STUDIES ON β-LACTAM ANTIBIOTICS. IV† STRUCTURE-ACTIVITY RELATIONSHIPS OF 7β-[(Z)-2-ALKOXYIMINO-2-(2-AMINO-4-THIAZOLYL)ACETAMIDO]-3-CEPHEM-4-CARBOXYLIC ACIDS

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In recent years, so called "third-generation cephalosporins" such as ceftizoxime,[1,2] cefotaxime,[3,4] cefmenoxime,[5] and ceftriaxone[6] which have significant antibacterial activity against both Gram-positive and Gram-negative bacteria have been developed successively.

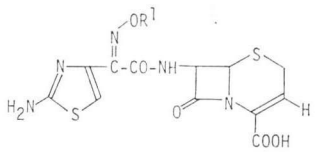
In our previous paper,[2] we have reported the synthesis, chemical properties, *in vitro* antibacterial activity, and structure-activity relationships of new cephalosporins prepared during the course of our studies on ceftizoxime (**Ib**) (Fig. 1).

Further extensive studies on the structure-activity relationships and on pharmacological properties of several cephems (**I**) (Fig. 1) having a 2-(2-amino-4-thiazolyl)-(*Z*)-2-alkoxyiminoacetyl group at the 7-position of the cephem nucleus will be described in this paper.

We here examined correlation of the lipophilic character caused by the alkyl chain length of the oxime ether moiety (R^1) with antibacterial activity and with urinary and biliary excretion after oral administration of the cephalosporins (**I**) to rats.

In this report, the structure-activity relationships associated with the *O*-alkyl chain of **I** were most advantageously dealt with by keeping the 3-substituent of the cephem nucleus as hydrogen similar to ceftizoxime (**Ib**).

Fig. 1. General structure of 7β-[(*Z*)-2-alkoxyimino-2-(2-amino-4-thiazolyl)acetamido]-3-cephem-4-carboxylic acids.



I

Ib ($R^1=CH_3$): Ceftizoxime

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Materials and Methods

The synthesis of 7β-[(*Z*)-2-alkoxyimino-2-(2-amino-4-thiazolyl)acetamido]-3-cephem-4-carboxylic acids was reported previously.[2]

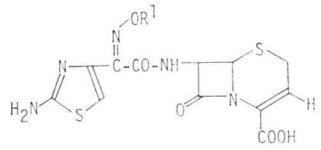
The *in vitro* antibacterial activity is given as the minimum inhibitory concentration (MIC) in mcg/ml, required to inhibit growth of the bacterial culture. MICs were determined by an agar dilution method using heart infusion agar (Difco) after incubation at 37°C for 20 hours and with an inoculum size of about 10^6 C.F.U./ml. *Escherichia coli* 28 is a cephalosporin-resistant strain.

Urinary and biliary excretion: Sprague Dawley rats were fasted overnight and orally dosed with 100 mg/kg of the test drugs. Urine samples were collected for 24 hours after dosing. For bile collection another group of rats was canulated with polystyrene tube into the bile duct and the test drugs were given orally at doses of 100 mg/kg. The samples were assayed by a disc-agar diffusion method using *Bacillus subtilis* ATCC 6633 as test organism and citrate agar as test medium.

Results and Discussion

The antibacterial activities of the synthesized compounds against Gram-positive and Gram-negative bacteria are shown in Table 1. Cephems with the methyl (**1b**, $R^1=CH_3$) or ethyl (**1c**, $R^1=CH_2CH_3$) oxime ether group have very high activity against a wide range of organisms. When the length of the alkyl chain of the oxime ether group is extended, the activity against Gram-positive bacteria tends to increase, while the activity against the Gram-negative organisms tends to decrease. The changes in activity are, however, not great in the compounds containing three or more carbon atoms in the alkyl group. Contrary to our expectation, the unsubstituted hydroxyimino derivative (**1a**, $R^1=H$) shows the

Table 1. Effect on antibacterial activity of changing the alkyl chain length in the oxime ether group.



