SYNTHESIS AND BIOLOGICAL ACTIVITY OF (CYCLOPENTENO-PYRIDINIUM)THIOMETHYLCEPHALOSPORINS†

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Substituted (cyclopentenopyridinium)thiomethyl groups were introduced as C-3 substituents of (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-oxyimino]acetamidocephalosporins. Structure-activity relationships of this class of cephalosporins are discussed on the basis of their MIC. The selected compounds, **3a** and **4a** (ME1221), having an acidic substituent, showed excellent *in vivo* efficacy and low toxicity.

The discovery of the aminothiazolylmethoxyimino side chain at C-7 was one of a breakthrough in the field of β-lactam antibiotics.[2] Since then many efforts aimed at synthesizing more effective analogs were made by many researchers. This has led to development of cefotaxime,[3] cefmenoxime,[4] ceftriaxone,[5] ceftazidime[6] and others by modifying C-3 and oxime substituents.

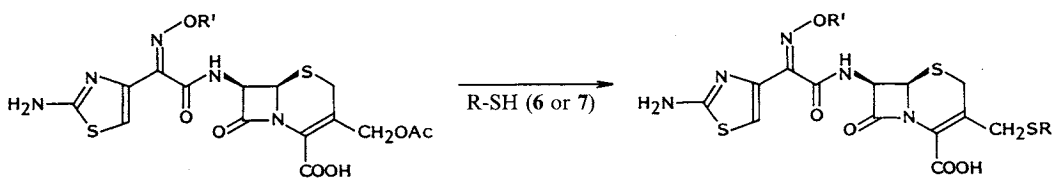
2,3-Cyclopentenopyridine was first introduced into the cephem nucleus as the C-3 methylene substituent of cefpirome.[7] We introduced substituted (cycloalkenopyridinium)thiomethyl groups as the C-3 substituents. In this paper the synthesis and biological activity of the title compounds are reported.

Chemistry

Introduction of (cyclopentenopyridinium)thiomethyl groups at C-3 was carried out by substitution of the acetoxy group in **1** with cyclopenteno- or cyclohexenopyridinethiones (Chart 1). Compounds **2** and **3** (Tables 1 and 2) were prepared from cefotaxime, and **4** and **5** (Tables 3 and 4) from substituted oxyimino compounds.

1-Methylpyridinethiones with cyclopenteno (**6a**~**6f**) and cyclohexeno (**6h** and **6i**) rings, 2,3-cyclopenteno-1*H*-pyridine-4-thione (**6j**), and 2,3-dihydro-1*H*-indolizine-5-thione (**6g**), used for preparation

Chart 1.

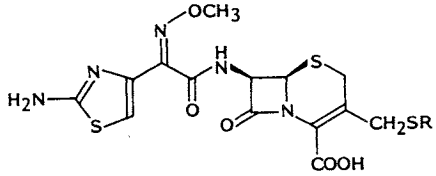


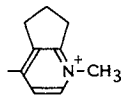
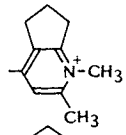
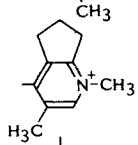
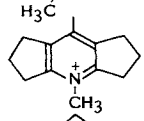
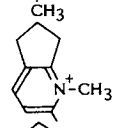
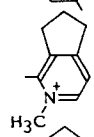
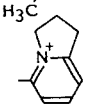
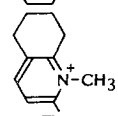
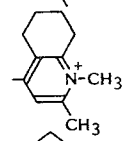
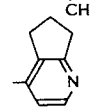
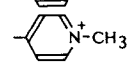
1 **2**~**5**

R : Cyclopenteno- or cyclohexenopyridinium shown in Tables 1 and 2.
R': Shown in Tables 3 and 4.

† A part of this work was presented at the 14th International Conference of Chemotherapy held in Kyoto in June 23~28, 1985.[1]

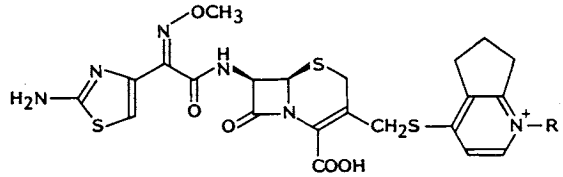
Table 1. Antibacterial activities of compounds **2** (MIC, μg/ml).



