there is an induction period (*ca.* 8 hours) in the catalysis under N_2 while no such period of time has been observed in the catalysis under H_2 (see Figure 2). In the presence of hydrogen (H_2), complex **2c** catalyzes the isomerization of *t*DC2B to give a mixture of *c*DC1B and *t*DC1B, and hydrogenation of *t*DC2B to give DCB (Figure 4). Again, the same results (as shown in Figure 4) have been obtained by using **1c** in place of **2c** under H_2 (**1c** immediately reacts with H_2 to form **2c** under the catalytic conditions). The rate of DCB formation from *c*DC1B (or *t*DC1B) with **2a** (or **2b**) (Figure 2) seems to be significantly faster than that from *t*DC2B with **2c** (Figure 4). It is apparent in Figure 4 that DCB is formed by the hydrogenation of *c*DC1B and *t*DC1B which are produced by the isomerization of *t*DC2B although it is not clear whether DCB is also formed by the direct hydrogenation of *t*DC2B (without going through the isomerization).

Finally, it would be mentioned that the hydrogenation and isomerization of the dicyanoolefins (*t*DC2B, *c*DC1B, *t*DC1B) investigated in this study are significantly slower than those of the monocyanoolefins (*trans*-$CH_3CH=CHCN$, *cis*-$CH_3CH=CHCN$, $CH_2=CHCH_2CN$) with $[IrL(CO)(PPh_3)_2]ClO_4$ (L = monocyanoolefin).[2]

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Synthetic beta-Lactam Antibiotics

I. Synthesis and Antibacterial Activity of 7-Amino-3-[1-(halo-substituted phenyl)-1H-tetrazole-5-yl]thiomethyl-3-cepheme-4-carboxylic acids

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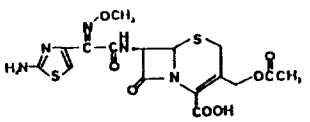
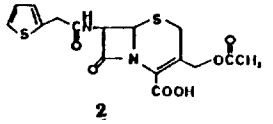
The syntheses of mercaptotetrazoles and cephalothin analogs are described. Their in vitro poetency was established. The compounds exhibited high antibacterial activity against Gram-positive bacteria and moderate activity against Gram-negative bacteria.

Introduction

The cephalosporin antibiotics represent the most important class of drugs against infectious diseases caused by bacteria. In recent years, several new cephalosporin antibiotics such as cefotaxime (1), ceftizoxime[1], and ceftazidime[2] with a broad spectrum of activity and increased activity against bacteria producing beta-lactamase have been developed[3]. Although these recently introduced cephalosporins are characterized by their excellent activity against a variety of Gram-positive and Gram-negative bacteria, they are relatively weak in anti-*Staphylococcal* activity as compared to older cephalosporins such as cephalothin (2) and cefazoline[4].



1



2

In the course of our extensive research on the modification of cephalosporin, our primary target in the present work was to obtain cephalothin analogs with an enhanced penetration ability into Gram-negative cells including *Pseudomonas aeruginosa* while retaining anti-*Staphylococcal* activity.

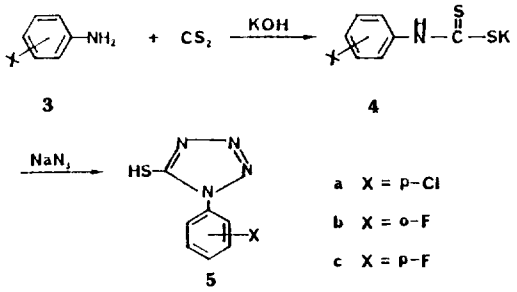
From the viewpoint of molecular modification, new cephalosporin derivatives having the fluoroaromatic group at the N_1-position of the mercaptotetrazole were of particular interest to us since the introduction of a fluorine atom does influence biological properties such as antimicrobial activity, pharmacokinetics and metabolism of those cephalosporins as well as it has been known that electron-withdrawing groups on the 3-position of cephalosporins enhance antibacterial activity[5].

To achieve this goal, starting from 7-aminocephalosporanic acid (7-ACA), several compounds were synthesized by introducing the heterocyclic-thio substituents containing a fluorine or chlorine atom at the 3-methyl position by displacement of the acetoxy group.

This paper describes the very simple and dependable preparation method of the mercaptotetrazol (5), the syntheses of the new cephalosporins represented by formular 9 and their in vitro antibacterial activities against 20 selected strains of Gram-positive and Gram-negative bacteria in comparison with cefotaxime (1).