I

Compounds R¹		MIC (mcg/ml)							
		S. aureus 209P JC-1	33	*E. coli* NIHJ JC-2	28	*K. pneumoniale* 20	*P. mirabilis* 18	*P. vulgalis* 1	*S. marcesense* 35
a	–H	0.39	1.56	0.10	0.10	≦0.025	≦0.025	0.10	12.5
b	$-CH_3$ (ceftizoxime)	6.25	12.5	≦0.025	0.05	≦0.025	≦0.025	≦0.025	1.56
c	$-CH_2CH_3$	3.13	6.25	0.05	0.10	≦0.025	≦0.025	≦0.025	0.78
d	$-CH_2CH_2CH_3$	1.56	3.13	0.20	0.10	≦0.025	0.10	0.10	3.13
e	$-CH(CH_3)_2$	3.13	6.25	0.39	0.20	0.05	0.10	≦0.025	3.13
f	$-CH_2(CH_2)_2CH_3$	1.56	1.56	0.39	0.39	0.20	0.20	0.05	6.25
g	$-CH_2CH(CH_3)_2$	0.78	3.13	0.78	0.20	0.10	0.20	0.10	6.25
h	$-CH_2(CH_2)_3CH_3$	0.78	3.13	1.56	0.39	0.10	0.78	0.10	6.25
i	$-CH_2(CH_2)_4CH_3$	1.56	3.13	1.56	0.39	0.05	0.78	0.10	12.5

best activity against *Staphylococcus aureus* 209P JC-1 and less activity against some Gram-negative bacteria including opportunistic pathogens, in comparison with ceftizoxime (**1b**). Furthermore, all of these compounds have excellent activity against *Escherichia coli* 28 which is a β-lactamase-producing strain.

From the data of Table 1, it is apparent that increasing lipophilicity of the alkyl side chain in the oxime ether moiety tends to increase the activity against Gram-positive bacteria, but decrease the activity against Gram-negative bacteria. However, the compounds still have appreciable activity against a wide range of bacteria.

In the next step, the influence of the alkyl chain length of **I** on the excretion after oral administration was studied. The urinary and biliary excretion of the cephalosporins (**Ia**~**i**) in rats were followed for 24 hours after an oral dose of 100 mg/ml (Table 2). The biliary excretion was found to increase with the alkyl chain length, whereas the urinary excretion tended to decrease. For example, the urinary and biliary recovery of ceftizoxime (**1b**) are 8.53% and 0.33% respectively, whereas the *n*-hexyl homolog (**Ii**) is exclusively excreted in bile (33.3%) without detectable amounts of **Ii** in the urine.

From our present results, there appears to be a correlation between not only antibacterial activity but also urinary and biliary recovery in rats with the lipophilic character of **I** caused by changes in the alkyl chain length of the oxime ether group.

Table 2. 24-Hour urinary and biliary recovery (%) of alkoxyimino analogs (**I**) after oral administration (100 mg/kg) in rats.

Compounds R¹		Number of carbon atoms	Recovery (%)	
			Urine	Bile
a	–H	0	5.10	1.68
b	$-CH_3$	1	8.53	0.33
c	$-CH_2CH_3$	2	6.61	2.98
d	$-CH_2CH_2CH_3$	3	2.84	5.47
e	$-CH(CH_3)_2$	3	2.75	9.55
f	$-CH_2(CH_2)_2CH_3$	4	0.18	7.83
g	$-CH_2CH(CH_3)_2$	4	0.91	7.77
h	$-CH_2(CH_2)_3CH_3$	5	*n.d.	22.84
i	$-CH_2(CH_2)_4CH_3$	6	*n.d.	33.25

* n.d.: not detected.

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References

1) TAKAYA, T.; T. KAMIMURA, H. KOJO, Y. MINE, S. NISHIDA, S. GOTO & S. KUWAHARA: Current Chemotherapy and Infectious Disease. Vol. 1, p. 255. The American Society for Microbiology, Washington, DC, 1980
2) TAKAYA, T.; H. TAKASUGI, T. MASUGI, T. CHIBA, H. KOCHI, T. TAKANO & H. NAKANO: Structure-activity relationships of sodium 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino) acetamido]-3-cephem-4-carboxylate (ceftizoxime) and its related compoumds. Nippon Kagaku Kaishi 1981: 785~804, 1981
3) BUCOURT, R.; R. HEYMES, A. LUTZ, L. PENASSE & J. PERRONT: Propriétés antibiotiques inattendues dans le domaine des cephalosporines. C. R. Acad. Sc. Paris, Serie D; 284: 1847~1849, 1977
4) HEYMES, R.; A. LUTZ & E. SCHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative: Pre-clinical study. Infection 5: 259~260, 1977
5) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977
6) REINER, R.; U. WELSS, U. BROMBACHER, P. LANZ, M. MONTAVON, A. FURLENMEIER, P. ANGEHRN & P. J. PROBST: Ro 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiotics 33: 783~786, 1980