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Synthesis of New Cephamycin Derivatives and a Novel Rearrangement between Isothiazolethioacetamides and 1,3-Dithietanecarboxamides

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A novel intramolecular rearrangement of isothiazolethioacetamides into 1,3-dithietanecarboxamides is described, together with the synthesis of a new cephamycin derivative (YM-09330)[2] having a 1,3-dithietane structure at the 7β-position. This compound showed strong antibacterial activity, especially against gram-negative organisms.

Keywords——cephamycin; rearrangement; dithietane; isothiazole; antibacterial activity; metal catalyst; interconversion

A grave limitation of early cephalosporins was their relatively poor activity against gram-negative organisms.

Recently however, cephamycin derivatives have been found with improved activity against such organisms because of their marked resistance to hydrolysis by β-lactamases.[3] We wish to report here the preparation of a new cephamycin derivative (YM-09330) having a 1,3-dithietane structure at the 7β side chain, as well as a novel intramolecular rearrangement between isothiazolethioacetamides and 1,3-dithietanecarboxamides.[4] In our labolatory, several kinds of 7β-heterocyclic thioacetamido cephamycin derivatives having polar substituents on the heterocyclic ring (such as a hydroxyl or amino group) have been prepared.[5] Among them, the 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetamido cephamycin derivative (**1**) showed the strongest antibacterial activity and widest spectrum against gram-negative organisms such as *indole-positive proteus, serratia, enterobacter, etc.* It was later found that **1** was readily converted into another compound (**2**) in dilute sodium hydrogen carbonate solution and that **2** thus formed exhibited strong antibacterial activity against gram-negative organisms, comparable to that of the original compound **1**. Compound **2** was assumed to be a 1,3-dithietane carboxamido cephamycin derivative, judging from the data described below. i) Compound **2** was found to be an isomer of **1** by elemental analysis. ii) In the NMR spectrum of **2**, the signal at δ 3.99 (2H, s, –C–H) in **1** was absent, and a new signal at δ 5.14 (1H, s, –C–H) was observed. iii) Alkylation of **2** with diazomethane gave the compound (**3**), which has two ester groups. iv) In the IR spectrum of **2**, new absorption bands at 3420 cm^{-1} and 3250 cm^{-1} due to a primary amido group were seen. The presence

1) Location: *Azusawa 1-1-8, Itabashi-ku, Tokyo 174, Japan.*
2) Code number assigned by Yamanouchi Pharmaceutical Co., Ltd. Part of this work was presented at the ACS/CSJ Chemical Congress, Honolulu, Hawaii, April, 1978.
3) *a*) D.D. Daust, H.R. Onishi, H. Wallick, D. Hendlin, and E.O. Stapley, *Antimicrobial Ag. Chemother.*, 3, 254 (1973); *b*) D.F. Mahoney, G.A. Koppel, and J.R. Turner, *ibid.*, **10**, 470 (1976); *c*) R. Nagarajan, L.D. Boeck, M. Gorman, R.L. Hamill, C.E. Higgens, M.M. Hoenn, W.M. Stark, and J.G. Whitney, *J. Am. Chem. Soc.*, **93**, 2308 (1971).
4) M. Iwanami, T. Maeda, F. Fujimoto, Y. Nagano, N. Nagano, A. Yamazaki, T. Shibanuma, K. Murase, K. Tamazawa, M. Aruga, and R. Ishikawa, Abstract of Papers, The ACS/CSJ Chemical Congress, Honolulu, Hawaii, April, 1979, p. MEDI 44.
5) M. Iwanami, T. Maeda, Y. Nagano, M. Fujimoto, N. Nagano, and A. Yamazaki, Jpn. Patent (Kokai) 3090 (1979).

of the primary amido group was confirmed by the reaction of **3** with PCl_5 under ice-cooling to afford a compound (**4**) having a cyano group.

It appears that the mechanism of this rearrangement was as follows; a hydrogen of the active methylene between the sulfur and the carbonyl group of the thioacetamido side chain was abstracted by a weak base such as sodium hydrogen carbonate, and the anion thus formed attacked the sulfur atom on the isothiazole ring, accompanied by –S–N– bond cleavage to give the 1,3-dithiethane ring. In order to confirm its structure, **4** was prepared by another synthetic route, as shown in Chart 1. These two samples of **4** gave identical NMR spectra, IR spectra and *Rf* values of TLC.

