STUDIES ON β-LACTAM ANTIBIOTICS V†

EFFECT ON ANTIMICROBIAL ACTIVITY OF 2- AND/OR 3-METHYL GROUP (S) IN A CEPHEM NUCLEUS

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Synthesis and effect on antibacterial activity of 2- and/or 3-methyl group(s) in the cephalosporins (**4a**~**c**) with (*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetyl side chain were described.

In a previous paper[1] we reported the influence on antibacterial activity of the substituents at the 3-position in the cephalosporin nucleus having (*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetyl side chain. Among these cephalosporins 3-methyl analog (**4d**) was found to have intrinsic activity against Gram-negative bacteria in spite of its poor activity against Gram-positive bacteria. Therefore we intended to improve the 3-methyl cephalosporin (**4d**) in antimicrobial activity, especially against Gram-positive bacteria.

In this paper we will describe the synthesis and *in vitro* antibacterial activity of the 2- and/or 3-methylcephalosporin analogs (**4a**~**c**) with the same 7-acyl group as ceftizoxime.[1,2]

Chemistry

Semisynthetic cephalosporins (**3a**~**c**) were prepared by acylation of 7β-aminocephalosporanic acid derivatives (**2a**~**c**) with (*Z*)-2-(2-formamido-4-thiazolyl)-2-(methoxyimino)acetic acid[1,3] followed by subsequent removal of the formyl group, as outlined in Scheme 1. Activation of the acid (**1**) with VILSMEIER reagent prepared from phosphoryl chloride ($POCl_3$) and dimethylformamide (DMF) was satisfactorily employed for the above coupling reaction. Acylation of the 7-aminocephalosporins (**2a**~**c**) was carried out in excellent yields under non-aqueous condition by trimethylsilylation using *N*-(trimethylsilyl)acetamide (MSA). Deprotection of the *N*-formyl cephems (**3a**~**c**) proceeded smoothly at room temperature in a methanolic solution containing conc. hydrochloric acid to give 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylic acid derivatives (**4a**~**c**). The structure of **3** and **4** was determined by analysis of NMR spectra (Table 1). Macroporous non-ionic adsorption resin was effectively employed for the purification of **4**.

Biological Activity

In vitro antimicrobial activity of **4** was listed in Table 2. Contrary to our expectation, introduction of a methyl group with lipophilic character into the 2α-position of the 3-methyl analog (**4d**) causes a significant decrease in activity of 2,3-dimethyl analog (**4c**). However, removal of the 3-methyl group

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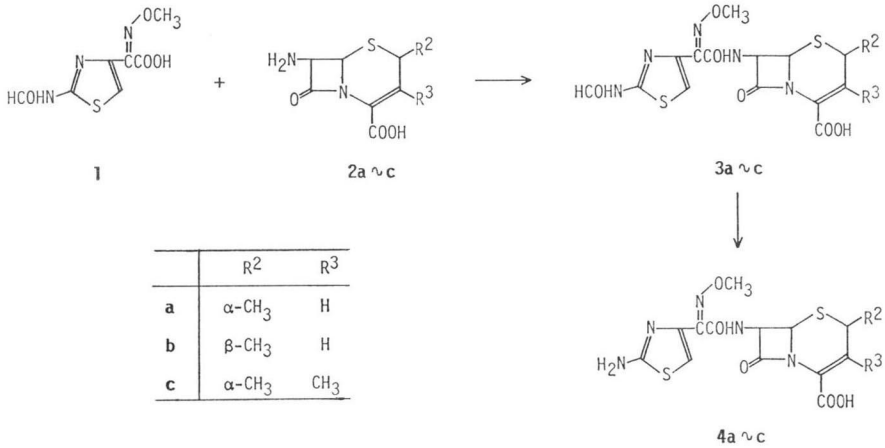
Scheme 1.



	R^2	R^3
a	α-CH_3	H
b	β-CH_3	H
c	α-CH_3	CH_3

Table 1. NMR spectral data of **3a**~**c** and **4a**~**c**.