Compound	R	*S.a.*	*E.c.*-1	*E.c.*-2	*K.p.*	*P.r.*	*E.cl.*	*P.a.*
2a		0.05	0.05	0.10	0.05	0.39	0.10	6.25
2b		0.10	0.20	0.20	0.20	0.39	0.20	6.25
2c		0.20	0.20	0.39	0.39	1.56	0.39	12.5
2d		0.20	0.78	0.78	0.78	1.56	0.78	12.5
2e		0.20	0.05	0.10	0.05	0.39	0.10	12.5
2f		0.78	0.39	0.39	0.39	3.13	0.39	50
2g		0.20	0.10	0.10	0.10	0.78	0.10	12.5
2h		0.10	0.05	0.10	0.10	1.56	0.10	25
2i		0.10	0.78	0.78	0.78	1.56	0.78	12.5
2j		0.05	0.78	1.56	0.39	0.39	0.78	12.5
2k		0.20	0.05	0.20	0.05	0.39	0.05	6.25
Cefpirome		0.39	0.05	0.20	0.05	0.78	0.10	3.13

Abbreviations: *S.a.*, *Staphylococcus aureus* 209P JC-1; *E.c.*-1, *Escherichia coli* NIHJ JC-2; *E.c.*-2, *E. coli* 255; *K.p.*, *Klebsiella pneumoniae* PCI 602; *P.r.*, *Providencia rettgeri* J-0026; *E.cl.*, *Enterobacter cloacae* G-0008; *P.a.*, *Pseudomonas aeruginosa* MB-3833.

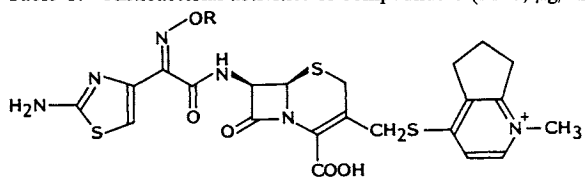
Table 2. Antibacterial activities of compounds **3** (MIC, μg/ml).

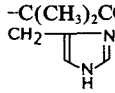
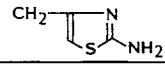


Compound	R	*S.a.*	*E.c.*-1	*E.c.*-2	*K.p.*	*P.r.*	*E.cl.*	*P.a.*
3a	$-CH_2COOH$	0.78	≦0.025	0.39	≦0.025	0.39	0.10	6.25
3b	$-CH_2CH{=}CH_2$	0.05	0.20	0.20	0.10	0.78	0.39	12.5
3c	$-CH_2CH_2OH$	0.10	0.20	0.20	0.20	1.56	0.39	12.5
3d	$-CH_2CONH_2$	0.10	0.10	0.20	0.20	0.39	0.20	12.5
3e	$-CH_2CH_2NMe_2$	0.39	0.20	0.20	0.20	1.56	0.39	25
3f	$\rightarrow O$	0.39	0.78	6.25	0.78	1.56	0.78	25
3g	$-CH_2CN$	0.10	0.05	0.10	0.05	0.78	0.10	6.25
3h	$-CH_2CH_2SO_3H$	0.78	≦0.025	0.39	≦0.025	0.78	0.05	12.5
3i	$-CH_2SCH_3$	0.10	0.20	0.20	0.20	3.13	0.78	12.5
3j		0.10	0.20	0.39	0.39	0.78	0.39	25
3k	$-CH_2CF_3$	0.20	0.20	0.39	0.39	0.78	0.20	12.5

Abbreviations: See the footnote of Table 1.

Table 3. Antibacterial activities of compounds **4** (MIC, μg/ml).



Compound	R	*S.a.*	*E.c.*-1	*E.c.*-2	*K.p.*	*P.r.*	*E.cl.*	*P.a.*
4a	$-CH_2COOH$	1.56	0.05	0.39	0.10	0.10	0.10	6.25
4b	$-CH_2CONH_2$	0.20	0.05	≦0.025	0.05	0.78	0.20	12.5
4c	$-CH_2CONHCH_3$	0.20	0.20	0.39	0.20	1.56	0.20	12.5
4d	$-C(CH_3)_2COOH$	1.56	0.39	1.56	0.20	1.56	0.39	3.13
4e		0.20	0.10	0.20	0.10	0.78	0.10	25
4f		0.05	0.78	0.78	0.78	0.78	0.78	12.5

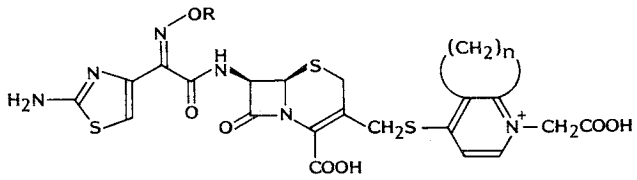
Abbreviations: See the footnote of Table 1.