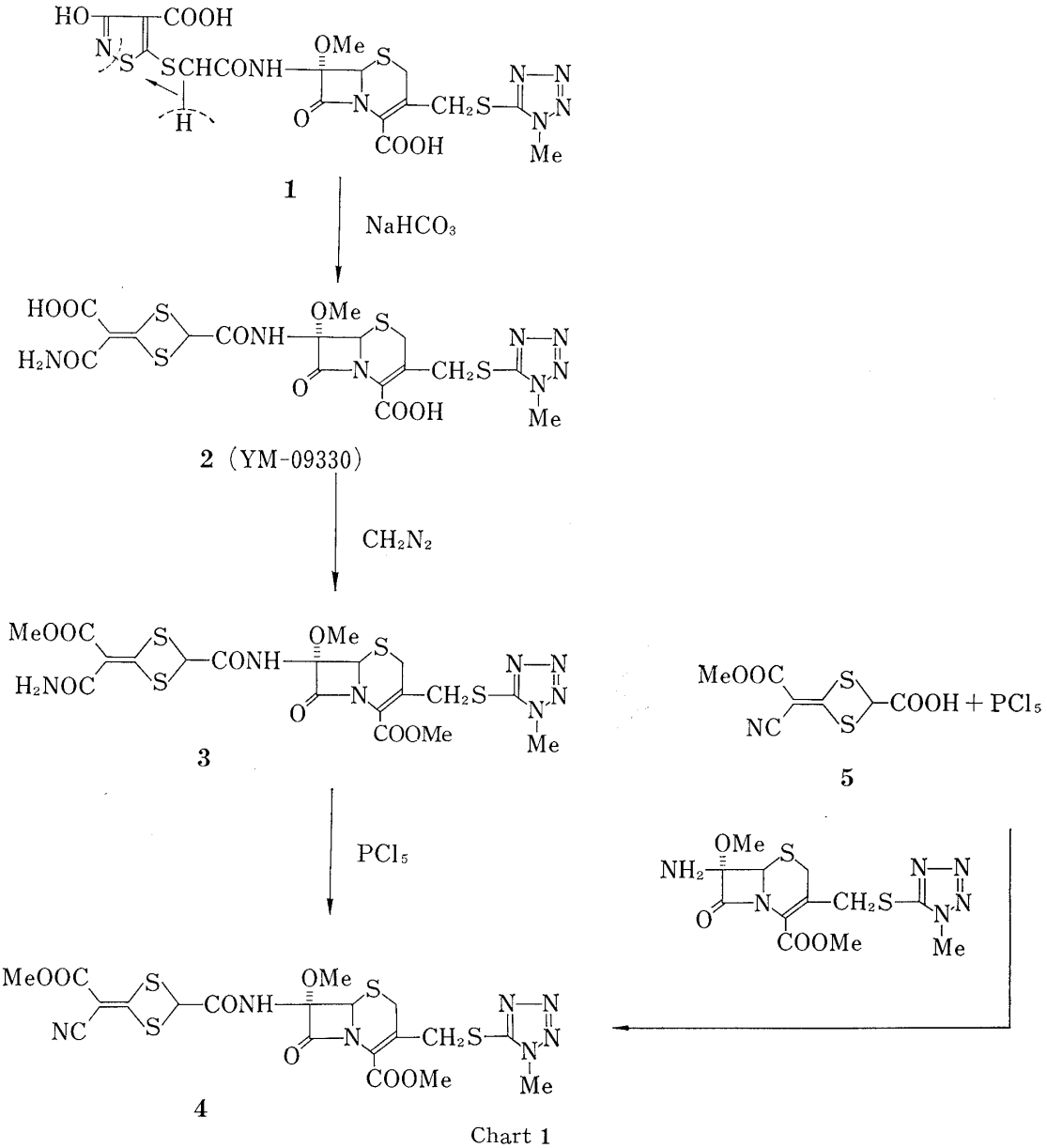

Chart 1

Previously, Chan *et al.*[6] reported the dimerization of 2-ethyl-3-isothiazolone in an ethanol solution of sodium methoxide. However, the intramolecular rearrangement of isothiazole-thioacetamides into 1,3-dithietanecarboxamides has not been reported. To investigate this reaction further, we prepared some model compounds (**7a**, **b**).

6) A.W. Chan, W.D. Grow, and I. Gosney, *Tetrahedron*, **26**, 1493 (1970).

Treatment of the isothiazolethioacetamide (**7a**) with aqueous sodium hydrogen carbonate solution gave an equilibrium mixture of **7a** and 1,3-dithietanecarboxamide (**8a**) in a ratio of 1 : 1. Another model compound (**7b**), an ester of isothiazolethioacetic acid having more acidic protons than **7a** in the isothiazole side chain, rearranged completely under the same conditions to afford the corresponding 1,3-dithietanecarboxylic acid (**8c**) *via* the ester (**8b**), which was hydrolyzed in the alkaline solution used for the rearrangement. Treatment of **7b** with triethylamine in ethanol solution to prevent this hydrolysis gave **8b** in good yield. On the other hand, the reverse rearrangement, **8a** to **7a**, occurred with strong bases, such as sodium hydroxides or sodium methoxide. These relationships are shown in Table I and Chart 2.

