H, N.

7-[2-Sulfonato-2-(1-methyl-3-pyridinio)acetamido]cephalosporanic acid (**13**)

To a mixed solution of 2-sulfonato-2-(1-methyl-3-pyridinio)acetic acid (0.33 g, 1.41 mmol) and *tert*-butyl 7-aminocephalosporanate[11] (0.47 g, 1.42 mmol) in CH_3CN - water (3:1, 37 ml), was added DCC (0.32 g, 1.55 mmol). The mixture was stirred at 0~5°C for 2.5 hours. Filtration and evaporation of the filtrate gave the cephalosporin ester as a pale yellow powder which subsequently was treated with trifluoroacetic acid (4 ml) at room temperature for 30 minutes. The reaction product was chromatographed and worked up in the usual way to yield the desired cephalosporin as a colorless powder (100 mg). IR (KBr disc) 1760 (β-lactam), 1605 ($-COO^-$), 1230 ($-SO_2-$), 1040 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 2.13 (3H, s, $-OCOCH_3$), 3.45 and 3.66 (2H, dd, 18 cps, C_2-H), 4.46 (3H, s, $N-CH_3$), 4.83 (2H, d, 3.0 cps, C_3-CH_2-), 5.20 (1H, d, 5 cps, C_6-H), 5.40 (1H, s, $-\underset{|}{C}\underline{H}CON-$), 5.76 (1H, d, 5 cps, C_7-H), 8.1~9.1 (4H, pyridinium-H). *Anal.* ($C_{18}H_{19}N_3O_9S \cdot 3H_2O$) C, H, N.

7-[2-Sulfonate-2-(1-methyl-3-pyridinio)acetamido]-3-(1-pyridinio)methyl-3-cephem-4-carboxylate (**18**)

A mixture of the cephalosporin (**13**) (90 mg, 0.177 mmol), isonicotinamide (43 mg, 0.35 mmol), KI (1.47 g, 8.85 mmol) in water (0.8 ml) was kept for 2 hours at 70°C. Working up in the usual way gave 18 mg of **18** as a colorless powder. IR (KBr disc) 1760 (β-lactam), 1680 (-CONH-), 1610 ($-COO^-$), 1225 ($-SO_2-$), 1040 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.26 and 3.68 (2H, dd, 18 cps, C_2-H), 4.46 (3H, s, N-

CH_3), 5.27 (1H, d, 5.0 cps, C_6–H), 5.40 (1H, s, –CHCON–), 5.83 (1H, d, 5.0 cps, C_7–H), 8.06~9.12 (9H, m, pyridinium-H).

Sodium 7-(sulfoacetamido)cephalosporanate

The reaction of 7–ACA (1.0 g, 3.68 mmol) with sulfoacetyl chloride (1.0 g, 6.34 mmol) was performed in the usual way to give the desired product (100 mg). IR (KBr disc) 1760 (β-lactam), 1670 (–CONH–), 1610 ($-COO^-$), 1230 ($-SO_2-$), 1050 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 2.18 (3H, s, $-OCOCH_3$), 3.50 and 3.68 (2H, dd, 18 cps, C_2–H), 3.98 (2H, s, $-CH_2CON-$), 4.83 and 4.93 (2H, dd, 13 cps, C_3–CH_2–), 5.25 (1H, d, 4.8 cps, C_6–H), 5.80 (1H, d, 4.8 cps, C_7–H).

7-(Sulfoacetamido)-3-pyridiniummethyl-3-cephem-4-carboxylic acid (**19**)

The replacement reaction of the acetoxy group of the preceding cephalosporanic acid (639 mg, 1 mmol) with pyridine (238 mg, 3 mmol) was carried out by the method similar to that described above giving **19** as a potassium salt (0.3 g). IR (KBr) 1766 (β-lactam), 1670 (–CONH–), 1620 ($-COO^-$), 1210 ($-SO_2-$), 1047 ($-SO_3^-$) cm^{-1}; NMR (D_2O) 3.20 and 3.61 (2H, dd, 18 cps, C_2–H), 3.93 (2H, s, $-CH_2CO-$), 5.15 (1H, d, 4.8 cps, C_6–H), 5.40 (2H, C_3–CH_2–), 5.67 (1H, d, 4.8 cps, C_7–H), 8.0~8.9 (5H, m, pyridinium-H).

Acknowledgments

The authors express their gratitude to Dr. E. OHMURA, Director of the division, Drs. K. MORITA and S. MORIMOTO for their kind encouragement and also to Mr. T. NISHIMURA for his valuable information relating this work. Thanks are also due to Messrs. F. KIRIKI and T. MATSUDA for their technical assistance.

References

1) NOMURA H.; T. FUGONO T. HITAKA, I. MINAMI, T. AZUMA, S. MORIMOTO & T. MASUDA: Semisynthetic β-lactam antibiotics. 6. Sulfocephalosporins and their antipseudomonal activities. J. Med. Chem. 17: 1312~1315, 1974
2) a) TSUCHIYA, K.; K. NAGATOMO & M. KONDO: Antibacterial activity of SCE-129. Abstract Papers of the 22nd Annual Meeting of Japan Society of Chemotherapy, p. 15, Sept. 1975
b) TSUCHIYA, K. & M. KONDO: Comparison of antipseudomonal activity among SCE-129, SBPC, GM and DKB. Abstract Papers of the 24th Annual Meeting of Japan Society of Chemotherapy, p. 86, June 1976
3) MORIMOTO, S.; H. NOMURA, T. FUGONO, I. MINAMI, T. ISHIGURO & T. MASUDA: Semisynthetic β-lactam antibiotics. 3. Structure-activity relationships of sulfopenicillins. J. Antibiotics 26: 146~152, 1973
4) MISIEK, M.; A. J. MOSES, T. A. PURSIANO, F. LEITNER & K. E. PRICE: *In vitro* activity of cephalosporins against *Mycobacterium tuberculosis* H37 Rv: Structure-activity relationships. J. Antibiotics 26: 737~744, 1973
5) CRAST, L. B.; R. G. GRAHAM & L. C. CHENEY: Synthesis of cephapirin and related cephalosporins from 7-(α-bromoacetamido)cephalosporanic acid. J. Med. Chem. 16: 1413~1415, 1973
6) STEDMAN, R. J.; A. C. SWIFT, L. S. MILLER, M. M. DOLAN & J. R. E. HOOVER: Semisynthetic penicillins. IV. Pyridylmethylpenicillins and related cephalosporins. J. Med. Chem. 10: 363~366, 1967
7) FLYNN, E. H. edited: Cephalosporins and penicillins, chemistry and biology. p. 549, Academic Press, New York and London, 1972
8) BRAIN, E. G. & J. H. C. MAYLER: Penicillins, Brit. Pat. 1004670, 1965
9) GRONOWITZ, S.; I. SJOGREN, L. WERNSTEDT & B. SJOBERG: Resolution and configuration of α-amino-3-thienylacetic and α-azido-3-thienylacetic acids. Arkiv. Kemi. 23: 129~143, 1964 (CA 62: 7615a)
10) MORIMOTO, S.; H. NOMURA, T. FUGONO, T. ISHIGURO & K. MAEDA: Semisynthetic β-lactam antibiotics. I. Acylation of 6-aminopenicillanic acid with the activated derivatives of α-sulfophenylacetic acid. J. Med. Chem. 15: 1105~1107, 1972
11) STEDMAN, R. J.: *t*-Butyl ester of aminocephalosporanic acid. J. Med. Chem. 9: 444, 1966