Synthesis

The mercaptotetrazoles (5a-5c) were prepared by the method shown in scheme 1. The syntheses of the 1-alkyl or aryl substituted mercaptotetrazoles have been already reported in several literatures.[6-8] But the known procedures are usually unreproducible, unpleasant to handle, and the yield was found not to be satisfactory. We have found that the potassium salt of dithiocarbamates (4), prepared in quantitative yields by reaction of the anilines (3a-3c) and carbon disulfide in the presence of two equimolar amounts of potassium hydroxide at room temperature, was reacted with sodium azide at reflux for 6 hr to afford the mercaptotetrazoles (5a-5c) in high yields. The reaction was one pot reaction without isolation of the intermediate (4). We tried other bases such as potassium carbonate instead of potassium hydroxide without success.

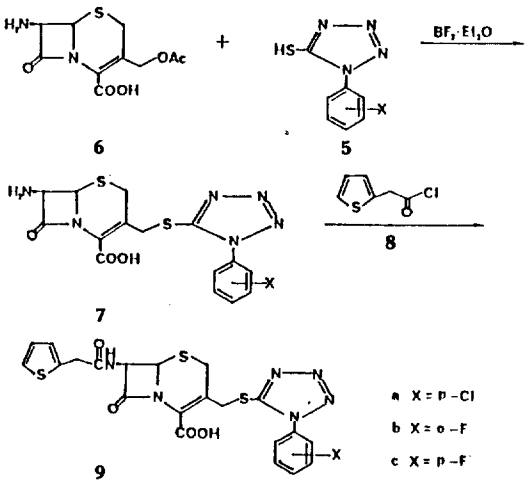


Scheme 1

The synthesis of cephalothin analogs is shown in scheme 2. The reaction was performed without any protection for the amino and carboxy groups of the 7-ACA (6).

Nucleophilic displacement of the 3′-acetoxy group of 6 by the mercaptotetrazoles (5a-5c) was carried out in anhydrous acetonitrile with the addition of borontrifluoride etherate at 50°C for 2hr to afford 7 in high yields.[9] The reaction in aqueous solution conditions was not attempted because of a low yield.[3].

For coupling reaction between 7 and thiophene acetic acid, we used the acid chloride method among several acylation reaction conditions at C-7 of the 7-ACA.[10-12] The thiopheneacetic acid was reacted with phosphorus pentachloride in methylene chloride at 0°C. Without isolation of the acid chloride (8), the 7-ACA derivatives (7a-7c) were reacted with 8 in the presence of excess amounts of N-(trimethylsilyl) acetamide at -20°C. This silylating agent can increase the solubility in solvents of low polarity such as methylene chloride and accelerate the reaction rate as well as protect the carboxy group of 7. Three new cephalothin analogs (9) were prepared in satisfactory yields.



Scheme 2

Antibacterial Activity and Disscussion

Minimum inhibitory concentrations (MICs) of the cephalosporins were determined by the standard two fold agar dilution method. Mueller Hinton Agar was generally used for bacteria. The size of inoculum used for MIC determination was adjusted to Mcfarland No. 0.5. The lowest concentration inhibiting the visible growth after 18hr incubation at 37°C was expressed as the MIC.

The MIC values of this series of cephalosporins against 20 selected strains of Gram-positive and Gram-negative bacteria in comparison with cefotaxime are shown in Table 1.

The data indicates that the cephalothin analogs (9a-9c) have excellent antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis* ATCC No. 6633, *Micrococcus luteus* ATCC No. 10240, *Staphylococcus aureus* ATCC No. 6538p (penicillin G sensitive) compared with cefotaxime. These cephalothin analogs showed moderate activity against *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis.* None of the compound prepared was as active as cefotaxime against Gram-negative bacteria including *Pseudomonas aeruginosa.* These three compounds had about the same range of antibacterial activity or better as cephalothin[13,14] against Gram-negative bacteria and improved activity against Gram-positive bacteria.