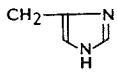
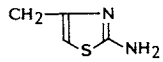
of the compounds in Table 1, were obtained by sulfurization of corresponding pyridone derivatives with phosphorous pentasulfide. 1-Substituted 2,3-cyclopenteno-1*H*-pyridine-4-thiones (**7a**~**7k**), used for preparation of **3**, were derived from 2,3-cyclopenteno-4-pyrone (**8**)[8] by the two methods outlined in Chart 2.

Antibacterial Activity

The antibacterial activities (MIC) of (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]cephalosporins having (cycloalkenopyridinium)thiomethyl groups at C-3 (**2a**~**2i**) are compared with those of cyclopentenopyridinylthiomethyl compound **2j**, the pyridinium compound **2k** and cefpirome[7] in Table 1. Among these compounds, **2a** possessing 1-methyl-2,3-cyclopentenopyridinium-4-ylthiomethyl group showed the best antibacterial activity, but possesed low solubility in water and relatively high acute

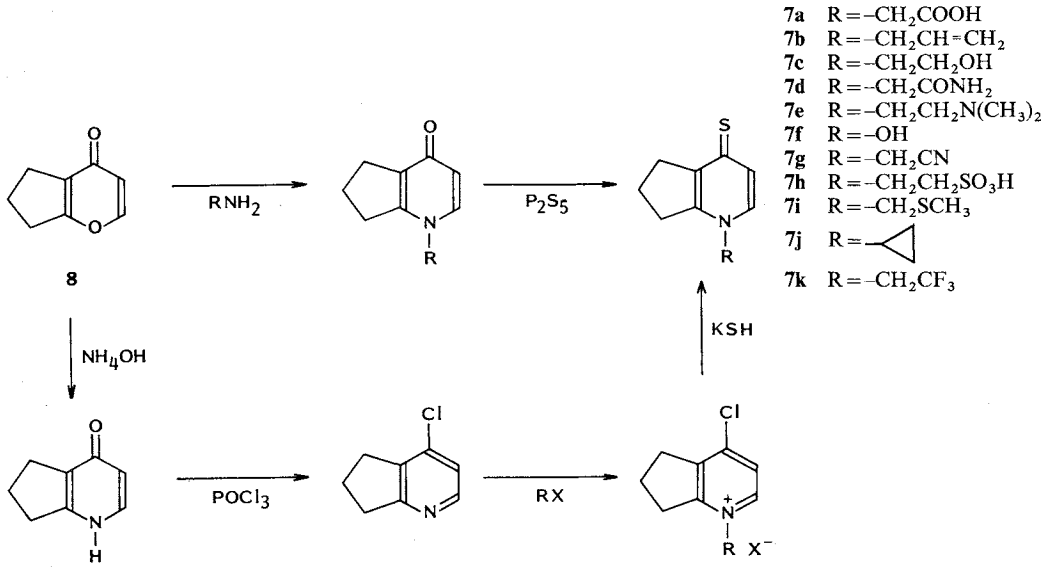
Table 4. Antibacterial activities of compounds **5** (MIC, μg/ml).



Compound	R	n	*S.a.*	*E.c.*-1	*E.c.*-2	*K.p.*	*P.r.*	*E.cl.*	*P.a.*
5a	$-CH_2CH_3$	3	0.78	0.20	1.56	0.20	0.78	0.39	12.5
5b	$-CH_2CH_2F$	3	0.78	0.05	≦0.025	0.05	1.56	0.20	12.5
5c	$-H$	3	0.20	0.05	0.39	0.10	0.39	0.20	25
5d	$-CH_2CONH_2$	3	1.56	0.05	0.78	≦0.025	0.39	0.05	6.25
5e	$-CH=CH_2$	3	0.78	0.20	1.56	0.20	0.78	0.39	25
5f	$-CHF_2$	3	0.78	0.05	0.39	0.05	0.78	0.20	12.5
5g		3	0.39	0.78	3.13	0.39	1.56	0.78	25
5h		3	0.78	0.05	0.78	0.05	0.39	≦0.025	6.25
5i		3	0.39	0.39	3.13	0.39	0.78	0.78	12.5
5j	$-CH_2CH_3$	0	0.39	0.10	1.56	0.05	0.20	0.20	12.5

Abbreviations: See the footnote of Table 1.

Chart 2.



toxicity (LD_{50} < 1 g/kg, mice, iv).