Table I. Rearrangement between Isothiazole (**7a, b**) and 1,3-Dithiethane (**8a, b, c**)

Starting material	Catalyst	Solvent	Time (hr)	Temp.	Product ratio
7a	$NaHCO_3$	H_2O	12	R.T.	**7a** : **8a** 1 : 1
7a	K_2CO_3	H_2O	12	R.T.	**7a** : **8a** 1 : 1
7a	K_2CO_3	H_2O	5	50°	**7a** : **8a** 1 : 1
7a	Et_3N	MeOH	12	R.T.	**7a** : **8a** 3 : 1
7a	H_2SO_4	MeOH	20 min	50°	**7a** : **8a** 1 : 6
7b	$NaHCO_3$	H_2O	5	R.T.	**8c** 100%
7b	Et_3N	EtOH	5	R.T.	**8b** 100%
7b	$EtNH_2$	EtOH	12	R.T.	**8a** 100%
7b	None	None	5 min	160—170°	**8b** 100%
8a	NaOH	H_2O	5	R.T.	**7a** 100%
8a	NaOMe	DMSO	12	R.T.	**7a** 100%
8b	NaOMe	EtOH	12	R.T.	**7b** 100%
8a	K_2CO_3	H_2O	12	R.T.	**7a** : **8a** 1 : 1

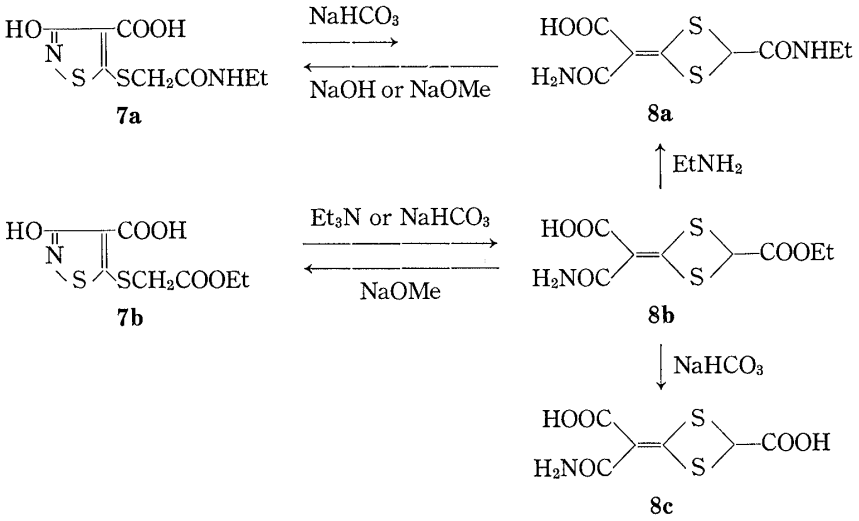

Chart 2

As shown in Table I, the rearrangement occurred reversibly in the presence of various bases. We next examined the effect of pH on the formation of **1** and **2**. At pH 8.8 the reversible interconversion of these compounds reached equilibrium after 80 min at 5° (Fig. 1). At this pH, the formation of **2** was predominant and the ratio of **2** to **1** was about 7: 3, but at pH 7—8, the position of equilibrium was shifted nearly completely in favor of **2** and the amount of **1** was negligible. The ratio of these compounds was determined by HPLC measurement using a reversed phase column.

As found in a model study, the ratio of **1** to **2** increased at high pH (above 9), but decomposition of the β-lactam ring became rapid at the same time.

However, Kikuchi *et al.* in our laboratories found that the conversion of **2** to **1** occurred nonenzymatically with Mg^{2+} as a catalyst under physiological conditions.[7] In order to utilize this reaction for the synthesis of **1** from **2**, the reaction conditions were examined. As anticipated, the reaction of **2** to **1** was greatly accelerated at pH 9 in the presence of a metal ion, such as Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+} *etc.*, as a catalyst. The formation of **1** reached 98% with Ni^{2+} at pH 8.8 and 5°, as shown in Fig. 2. The effect of Mg concentration on the formation of **1** from **2** is shown in Fig 3.

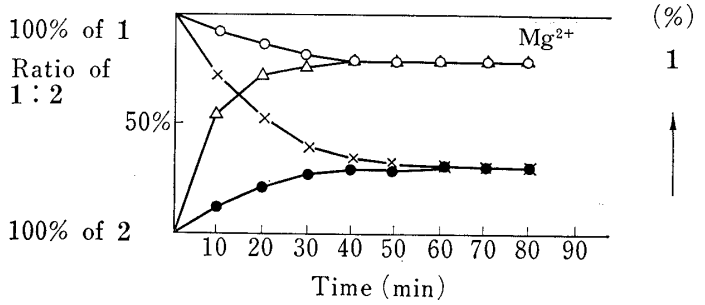

Fig. 1. Equilibrium between **1** and **2** in the Presence and Absence of Mg^{2+}

Sample conc.: 25 mg/ml; Mg^{2+} conc.; 40 mM; pH: 8.8; Temp.; 5°.

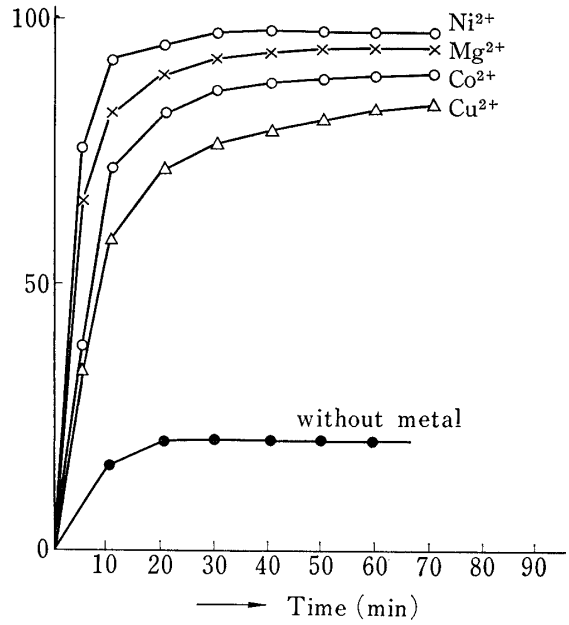

Fig. 2. Effect of Metal Ions on the Formation of **1** from **2**

Sample conc.: 25 mg/ml, Mg^{2+} conc.: 80 mM, pH: 8.8—8.9, Temp.; 5°.