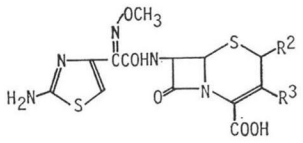
Compounds			NMR (DMSO-d_6, ppm)									
	R^2	R^3	CONH 1H,d J=8Hz	Ring proton of C_5 1H,s	C_7–H 1H,dd J=5,8Hz	C_6–H 1H,d J=5Hz	C_3–H or C_3–CH_3	C_2–H 1H	C_2–CH_3 3H,d J=7Hz	OCH_3 3H,s	CHONH 1H,br.s	CHO 1H,s
3a	α-CH_3	H	9.68	7.40	5.94	5.14	6.58 1H,d J=7Hz	3.90 m	1.46	3.90	12.54	8.52
3b	β-CH_3	H	9.70	7.45	5.87	5.31	6.31 1H,d J=2Hz	4.00 m	1.44	3.93	12.70	8.57
3c	α-CH_3	CH_3	9.53	7.33	5.78	5.17	2.02 3H,s	3.53 m	1.48	3.87	12.50	8.57
4a	α-CH_3	H	9.62	6.77	5.89	5.12	6.57 1H,d J=7Hz	3.90 m	1.44	3.84	—	—
4b	β-CH_3	H	9.60	6.73	5.80	5.28	6.28 1H,d J=2Hz	3.90 m	1.42	3.92	—	—
4c	α-CH_3	CH_3	9.63	6.76	5.73	5.18	1.98 3H,m	3.57 q J=7Hz	1.44	3.82	—	—

from the dimethyl analog (**4c**) results in a dramatic enhancement in activity of 2α-methyl analog (**4a**: FR-13374)[4]. In contrast, 2β-methyl enantiomer (**4b**) exhibits remarkably less activity than the 2α-methyl enantiomer (**4a**) does. This fact may imply that configuration of the substituent at the 2-position in a cephem nucleus is very important for antibacterial activity. Furthermore, removal of the 2-methyl group from **4a** leads to non-substituted analog (**4e**), ceftizoxime[1,2] which gives rise to a further slight improvement in activity. Thus, improvement of the 3-methyl analog (**4d**) in antimicrobial activity against both Gram-positive and Gram-negative bacteria was achieved.

Experimental

NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a JEOL-MH 100 NMR spectrometer using Me_4Si as an internal standard. IR spectra were taken on a Hitachi 260–10 spectrophotometer or a Shimadzu IR-420 spectrophotometer.

Table 2. Antibacterial activity of **4**.



Compounds			MIC (μg/ml)						
			S. aureus		*E. coli*		*K. pneumoniae*	*P. mirabilis*	*P. vulgaris*
	R^2	R^3	6	32	32	28*	12	1	2
4a	α-CH_3	H	12.5	6.25	0.1	0.1	0.1	≦0.025	0.05
4b	β-CH_3	H	100	25	3.13	3.13	3.13	0.39	0.39
4c	α-CH_3	CH_3	>100	>100	12.5	6.25	6.25	1.56	3.13
4d	H	CH_3	100	100	1.56	0.78	0.39	0.2	0.2
4e	H (ceftizoxime)	H	6.25	3.13	0.1	0.1	0.05	≦0.025	≦0.025

* Cephalosporinase producer.

Materials

The following compounds were prepared according to the methods of the literature: **2a**,[5] **2b**,[5] **2c**,[6] **4d**,[1] and **4e**.[1]

General Procedure for Acylation of the 7β-Aminocephalosporanic Acids (**2a**~**c**)