Next we introduced various substituents into pyridinium nitrogen to improve these properties and gave compounds **3a**~**3k** (Table 2). Although **3a** with acidic substituent showed slightly decreased activity against *Staphylococcus aureus* to compare with those of **2a**, the solubility in water was improved and showed low acute toxicity (LD_{50} > 3 g/kg, mice, iv).

Several substituents, mainly carboxylic acid and azole derivatives, were introduced into oxime oxygen of **2a** to replace methyl group and gave compounds **4a**~**4f** (Table 3). Introduction of carboxymethyl group

(**4a**) maintained the same Gram-negative activity including anti-pseudomonal activity, but decreased activity against *S. aureus* to compare with those of **2a**. Compound **4a** showed improved water solubility and low acute toxicity ($LD_{50}>3$ g/kg, mice, iv). Among two azole derivatives, **4f** showed good anti-staphylococcal activity, but had low water solubility.

The methyl group of oxime substituent in **3a** was replaced by some substituents and compounds **5a**~**5i** were obtained (Table 4). The hydroxyl derivative **5c** possesed improved activity against *S. aureus*. The fluorinated analogs **5b** and **5f** were as active as **3a** except against *Proteus rettgeri*. The ethyl derivative **5a** decreased anti-Gram-negative activity to compare with both **3a** and non-cyclopenteno compound **5j** (ME1220).[1] It was noteworthy that introduction of cyclopenteno ring into *N*-carboxymethylpyridinium substituent (**5j**→**5a**) decreased anti-staphylococcal activity, while introduction into *N*-methylpyridinium substituent (**2k**→**2a**) did not.

Further Evaluation of Compounds **3a** and **4a**

Compounds **3a** and **4a** (ME1221), with acidic substituent, were selected for further evaluation on the basis of MIC, solubility in water and acute toxicity. Their antibacterial activities against the β-lactamase producing strains are shown in Table 5. Compound **4a** was less active against Gram-positive bacteria than

Table 5. Antibacterial activities of **3a** and **4a** against β-lactamase producing strains (MIC, μg/ml).

Test organism	Type of β-lactamase[a]	**3a**	**4a**	Ceftazidime	Cefpirome
Staphylococcus aureus 606	PCase	1.56	3.13	6.25	0.78
Escherichia coli W3630 RGN823	PCase(IIIa)	0.05	0.05	0.20	0.20
E. coli W3630 RGN238	PCase(Va)	0.39	0.10	0.20	0.10
E. coli GN206	CSase(Ib)	0.10	0.10	0.39	0.05
Klebsiella pneumoniae GN69	PCase(IV)	≦0.025	≦0.025	0.10	0.05
Proteus mirabilis GN79	PCase(IIb)	0.39	0.20	0.20	0.39
Proteus vulgaris GN76	CSase(Ic)	0.10	≦0.025	0.05	0.20
Morganella morganii 1510	CSase(Ia)	6.25	1.56	12.5	0.39
Providencia rettgeri GN624	CSase(Ia)	0.78	≦0.025	0.78	0.20
Enterobacter cloacae GN7471	CSase(Ia)	6.25	0.78	3.13	0.20
Serratia marcescens GN10857	CSase(Ia)	6.25	0.78	0.78	1.56
Pseudomonas aeruginosa GN10362	CSase(Id)	25	6.25	1.56	3.13
P. aeruginosa M-0148	PCase(V)	25	6.25	1.56	6.25

[a] PCase: Penicillinase,[21] CSase: cephalosporinase.[21]

Table 6. *In vivo* activities of **3a** and **4a**.

Test organism	Challenge dose (cfu/mouse)	ED_{50}, mg/mouse (MIC μg/ml)			
		3a	**4a**	Ceftazidime	Cefpirome
Staphylococcus aureus Smith(I)	4.6×10^7	0.02	0.04	—	0.02
		(0.78)	(1.56)	—	(0.39)
Escherichia coli GN206	3.1×10^6	0.26	0.06	0.25	0.04
		(0.10)	(0.10)	(0.39)	(0.05)
Klebsiella pneumoniae PCI602	6.4×10^5	1.2	0.60	1.5	1.2
		(≦0.025)	(0.05)	(0.20)	(0.10)
Proteus mirabilis GN79	4.6×10^7	0.78	0.64	0.38	0.50
		(0.39)	(0.20)	(0.20)	(0.39)
Pseudomonas aeruginosa GN10362	6.0×10^4[a]	0.13	0.19	0.16	0.82
		(25)	(6.25)	(1.56)	(3.13)

[a] Twice dosing at 1 and 3 hours after challenge.