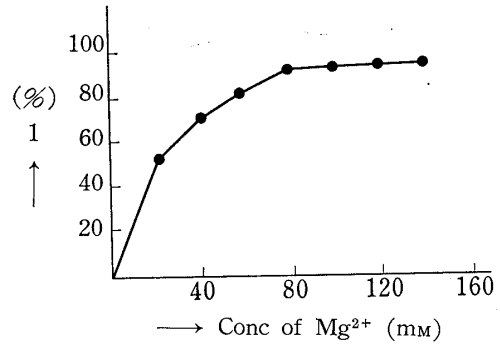

Fig. 3. Effect of Mg^{2+} Concentration on the Formation of **1** from **2**

Sample conc.: 25 mg/ml, pH: 8.8, Temp.: 5°, Time: 60 min.

We have so far described the rearrangement under basic conditions. However, it is interesting to note that the rearrangement could also occur under acidic conditions or on heating in some model compounds. Isothiazole (**7a**) was warmed at 50° in ethanol solution with a small amount of H_2SO_4 for 20 min to give 1,3-dithietane (**8a**) in good yield. Similarly, isothiazole (**7b**) when subjected to thermolysis at 160—170° for 5 min gave the corresponding 1,3-dithietane (**8b**) in a yield of 70%. The mechanisms of these reactions and of the reverse reaction catalyzed by metal ions will be reported in the near future.

The synthetic route to **1** is shown in Chart 3. Compound (**1**) was prepared from the 7β-bromoacetamido cephamycin derivative (**12**) and trisodium 4-carboxy-3-hydroxy-5-mercapto-

7) A. Tachibana, M. Komiya, Y. Kikuchi, K. Yano, and K. Mashimo, "Abstracts of Papers, 11th International Congress of Chemotherapy and 19th Interscience Conference on Antimicrobial Agents and Chemotherapy," Boston, Massachusetts, USA, 1979, p. 563.

isothiazole (**11**) in a yield of 70%. Compound (**11**) was prepared from 4-cyano-5-ethylthio-3-hydroxyisothiazole (**9**) in two steps. Hydrolysis of **9** was carried out in sodium hydroxide solution under reflux for 13 hr to afford the carboxylic acid (**10**), from which **11** was obtained by reduction with sodium in liquid ammonia in an overall yield of 60% (Chart 3).