Among three compounds, no significant difference in activity against 20 species was observed. It can therefore be presumed that a fluorine atom does not influence an-

Table 1. Antibacterial Activity of Three Cephalosporins in Comparison with Cefotaxime

No.		ATCC No.	1	9a	9b	9c
1	*Bacillus cereus*	27348	256	64	64	64
2	*Bacillus subtilis*	6633	4	0.032	0.032	0.032
3	*Micrococcus luteus*	9341	0.062	32	32	32
4	*Micrococcus luteus*	10240	1	0.125	0.125	0.125
5	*Staphylococcus aureus*	6538P	4	0.125	0.062	0.125
6	*Staphylococcus epidermidis*	12228	2	64	64	64
7	*Acinetobacter calcoaceticus*	15473	16	256	256	256
8	*Bordetella bronchiseptica*	4617	128	256	256	256
9	*Enterobacter aerogenes*	29751	8	256	256	256
10	*Escherichia coli*	10536	0.016	16	16	16
11	*Escherichia coli*	25922	0.032	32	16	32
12	*Escherichia coli*	31030	0.25	64	128	128
13	*Klebsiella pneumoniae*	10031	0.016	2	1	0.5
14	*Proteus mirabilis*	25933	0.016	8	16	8
15	*Providencia rettgeri*	9919	16	128	64	128
16	*Pseudomonas aeruginosa*	10145	16	256	256	256
17	*Pseudomonas aeruginosa*	25619	0.5	256	256	256
18	*Pseudomonas aeruginosa*	27853	128	256	256	256
19	*Salmonella typhimurium*	14028	0.25	64	32	64
20	*Serratia marcescens*	27117	0.25	256	256	256

timicrobial activity in this series.

Although the variations of the side chain of the mercaptotetrazole did not cause significant change in antibacterial activity against Gram-negative bacteria, further modifications to optimize activity in this series and alternative approaches to improve the biological activity of the cephalosporins are under investigation and will be the subject of further publications.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR-spectra were recorded at 60 MHz on JEOL PMX 60SI NMR and at 200 MHz on Bruker AM 200 NMR using TMS as an internal standard. IR spectra were taken on Analect Instrument fx-6160 FT-IR. Mass spectra were obtained by use of Hewlett-Packard 5985 GC-Mass spectroscopy. Elementary analysis were carried out at KAIST. No effort was made to improve the yields.

1-(4′-Chlorophenyl)-1H-tetrazole-5-thiol (5a): To a solution of 4-chloroaniline (12.7g, 0.10 mol) (3a) in 60 m*l* of water were added potassium hydroxide (11g, 0.20 mol) and carbon disulfide (7.6g, 0.10 mol) at 20°C. After being stirred for 1hr at the same temperature, sodium azide (7.8g, 0.12 mol) in 20 m*l* of water was added to the above solution. The reaction mixture was refluxed for 6hr. The aqueous solution was washed with ethyl acetate once and acidified to pH 2 with 1N HCL. The precipitate was filtered and washed with water and acetone. The crude solid was recrystallized from diisopropyl ether to afford 17.3g (81%) of 5a; mp 156°C; NMR (CF_3COOD) ppm 7.1-8.0 (4H,m).

1-(2′-Fluorophenyl)-1H-tetrazole-5-thiol (5b): 5b was obtained (83%) from 2-fluoroaniline (3b) by a similar procedure as described for the preparation of 5a; mp 146°C (lit[6]. 148-148.5°C); MS 196(M^+); NMR (CF_3COOD) ppm 7.2-7.9 (4H,m).

1-(4′-Fluorophenyl)-1H-tetrazole-5-thiol (5c): 5c was obtained (88%) from 4-fluoroaniline (3c) by a similar procedure as described for the preparation of 5a; mp 157°C (lit[6]. 157-158°C); MS 196(M^+); NMR(CF_3COOD) ppm 7.2-7.9 (4H,m).

7-Amino-3-[1-(4′-chlorophenyl)-1H-tetrazole-5-yl]thiomethyl-3-cepheme-4-carboxylic acid (7a): To a stirred solution of boron trifluoride etherate (7.85g, 55.5 mmol) in 100 m*l* of anhydrous acetonitrile under N_2 were added successively 3.6g (18.5 mmol) of 5a and 5.0g (18.5 mmol) of 7-amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (6, 7-ACA) at room temperature. The resulting solution was allowed to react at 50°C for 2hr. After cooling it, the residue in the solution was filtered off and washed with 25 m*l* of acetonitrile. The filtrate was diluted with 50 m*l* of water and adjusted to pH 4.0 by addition of ammonium hydroxide solution (28%) at 0°C. The precipitate was filtered and washed with water and acetone. The solid was dried in vacuo to give 6.5g (83%) of 7a; mp 199-201°C (decom); IR(KBr) 1804, 1617 cm^-; NMR(CF_3COOD) ppm 7.5-7.6(4H, m), 5.4(2H, brs), 4.5(2H, s), 3.9(2H, s).

Anal Calcd for $C_{15}H_{13}N_6CLO_3S_2$: C 42.4, H 3.08, N 19.8.
Found : C 42.2, H 3.05, N 19.7.