Table 7. Pharmacokinetic parameters in rats.

	3a	4a	Ceftazidime
$T_{1/2}\beta$ (minutes)	57.3±9.3	65.6±26.6	48.1±7.7
$AUC_{0-\infty}$ (μg·minutes/ml)	2,647±554	4,431±1,562	2,570±620
Vd (liter/kg)	0.53±0.05	0.38±0.07	0.48±0.07

Dose: 20 mg/kg, iv: mean±SD (n=3).

3a and cefpirome, but showed good activity against Gram-negative cephalosporinase-producing strains. As shown in Table 6, both **3a** and **4a** possessed comparable *in vivo* activity to those of reference compounds. It was noteworthy that *in vivo* anti-pseudomonal activity of **3a** and **4a** were almost same as that of ceftazidime in spite of their higher MIC values.

The pharmacokinetic parameters of **3a** and **4a** are shown in Table 7. AUC of compound **4a** was higher than that of **3a** and ceftazidime.

Experimental

Methods

NMR spectra were recorded on Varian T-60, Varian XL100 or Jeol GX400 spectrometer using TMS as a reference. MICs of synthesized cephalosporins were determined in 2-fold agar dilution method using sensitivity disk agar (modified Mueller-Hinton), and inoculum size was 10^6 cfu/ml according to the method of Japan Society of Chemotherapy. For determination of *in vivo* activities, eight male ICR mice per group were used. The animals were inoculated intraperitoneally with pathogens, and were intravenously given test compounds dissolved in distilled water 1 hour after challenge unless otherwise stated. The ED_{50} values (mg/mouse) were determined by the probit method from survival mice rate after 1 week. The pharmacokinetic parameters were taken after iv dosing of 20 mg/kg to three rats. Serum concentration of antibiotics was determined by HPLC of Nucleosil C_{18} with MeOH-2% AcOH as a developing solvent.

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(2,3-cyclopenteno-1-carboxymethylpyridinium-4-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (3a)

To a solution of cefotaxime (sodium salt, 720 mg) in 12 ml of 50% aqueous acetonitrile solution were added 350 mg of 1-carboxymethyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**7a**) and 2.25 g of sodium iodide. This solution was adjusted to pH 7 with aq $NaHCO_3$. The solution was stirred at 70°C for 8 hours, concentrated and added to 70 ml of acetone. The precipitate was collected, dissolved in water and chromatographed on a column of Diaion HP-20. The column was washed with water and eluted with 15% aq acetone. The fractions contained **3a** were collected, concentrated and lyophylized to give 560 mg of **3a**: ^{1}H NMR (D_2O) δ 2.16 (2H, m), 2.85 (2H, t), 3.03 (2H, t), 3.48 (2H, ABq), 3.86 (3H, s), 4.18 (2H, ABq), 4.81 (2H, s), 5.07 (1H, d), 5.63 (1H, d), 7.52 (1H, d), 8.05 (1H, d); SI-MS *m/z* 605 $(M+H)^+$.

Compounds 2a~2k and 3b~3k

These compounds were prepared in an analogous way of **3a** from cefotaxime and corresponding pyridinethiones (**6** and **7**).

Compounds 4a~4f

These compounds were prepared in an analogous procedure for **3a** from corresponding (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(substituted oxyimino)acetamido]-3-acetoxymethylceph-3-em-4-carboxylic acid sodium salts[9~11] and 1-methyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**6a**). **4a**: ^{1}H NMR (D_2O) δ 2.16 (2H, m), 2.85 (2H, m), 3.11 (2H, t), 3.47 (2H, ABq), 3.90 (3H, s), 4.16 (2H, ABq), 4.42 (2H, s), 5.05 (1H, d), 5.61 (1H, d), 6.82 (1H, s), 7.54 (1H, d), 8.03 (1H, d); SI-MS *m/z* 605 $(M+H)^+$.

Compounds **5a**~**5i**

These compounds were prepared in an analogous way of **3a** from corresponding (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(substituted oxyimino)acetamido]-3-acetoxymethylceph-3-em-4-carboxylic acid sodium salts[3,9~15] and 1-carboxymethyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**7a**).