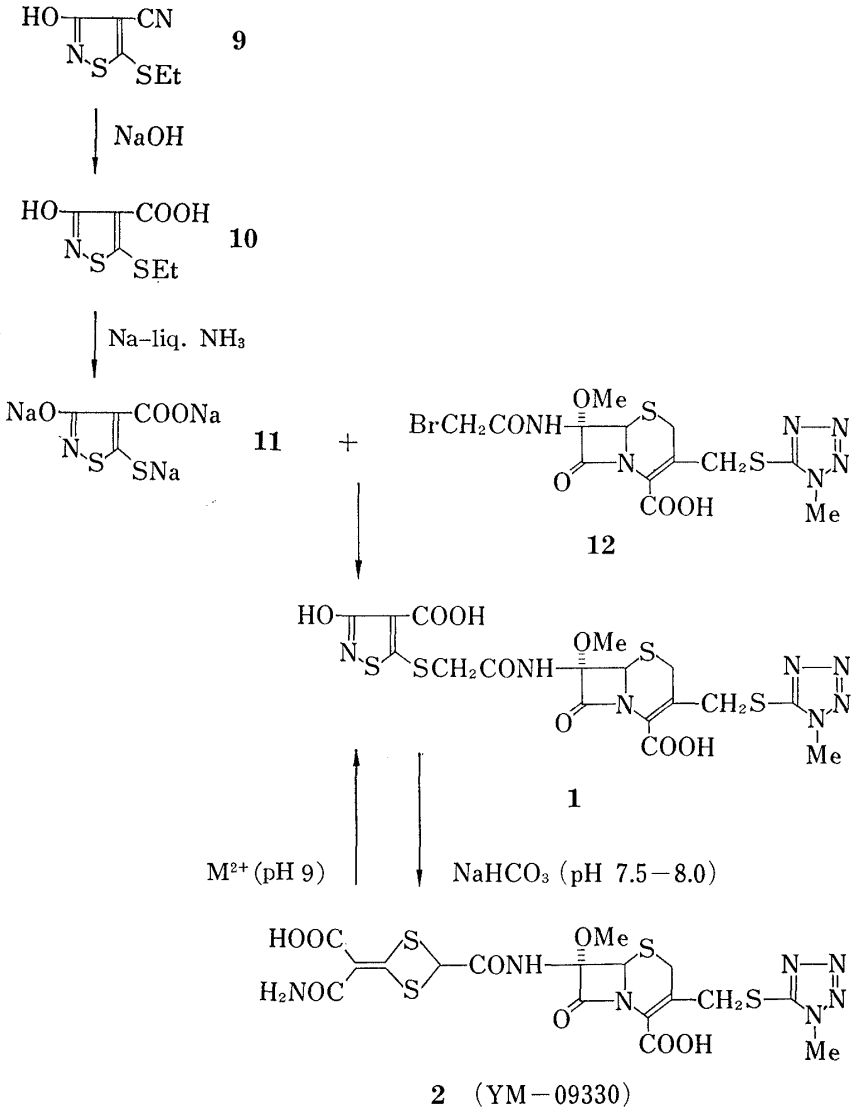

Chart 3

TABLE II. MIC Values of **2** (YM-09330), Cefmetazole (CMZ), Cefoxitin (CFX) and Cefazolin (CEZ)

Organism	MIC (mcg/ml)			
	YM-09330	CMZ	CFX	CEZ
Staph. aureus 209p	6.25	0.78	1.56	0.19
E. coli NIHJ	0.09	0.78	1.56	3.13
E. coli Ebara	0.19	0.78	0.78	25
Kleb. pneumoniae V-17	0.09	0.78	12.5	100
Proteus vulgaris OXK–U.S.	0.78	3.13	6.25	6.25
Proteus rettegeri Y-1	0.19	50	50	100
Serratia marcescens	0.39	6.25	50	100
Enterobacter aerogenes NY-2	12.5	100	100	100

The resulting compound (**1**) was converted to **2** (YM-09330) in an aqueous sodium hydrogen carbonate solution as described above.

The *in vitro* antibacterial activity of **2** (YM-09330) is presented in Table II.

It can be seen that **2** (YM-09330) has moderate antibacterial activity against gram-positive organisms. However, it has strong antibacterial activity against gram-negative organisms; it is at least 2—4 times more active than cefmetazole (CMZ)[8] or cefoxitin (CFX),[9] and is far more active than cefazolin.[10] In view of its excellent minimum inhibitory concentration (MIC), low toxicity, good protection in mice and good pharmacokinetics in animals, **2** (YM-09330) was selected for further study, and clinical trials are in progress.

Experimental

All melting points are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL MH-100 spectrometer (100 MHz) using Me_4Si as an internal standard. The following abbreviations are used; s, singlet, d, doublet, t, triplet, q, quartet. Silica gel F_{254} (Merck) TLC plates were used. For column chromatography, silica gel (Wakogel C-200) was used. High performance liquid chromatography (HPLC) was carried out using a Waters model 440 liquid chromatograph. Organic solvents used were dried over anhyd. $MgSO_4$ and all concentrations by evaporation were carried out *in vacuo*.

4-Carboxy-5-ethylthio-3-hydroxyisothiazole (10)——A mixture of 28.6 g of NaOH in 130 ml of H_2O and 26.0 g of 4-cyano-5-ethylthio-3-hydroxyisothiazole[11] was heated under reflux for 13 hr. When the reaction was over, 200 ml of H_2O was added. The reaction mixture was adjusted to pH 1.0 with 20 ml of conc. HCl and the precipitate was collected by filtration (21 g). This product (**10**) was recrystallized from MeOH. mp 201—203.5°.

Trisodium 4-Carboxy-3-hydroxy-5-mercaptoisothiazole (11)——Liquid NH_3 (150 ml) was cooled to −40° and 15 g of **10** was added portionwise at the same temperature with stirring. After stirring at room temperature for 1 hr, 80 ml of EtOH was added and NH_3 was evaporated off by bubbling N_2 gas through the solution for 1 hr. Next, 200 ml of EtOH was added to the reaction mixture. A small amount of insoluble material was filtered off and 25 ml of 50% aqueous EtOH was added to the filtrate. The white precipitate was collected by filtration (16.0 g).

7β-[4-(Carbamoyl Carboxymethylene)-1,3-dithietan-2-yl]-carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic Acid (2) (YM-09330)——Twenty-one g of **11** in 120 ml of H_2O was added dropwise with stirring to a solution of 35 g of 7β-bromoacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid (**12**) in 120 ml of MeOH at 5—10°, then a solution of 28 g of $NaHCO_3$ in 100 ml of H_2O was added. The reaction mixture was adjusted to pH 8.0 with dilute HCl. After stirring at room temperature for 5 hr, the conversion was checked by HPLC (the ratio of **1** to **2** was about 5: 95). When the reaction was over, a small amount of insoluble material was filtered off and the filtrate was poured into 200 ml of ice-water containing 45 ml of conc. HCl. The precipitate was collected by filtration (35 g). The crude product (**2**) was recrystallized from a mixture of MeOH: CH_2Cl_2 (1: 1). NMR (d_6-DMSO) δ: 3.40 (3H, s, OCH_3), 3.61 (2H, AB q, J=11 Hz, C_2 CH_2–), 3.92 (3H, s, NCH_3), 4.30 (2H, AB q, J=12 Hz, CH_2S–), 5.11, 5.14 (2H, s, C_6 H; –C(H)–H). IR ν_{max}^{KBr} cm^{-1}: 3420, 3250 (amide NH), 1770 (lactam C=O). *Anal.* Calcd for $C_{17}H_{17}N_7O_8S_4$: C, 35.48; H, 2.98; N, 17.03; S, 22.27. Found: C, 35.28; H, 3.21; N, 16.66: S, 21.83.