To a solution of DMF (7.7 mmole) in THF (25 ml) was dropwise added $POCl_3$ (7.7 mmole) at -10~$0°C$ under stirring, and the mixture was stirred at this temperature for 20~30 minutes to prepare VILSMEIER reagent. To the above mixture was added the *N*-formyl acid (**1**) (7 mmole) under ice-cooling and the mixture was stirred at this temperature for 30 minutes to produce an activated acid solution of **1**. To a solution of the 7β-aminocephalosporanic acid (**2a**~**c**) (7 mmole) and MSA (42 mmole) in AcOEt (30~40 ml) was added the above activated acid solution at $-20°C$, and the reaction mixture was stirred at -20~$0°C$ for one hour. To the reaction mixture was added a mixture of AcOEt and H_2O, and the AcOEt layer was separated. After H_2O was added to the AcOEt layer, the mixture was adjusted to pH 7.5 with saturated $NaHCO_3$ solution. The separated aqueous layer was acidified to pH 2.0 with 10% HCl and the acidified solution was extracted with AcOEt. The AcOEt layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the residue was triturated with Et_2O to afford a *N*-formylcephalosporin (**3**).

General Procedure for Deformylation of the *N*-Formylcephalosporins (**3a**~**c**)

To a mixture of **3** (5 mmole) in MeOH (20~30 ml) and THF (10~30 ml) was added conc. HCl (15~20 mmole) at room temperature and the mixture was stirred at this temperature for 2~5 hours. The reaction mixture was evaporated and the residue was dissolved in a saturated $NaHCO_3$ solution. The solution was washed with AcOEt and the aqueous layer was acidified to pH 2.5~3.0 with 10% HCl under ice-cooling. The precipitate was filtered, washed with cold H_2O, and dried (P_2O_5) to afford **4**.

Purification of **4**

To a suspension of the crude **4** in H_2O (10 times volume of **4**) was adjusted to pH 6 with a saturated $NaHCO_3$ solution under ice-cooling, and the solution was subjected to column chromatography on macroporous non-ionic adsorption resin Diaion HP-20 (Mitsubishi Chem. Ind. Ltd.). The column was eluted with H_2O and the eluate was acidified to pH 2.5 with 10% HCl under ice-cooling. The precipitate was filtered, washed with H_2O and dried *in vacuo* (P_2O_5) to afford pure **4**.

Antibiotic Susceptibility

All the *in vitro* antibacterial activity is given as the minimum inhibitory concentration (MIC) in μg/ml, required to prevent growth of the bacterial culture. MIC was determined by agar dilution method

using heart infusion agar (Difco) after incubation at 37°C for 20 hours and the inoculum size about 10^5 C.F.U./ml. *Escherichia coli* 28 is a cephalosporin-resistant strain.

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References

1) TAKAYA, T.; H. TAKASUGI, T. MASUGI, T. CHIBA, H. KOCHI, T. TAKANO & H. NAKANO: Structure-activity relationships of sodium 7β-[(*Z*)-2-amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-cephem-4-carboxylate (ceftizoxime) and its related compounds. Nippon Kagaku Kaishi 1981: 785～804, 1981
2) TAKAYA, T.; T. KAMIMURA, H. KOJO, Y. MINE, S. NISHIDA, S. GOTO & K. KUWAHARA: Ceftizoxime (FK 749), a new parenteral cephalosporin: *in vitro* and *in vivo* antibacterial activities. Current Chemotherapy and Infectious Disease, Vol. 1, p. 255, The American Society for Microbiology, Washington, DC, 1980
3) TAKAYA, T.; T. MASUGI, H. TAKASUGI & H. KOCHI: 3,7-Disubstituted syn-isomers of 3,7-cephem-4-carboxylic acid derivatives. Belg. 852,427, Sep. 14, 1977 (Chem. Abst. 89: 59892h, 1978)
4) MINE, Y.; T. MURAKAWA, T. KAMIMURA, T. TAKAYA, M. NISHIDA, S. GOTO & S. KUWAHARA: A new parenteral cephalosporin. 1. *In vitro* and *in vivo* antimicrobial activities. 17th Intersci. Conf. Antimicrob. Agents & Chemother., Abstract No. 147, New York, October 12～14, 1977
5) TAKAI, K.; Unpublished results
6) WRIGHT, I. G.; C. W. AHBROOK, T. GOODSON, G. V. KAISHER & E. M. VAN HEYNIGEN: Chemistry of cephalosporin antibiotics. 23. 2-Methyl- and 2-methylenecephalosporins. J. Med. Chem. 14: 420～426, 1971