2,3-Cyclopenteno-1-methyl-1*H*-pyridine-4-thione (**6a**)

A mixture of 2.05 g of 2,3-cyclopenteno-4-pyrone,[8] 20 ml of dioxane and 20 ml of 40% aqueous methylamine in a sealed tube was heated at 100°C for 15 hours. The reaction mixture was concentrated, and the residue was suspended in 50 ml of ethyl acetate. After the mixture was stirred for 1 hour at room temperature, the precipitate was filtered and dried to give 1.63 g of 2,3-cyclopenteno-1-methyl-4(1*H*)-pyridone. The pyridone (1.18 g) was mixed well with 1.78 g of phosphorous pentasulfide and heated at 140°C for 2 hours. To the cooled reaction mixture was added 1 N NaOH to adjust pH 7.5. The mixture was extracted with chloroform. The chloroform layer was dried over $MgSO_4$ and concentrated. The residue was washed with ethyl acetate to give 840 mg of **6a**, which was recrystallized from chloroform; yellow needles; mp 213~213.5°C; ^{1}H NMR ($CDCl_3$) δ 2.16 (2H, m), 2.99 (2H, t), 3.03 (2H, t), 3.71 (3H, s), 7.07 (1H, d), 7.26 (1H, d).

Anal Calcd for $C_9H_{11}NS$: C 65.41, H 6.71, N 8.48, S 19.40.
Found: C 65.36, H 6.65, N 8.40, S 19.51.

Compounds **6b**, **6c**, **6d**, **6i**, **6j**, **7e**, **7j** and **7k**

These compounds were prepared in an analogous way of **6a** from corresponding pyrones[8,16] and appropriate amines. 2,3-Cyclopenteno-1,6-dimethyl-1*H*-pyridine-4-thione (**6b**): MP 250°C (dec); ^{1}H NMR ($CDCl_3$) δ 2.12 (2H, m), 2.34 (3H, s), 2.98 (2H, t), 3.06 (2H, t), 3.62 (3H, s), 7.23 (1H, s). 2,3-Cyclopenteno-1,5-dimethyl-1*H*-pyridine-4-thione (**6c**): MP >250°C; ^{1}H NMR (DMSO-d_6) δ 2.07 (2H, m), 2.15 (3H, s), 2.81 (2H, t), 3.07 (2H, t), 3.69 (3H, s), 7.71 (1H, s). 2,3-5,6-Dicyclopenteno-1-methyl-1*H*-pyridine-4-thione (**6d**): MP >250°C; ^{1}H NMR ($CDCl_3$) δ 2.11 (4H, m), 2.87 (4H, t), 3.06 (4H, t), 3.64 (3H, s). 2,3-Cyclohexeno-1,6-dimethyl-1*H*-pyridine-4-thione (**6i**): MP 206~207°C; ^{1}H NMR ($CDCl_3$) δ 1.60~2.0 (4H, m), 2.36 (3H, s), 2.70 (2H, t), 2.86 (2H, t), 3.61 (3H, s), 7.42 (1H, s). 2,3-Cyclopenteno-1*H*-pyridine-4-thione (**6j**): MP 147~148.5°C; ^{1}H NMR ($CDCl_3$) δ 2.01 (2H, m), 2.74 (2H, t), 2.93 (2H, t), 7.04 (1H, d), 7.39 (1H, d). 2,3-Cyclopenteno-1-(2-dimethylamino)ethyl-1*H*-pyridine-4-thione (**7e**): MP 169~171°C; ^{1}H NMR ($CDCl_3$) δ 2.22 (6H, s), 1.95~2.3 (2H, m), 2.60 (2H, t), 2.85~3.25 (4H, m), 3.92 (2H, t), 7.14 (1H, d), 7.28 (1H, d). 2,3-Cyclopenteno-1-cyclopropyl-1*H*-pyridine-4-thione (**7j**): MP 157~159.5°C; ^{1}H NMR ($CDCl_3$) δ 1.07 (2H, m), 2.04 (2H, m), 2.76 (2H, t), 3.17 (2H, t), 3.55 (1H, m), 7.00 (1H, d), 7.48 (1H, d). 2,3-Cyclopenteno-1-(2,2,2-trifluoro)ethyl-1*H*-pyridine-4-thione (**7k**): ^{1}H NMR ($CDCl_3$) δ 2.19 (2H, m), 3.02 (2H, t), 3.07 (2H, t), 4.40 (2H, m), 7.14 (1H, d), 7.34 (1H, d).