7β-(4-Carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic Acid (1) Method a——Compound **11** (3.6 g in 100 ml of H_2O) was added dropwise with stirring to a solution of 6.0 g of 7β-bromoacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)-thiomethyl-Δ^3-cephem-4-carboxylic acid (**12**) in 100 ml of MeOH at 0—5°. The reaction mixture was adjusted to pH 8.0 with dilute HCl. After stirring at room temperature for 30 min, the reaction mixture was adjusted to pH 2.0 with 4 N HCl and extracted with 200 ml of a mixture of *n*-BuOH: AcOEt (1: 4). The organic layer was washed twice with 50 ml of H_2O and satd. NaCl solution. The extract was concentrated and the residue was triturated with ether. The white precipitate of **1** was collected by filtration (5.6 g). This crude product (**1**) was recrystallized from a mixture of methyl ethyl ketone: acetone (1: 1). NMR (d_6-DMSO) δ: 3.41 (3H, s, OCH_3), 3.58 (2H, AB q, J=12 Hz, C_2 CH_2–), 3.93 (3H, s, NCH_3), 3.99 (2H, s, $-CH_2CO$), 4.28 (2H,

8) A semisynthetic cephamycin given the code number CS-1170; H. Nakano, H. Yanagisawa, B. Shimizu, M. Kaneko, M. Nagano, and S. Sugawara, *J. Antibiotics.*, **29**, 554 (1976).
9) A semisynthetic cephamycin; J. Birnbaum, O. Staplay, A.K. Miller, H. Wallich, D. Hendlin, and W.B. Woodruff, *J. Antimicrobial Ag. Chemother.*, **4**, 15 (1978).
10) A semisynthetic cephalosporin; M. Nishida, T. Matsubara, T. Murakawa, Y. Mine, and Y. Yokota, *J. Antibiotics.*, **23**, 137 (1970).
11) W.R. Hatchard, *J. Org. Chem.*, **28**, 2163 (1963).

AB q, $J=13$ Hz, CH_2S–), 5.10 (1H, s, C_6–H). *Anal.* Calcd for $C_{17}H_{17}N_7O_8S_4$: C, 35.48; H, 2.98; N, 17.03; S, 22.27. Found: C, 35.29; H, 2.99; N, 16.76; S, 22.83.

Method b——Compound **2** (20 g) was added portionwise to a solution of 40 g of $NaHCO_3$ and 8.0 g of $MgSO_4$ in 200 ml of H_2O at room temperature. The mixture was then cooled to 0—5° in an ice bath. About 55 ml of 2.8% of aqueous NH_3 was added and the mixture was adjusted to pH, 9.0. After stirring at the same temperature for 1 hr, the conversion was checked by HPLC (the ratio of **1** to **2** was about 95: 5). The mixture was poured into 200 ml of ice-water containing 80 ml of conc. HCl with vigorous stirring, and the precipitate of **1** was extracted with a mixture of *n*-BuOH: AcOEt (1: 4). The extract was concentrated and the residue was triturated with ether. The white precipitate of **1** was collected by filtration (16 g). The crude product (**1**) was recrystallized from a mixture of methyl ethyl ketone: acetone (1: 1).

4-(Cyano Methoxycarbonylmethylene)-1,3-dithietane-2-carboxylic Acid (5)——A solution of 2.0 g of sodium 2,2-bis(cyano methoxycarbonyl)ethylene-1,1-dithiolate in 20 ml of MeOH was treated with 2.0 g of sodium dibromoacetate. After stirring at room temperature for 3 hr, the reaction mixture was diluted with 20 ml of H_2O and adjusted to pH 2 with 10% HCl. The mixture was extracted with 100 ml of AcOEt. Concentration of the extract gave the product (**5**). This product was used immediately for the next reaction without further purification, because it is unstable and decomposes slowly at room temperature. NMR (d_6-DMSO) δ: 3.88 (3H, s, $COOCH_3$), 5.20 (1H, s, –C–H).

Methyl 7β-[4-(Cyano Methoxycarbonylmethylene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylate (4)——A mixture of 600 mg of **5** and 600 mg of PCl_5 was stirred at 5—10° for 30 min. The reaction mixture was added dropwise to a solution of 700 mg of methyl 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylate (**6**) and 1.0 ml of pyridine in 10 ml of CH_2Cl_2 at −25——20°. After stirring at room temperature for 1 hr, 10 ml of CH_2Cl_2 and 10 ml of H_2O were added successively. After removal of the solvent, the residue was purified by silica gel column chromatography using $CHCl_3$: acetone (2: 1) to afford 300 mg of **4**. NMR ($CDCl_3$) δ: 3.59 (3H, s, C_7–OCH_3), 3.50 (2H, C_2 CH_2–), 3.82, 3.90, 3.94 (9H, s, $-NCH_3$, $COOCH_3$, $COOCH_3$), 4.40 (2H, AB q, $J=13$ Hz, CH_2S–), 5.08, 5.22 (2H, s, C_6–H, –C–H). IR ν_{max}^{KBr} cm^{-1}: 2200 (CN), 1780 (lactam C=O).