7-Amino-3-[1-(2′-fluorophenyl)-1H-tetrazole-5-yl] thiomethyl-3-cepheme-4-carboxylic acid (7b): 7b was obtained (93%) from 5b and the 7-ACA by a similar procedure as described for the preparation of 7a; mp 210-211°C (decom); IR (KBr.) 1802, 1617 cm^{-1}; NMR (CF_3COOD) ppm 7.1-7.6 (4H, m), 5.4 (2H, brs), 4.5 (2H, s), 3.9 (2H, s).

Anal Calcd for $C_{15}H_{13}N_6FO_3S_2$: C 44.1, H 3.18, N 20.5.
Found : C 43.7, H 3.17, N 20.3.

7-Amino-3-[1-(4′-fluorophenyl)-1H-tetrazole-5-yl] thiomethyl-3-cepheme-4-carboxylic acid (7c): 7c was obtained

(87%) from 5c and the 7-ACA by a similar procedure as described for the preparation of 7a; mp 205-210°C (decom); IR (KBr) 1804, 1617 cm^{-1}; NMR (CF_3COOD) ppm 7.1-7.6 (4H, m), 5.4 (2H, brs), 4.5 (2H, s), 3.9 (2H, s).

Anal Calcd for $C_{15}H_{13}N_6FO_3S_2$: C 44.1, H 3.18, N 20.5.
Found : C 43.6, H 3.17, N 20.2.

7ß-[2-Thiophene)acetamido]-3-[1-(4′-chlorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cephem-4-carboxylic acid (9a): To 2-thiopheneacetic acid (0.28 g, 2.0 mmol) in 20 m*l* of methylene chloride was added phosphorus pentachloride (0.5 g, 2.4 mmol) at 0°C and the mixture was stirred for 1 hr at the same temperature. The resultant solution was evaporated in vacuo. The residue was dissolved in 5 m*l* of methylene chloride to produce the acid chloride solution (8) of thiopheneacetic acid. The above acid chloride solution was added dropwise to the mixture of 7a (0.85 g, 2.0 mmol) and N-(trimethylsilyl) acetamide (1.5 g, 12 mmol) in 20 m*l* of methylene chloride at -20°C. The reaction mixture was stirred for 2 hr at -15°C. After removing the solvent, the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated and the aqueous layer was washed with ethyl acetate once. To the combined ethyl acetate solution was added water and the solution was adjusted to pH 7.0 with a saturated sodium bicarbonate solution. The aqueous solution was separated and acidified to pH 2.0 with 10% hydrochloric acid solution. The organic compound was extracted with ethyl acetate three times. The combined ethyl acetate solution was washed with water, brine and dried over magnesium sulfate. After evaporation of the solvent, the crude product was triturated with n-hexane to afford 0.58g (53%) of 9a; mp 92-93°C (decom); IR(KBr) 1786, 1718, 1683, 1512 cm^{-1}; NMR (DMSO-d_6) 9.0-9.15 (1H, d), 7.3-7.9 (4H, m), 6.9-7.0 (2H, m), 5.7-5.9 (1H, m), 5.0 (1H, d), 4.4-4.5 (2H, d), 3.9 (2H, s) 3.8 (2H, s).

7ß-[(2-Thiophene)acetamido]-3-[1-(2′-fluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cepheme-4-carboxylic acid (9b): 9b was obtained (61%) from 7b and 8 by a similar procedure as described for the preparation of 9a. The crude product was triturated with ethyl ether; mp 107-109°C (decom); IR(KBr) 1782, 1722, 1685 cm^{-1}; NMR (DMSO-d_6) 9.0-9.1(1H, d), 7.3-7.7 (4H, m), 6.9-7.0 (2H, d), 5.7 (CH, q), 5.0 (1H, q), 4.4-4.5 (2H, d), 3.9 (3H, s), 3.8 (2H, s).

7ß-[(2-Thiophene)acetamido]-3-[1-(4′-fluorophenyl)-1H-tetrazol-5-yl] thiomethyl-3-cepheme-4-carboxylic acid (9c): was obtained (48%) from 7c and 8 by a similar procedure as described for the preparation of 9a; mp 124-126°C (decom); IR(KBr) 1778, 1718, 1511 cm^{-1} NMR (DMSO-d_6) 9.0-9.15 (1H, d), 7.4-7.7 (4H, m), 6.9-7.0 (2H, d), 5.6-5.8 (1H, q), 5.0-5.1 (1H, d), 4.4-4.5 (2H, d), 3.9 (2H, s), 3.7 (2H, 2).

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