5,6-Cyclopenteno-1-methyl-1*H*-pyridine-2-thione (**6e**)

To a solution of 5,6-cyclopenteno-2(1*H*)-pyridone[17] (700 mg) in 5 ml of MeOH was added 280 mg of KOH. The solution was evaporated to dryness. To a suspension of this potassium salt in 10 ml of acetone was added 2 g of methyliodide, and the mixture was stirred at 50°C for 3 hours. The reaction mixture was evaporated, dissolved in 30 ml of chloroform and washed with 20 ml of water. The solvent layer was dried over $MgSO_4$ and evaporated to give 710 mg of 5,6-cyclopenteno-1-methyl-2(1*H*)-pyridone. The pyridone (505 mg) was mixed well with 743 mg of phosphorous pentasulfide and heated at 170°C for 2 hours. To the cooled reaction mixture was added 5.29 ml of 3 N NaOH. The mixture was diluted with water and extracted with 30 ml of chloroform. The chloroform layer was concentrated and chromatographed over silica gel with chloroform-MeOH (5:1) as a developing solvent to give 380 mg of **6e**, which was recrystallized from acetonitrile; pale yellow needles; mp 168~169°C; ^{1}H NMR ($CDCl_3$) δ 2.21 (2H, m), 2.85 (2H, t), 3.05 (2H, t), 3.99 (3H, s), 7.11 (1H, d), 7.60 (1H, d).

3,4-Cyclopenteno-1-methyl-1*H*-pyridine-2-thione (**6f**)

This was prepared in the same manner from 3,4-cyclopenteno-2(1*H*)-pyridone, which was obtained from 6-chloro-3,4-cyclopenteno-2(1*H*)-pyridone[18] by hydrogenation with 10% Pd-C: MP 95.5~98°C; ^{1}H

NMR ($CDCl_3$) δ 2.10 (2H, m), 2.96 (2H, t), 3.04 (2H, t), 4.03 (3H, s), 6.60 (1H, d), 7.65 (1H, d).

2,3-Dihydro-1*H*-indolizine-5-thione (**6g**)

This was prepared in the same way of **6e** by sulfulization of 2,3-dihydro-5(1*H*)-indolizinone:[19] MP 85~86°C; ^{1}H NMR ($CDCl_3$) δ 2.11 (2H, m), 3.22 (2H, t), 4.55 (2H, t), 6.57 (1H, d), 7.18 (1H, t), 7.50 (1H, d).

5,6-Cyclohexeno-1-methyl-1*H*-pyridine-2-thione (**6h**)

This was prepared in an analogous way of **6e** from 5,6-cyclohexeno-2(1*H*)-pyridone:[17] MP 149.5~150.5°C; ^{1}H NMR ($CDCl_3$) δ 1.80 (2H, m), 1.85 (2H, m), 2.61 (2H, t), 2.77 (2H, t), 4.07 (3H, s), 6.93 (1H, d), 7.63 (1H, d).

1-Carboxymethyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**7a**)

2,3-Cyclopenteno-4-pyrone (5.5 g) was mixed with 50 ml of concd NH_4OH, and heated at 100°C for 3 hours in a sealed tube. After cooling, the crystals were collected, washed with a small amount of water and dried to afford 5.23 g of 2,3-cyclopenteno-4(1*H*)-pyridone. To 6.3 g of this pyridone was added 7 ml of phosphorous oxychloride, and this mixture was heated at 135°C for 1 hour. To the cooled reaction mixture was added 60 ml of 10% aq HCl, and washed with 60 ml of ethyl ether. The ethereal layer was extracted with 30 ml of 10% aq HCl. The aq layers were combined, made alkaline by 20% aq NaOH and re-extracted with 150 ml × 3 of ethyl ether. The combined ethereal layer was dried over $MgSO_4$ and concentrated to give 6.25 g of 4-chloro-2,3-cyclopentenopyridine.[20] This compound (1.2 g) was mixed with 1.2 ml of ethyl bromoacetate, and heated at 60°C for 1 hour. The solidified reaction mixture was triturated with ethyl ether. Crystals were collected and dried to give 2.27 g of 4-chloro-2,3-cyclopenteno-1-ethoxycarbonylmethylpyridinium bromide.

Hydrogen sulfide was introduced into the solution of 1.8 g of KOH and 25 ml of H_2O until the red color of phenolphthalein disappeared. To this potassium hydrogen sulfide solution was added 1.6 g of 4-chloro-2,3-cyclopenteno-1-ethoxycarbonylmethylpyridinium bromide. The mixture was stirred at room temperature for 15 minutes. The crystalline products were collected and dried to afford 2,3-cyclopenteno-1-ethoxycarbonylmethyl-1*H*-pyridine-4-thione.