Methyl 7β-[4-(Carbamoyl Methoxycarbonylmethylene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylate (3)——An ethereal solution of 3% diazomethane was added slowly to a solution of 6.0 g of **2** in 25 ml of ether with stirring until the evolution of N_2 ceased. After concentration, the residue was dissolved in 300 ml of $CHCl_3$ and a small amount of insoluble material was filtered off. After removal of the solvent, the residue was purified by silica gel column chromatography using a mixture of $CHCl_3$: acetone (2: 1) as an eluent to give the product (**3**) (3.0 g). NMR ($CDCl_3$) δ: 3.50 (2H, C_2–CH_2), 3.53, 3.58 (3H, s, C_7–OCH_3), 3.77, 3.89, 3.95 (9H, s, NCH_3, $COOCH_3$, $COOCH_3$), 4.40 (2H, AB q, $J=14$ Hz, CH_2S–), 4.96, 5.07, 5.12, 5.22 (2H, s, C_6–H, –C–H). IR ν_{max}^{KBr} cm^{-1}: 2100 (CN), 1780 (lactam C=O).

Methyl 7β-[4-(Cyano Methoxycarbonylmethylene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylate (4)——A solution of 120 mg of **3** in 2.2 ml of $CHCl_3$ was cooled at −15°, and 0.075 ml of pyridine and 125 mg of PCl_5 was added successively. After stirring at room temperature for 10 min, the solution was diluted with 30 ml of $CHCl_3$ and the reaction mixture was washed with 10 ml of 1 N HCl and 20 ml of H_2O successively. After concentration, the residue was purified by silica gel column chromatography using a mixture of $CHCl_3$: acetone (10: 3) as an eluent to give the product (**4**) (50 mg). The spectral data for this product were in complete accord with those for the authentic sample (**4**) described above.

4-Carboxy-5-ethoxycarbonylmethylthio-3-hydroxyisothiazole (7b)——Compound **11** (14.9 g in 100 ml of H_2O) was added dropwise to a solution of 8.4 g of ethyl bromoacetate in a mixture of 50 ml of MeOH and 30 ml of H_2O at 10°. The reaction mixture was adjusted to pH 7 throughout the reaction by adding 10% HCl. After stirring for 30 min at the same temperature, the reaction mixture was acidified with conc. HCl and the precipitate that separated was collected by filtration (11 g). The product was recrystallized from EtOH. mp 160—162°. NMR (d_6-DMSO) δ: 1.26 (3H, t, $J=7.1$ Hz, $-CH_2C\underline{H}_3$), 4.08 (2H, q, $J=7.1$ Hz, $-C\underline{H}_2CH_3$). IR ν_{max}^{KBr} cm^{-1}: 3250 (–OH), 1700 (ester C=O). MS *m/e*: 263 (M^+).

2-(Carbamoyl Carboxymethylene)-4-ethoxycarbonyl-1,3-dithietane (8b)——Triethylamine (25 ml) was added to a suspension of 2.5 g of **7b** in 30 ml of EtOH, giving a clear solution. The solution was stirred at room temperature for 5 hr then 10 ml of H_2O was added. The product (**8b**) that separated was collected by filtration and washed with H_2O (2.1 g). mp 232—234° (dec.). NMR (d_6-DMSO) δ: 1.26 (3H, t, $J=7.6$ Hz, $-CH_2C\underline{H}_3$), 4.20 (2H, q, $J=7.6$ Hz, $-C\underline{H}_2CH_3$), 5.13 (1H, s, –C–H). IR ν_{max}^{KBr} cm^{-1}: 3420, 3260 (amide NH), 1718 (ester C=O). MS *m/e*: 263 (M^+).

4-Carboxy-5-ethylcarbamoylmethylthio-3-hydroxyisothiazole (7a) Method a——Compound **11** (18 g in 50 ml of H_2O) was added dropwise to a solution of 10 g of *N*-ethylbromoacetamide in a mixture of 20 ml of MeOH and 10 ml of H_2O at 10—15°. After stirring at room temperature overnight, the reaction mixture was diluted with 50 ml of H_2O and acidified with 10% HCl. The product (**7a**) that separated was collected by filtration (13 g). It was recrystallized from MeOH. mp 210—212° (dec.). NMR (d_6-DMSO) δ: 1.08 (3H, t, $J=7.8$ Hz, $-CH_2C\underline{H}_3$), 3.17 (2H, q, $J=7.8$ Hz, $-C\underline{H}_2CH_3$), 3.87 (2H, s, CH_2S–). IR ν_{max}^{KBr} cm^{-1}: 3250 (–OH), 1700 (ester C=O). MS *m/e*: 262 (M^+).

7a from 8a Method b——A solution of 0.2 g of **8a** dissolved in 2 ml of H_2O containing 1.7 ml of 1 N NaOH was stirred for 5 hr. When the reaction was over, the reaction mixture was acidified with 10% HCl to give 0.12 g of **7a**. mp 210—212°. The product was identified by comparison of the spectral data with those for **7a** prepared by Method a.

7a from 8a Method c——A solution of 131 mg of **8a** in 3 ml of DMSO was treated with 1.07 ml of 1 N MeONa at room temperature. After stirring at room temperature overnight, the reaction mixture was adjusted to pH 1.4 with 6 N HCl and the crystals that separated were collected by filtration (95 mg). mp 210—212°. The product was identified by comparison of the spectral data with those for the product of Method a.

2-(Carbamoyl Carboxymethylene)-4-ethylcarbamoyl-1,3-dithietane (8a) Method a——A mixture of 2.0 g of **7a** and 2.9 g of 70% ethylamine in 38 ml of MeOH was stirred at room temperature for 4 hr. When the reaction was over, the solvent was evaporated off and 20 ml of H_2O was added to the residue. The mixture was then acidified with 6 N HCl to pH 2. The white crystals of **8a** that separated were collected by filtration (1.3 g). mp 290—293° (dec.). NMR (d_6-DMSO) δ: 1.06 (3H, t, J=8.0 Hz, $-CH_2\underline{CH_3}$), 3.15 (2H, q, J=8.0 Hz, $-\underline{CH_2}CH_3$), 4.94 (1H, s, $-\underline{C}-H$). IR ν_{max}^{KBr} cm^{-1}: 3440, 3250 (amide NH), 1680 (ester C=O). MS m/e: 262 (M^+).

8a Method b——The isothiazole (**7a**) (2.0 g) was suspended in 50 ml of EtOH containing one drop of conc. H_2SO_4 and the mixture became turbid again on completion of the reaction. After cooling the mixture, the precipitate was collected by filtration (1.2 g). The product was identified by comparison of spectral data with those for **8a** prepared by Method a.

2-(Carbamoyl Carboxymethylene)-4-carboxy-1,3-dithietane (8c)——A solution of 10 g of **7b** and 20.3 g of $NaHCO_3$ in 254 ml of H_2O was stirred at 19—24° for 3 hr. When the reaction was over, the solution was adjusted to pH 1.4 with 6 N HCl and the crystals of **8c** that separated were collected by filtration (7.2 g). mp 175—178° (dec.). NMR (d_6-DMSO) δ: 5.09 (1H, s, $-\underline{C}-H$), 7.58 (2H, $CONH_2$), 10.8 (COOH). IR ν_{max}^{KBr} cm^{-1}: 3440, 3180 (amide NH), 1735 (ester C=O). MS m/e: 262 (M^+).

8b —By Thermal Rearrangement——The isothiazole (**7b**) (1.0 g) was heated at 160—170° for 10 min. After cooling the product was washed with ethanol to give 0.7 g of **8b**. mp 232—234°. The product was identified by comparison of the spectral data with those for **8b** prepared by Method a.

Measurement of Product Ratio (1 : 2)——The sample solution was analyzed under the operating conditions mentioned below. Column, 15 × 0.4 cm i.d.; packing, Lincosorb RP-18 (Merck); mobile phase, 0.05 M KH_2PO_4–MeOH (4 : 1); column temperature, 20°; flow rate, 1.0 ml/min; injection volume, 2 μl.

a) Sample Solution A (without $MgSO_4$): A solution containing 500 mg of **2** (or **1**) and 500 mg of $NaHCO_3$ dissolved in about 10 ml of H_2O was adjusted to pH 8.8 with 2.7% NH_3 aqueous solution at 5°. The volume of the solution was adjusted to 20 ml with H_2O, and a 0.1 ml aliquot of the solution was diluted with 1.0 ml of solvent A. The peak area was measured with an integrator. The retention times of **2** and **1** were about 1.9 min and 3.5 min, respectively.

b) Sample Solution B (with $MgSO_4$): A sample solution containing 500 mg of **2** (or **1**), 500 mg of $NaHCO_3$ and $MgSO_4$ (192 mg, 80 mM) dissolved in about 10 ml of H_2O was adjusted to pH 8.8 with 2.7% NH_3 aqueous solution at 5°. The volume of the solution was adjusted to 20 ml with H_2O. The product ratio was determined by the method described above.

c) Sample Solution C (with Various Metals): $NiSO_4$, $FeSO_4$, $CoSO_4$, or $CuSO_4$ (80 mM) was used instead of $MgSO_4$ and other operations were carried out as described above.

d) Sample Solution D (Effect of $MgSO_4$): $MgSO_4$ (40—160 mM) was added to the sample solution containing **2** and $NaHCO_3$, and other operations were carried out as described above. The data are presented in Figs. 1—3.

Measurement of the Minimum Inhibitory Concentration (MIC)——The MICs were determined by the standard method of the Chemotherapeutic Society of Japan.[12] The data are presented in Table II.

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12) Standard method of MIC measurement; *Chemotherapy*, **22**, 1126 (1974).