To the DMF (4 ml) solution of 712 mg of the ester was added 4 ml of 1 M NaOH. The mixture was stirred at room temperature for 1 hour, diluted with 10 ml of H_2O, acidified with 1 M HCl and concentrated. The crystals were collected and dried to give 505 mg of **7a**: MP 148~149.5°C; ^{1}H NMR (CD_3OD) δ 1.95~2.4 (2H, m), 2.8~3.3 (4H, m), 4.90 (2H, s), 7.30 (1H, d), 7.56 (1H, d).

Compounds **7b**, **7c**, **7g**, **7h** and **7i**

These compounds were prepared in an analogous way. 1-Allyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**7b**): MP 158~159°C; ^{1}H NMR (CD_3OD) δ 1.9~2.15 (2H, m), 2.8~3.2 (4H, m), 4.4~4.6 (4H, m), 4.4~4.6 (2H, m), 5.16 (1H, d), 5.58 (1H, d), 5.7~6.3 (1H, m), 7.10 (1H, d), 7.35 (1H, d). 2,3-Cyclopenteno-1-(2-hydroxy)ethyl-1*H*-pyridine-4-thione (**7c**): MP 168~170°C; ^{1}H NMR ($CDCl_3$) δ 1.88~2.35 (2H, m), 2.67~3.2 (4H, m), 3.83 (2H, t), 4.17 (2H, t), 7.28 (1H, d), 7.59 (1H, d). 1-Cyanomethyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**7g**): ^{1}H NMR ($CDCl_3+CD_3OD$) δ 2.22 (2H, m), 3.04 (2H, t), 3.18 (2H, t), 3.85 (2H, s), 7.36 (1H, d), 7.40 (1H, d). 2,3-Cyclopenteno-1-(2-sulfo)ethyl-1*H*-pyridine-4-thione (**7h**): ^{1}H NMR (CD_3OD) δ 2.39 (2H, m), 3.08 (2H, t), 3.45 (2H, t), 3.54 (2H, t), 4.79 (2H, t), 7.77 (1H, d), 8.43 (1H, d). 2,3-Cyclopenteno-1-methylthiomethyl-1*H*-pyridine-4-thione (**7i**): ^{1}H NMR ($CDCl_3$) δ 2.15 (3H, s), 2.17 (2H, m), 3.04 (2H, t), 3.09 (2H, t), 4.84 (2H, t), 7.14 (1H, d), 7.34 (1H, d).

1-Carbamoylmethyl-2,3-cyclopenteno-1*H*-pyridine-4-thione (**7d**)

2,3-Cyclopenteno-1-ethoxycarbonylmethyl-1*H*-pyridine-4-thione (240 mg) was suspended with 2 ml of conc NH_4OH, and stirred for 1 hour under ice-cooling. The reaction mixture was concentrated to dryness to give 200 mg of **7d**: ^{1}H NMR (CD_3OD+D_2O) δ 2.03 (2H, m), 2.76 (2H, t), 2.92 (2H, t), 4.68 (2H, s), 7.05 (1H, d), 7.26 (1H, d).

2,3-Cyclopenteno-1-hydroxy-1*H*-pyridine-4-thione (**7f**)

4-Chloro-2,3-cyclopentenopyridine (920 mg) was dissolved in 4 ml of AcOH and warmed to 90℃. To this mixture was added 1.1 ml of 30% hydrogen peroxide. This mixture was stirred at 90℃ for 4 hours, diluted with 4 ml of H_2O, neutralized with aq sodium thiosulfate and concentrated. To the residue were added aq $NaHCO_3$ and brine, and this mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was dried over $MgSO_4$ and concentrated. The resulting crystals were triturated with ethyl ether, collected by filtration and dried to give 700 mg of 4-chloro-2,3-cyclopentenopyridine-1-oxide.

Hydrogen sulfide was introduced into the solution of 717 mg of KOH and 10 ml of H_2O until the red color of phenolphthalein disappeared. To this potassium hydrogen sulfide solution was added 340 mg of 4-chloro-2,3-cyclopentenopyridine-1-oxide, and this mixture was heated at 100℃ in a sealed tube overnight, concentrated, diluted with 10 ml of H_2O and acidified with concd HCl. The resulting precipitate was collected and dried to give 220 mg of **7f**: MP 138℃ (dec); ^{1}H NMR ($CDCl_3+CD_3OD$) δ 2.1~2.45 (2H, m), 2.9~3.4 (4H, m), 7.28 (1H, d), 7.73 (1H, d).

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