SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 3-(2-PROPENYL)-CEPHALOSPORINS

II. PREPARATION OF PRODRUGS

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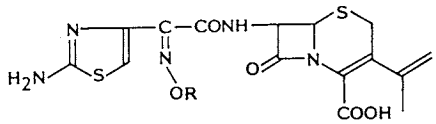
Three prodrug esters (**2a**~**2c**) of 3-(2-propenyl)cephem (**1a**) have been prepared and their oral absorption was determined in rats and mice. While pivaloyloxymethyl ester (**2a**) did not improve the oral absorption of the parent cephem **1a**, [(1-methyl)ethoxycarbonyloxy]ethyl ester (**2b**) and (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester (**2c**) improved oral absorption by a factor of five.

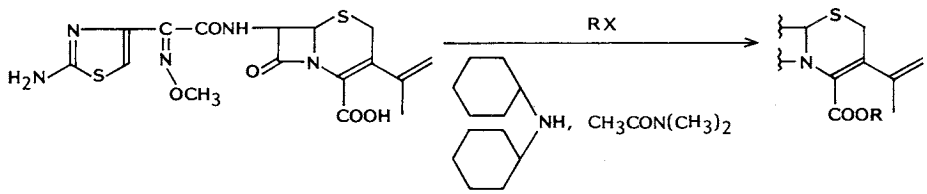
In the previous paper we described the synthesis and biological activity of 3-(2-propenyl)cephems (**1a**~**1c**).[1] These cephems showed a similar degree of antibacterial activity as cefixime, but were poorly absorbed orally and subcutaneously.

When drugs, despite their high pharmacological activities are not sufficiently effective in practice due to their poor bioavailability, the prodrug approach is often used to overcome such problem; for example, some ampicillin esters are used clinically as prodrugs.[2]

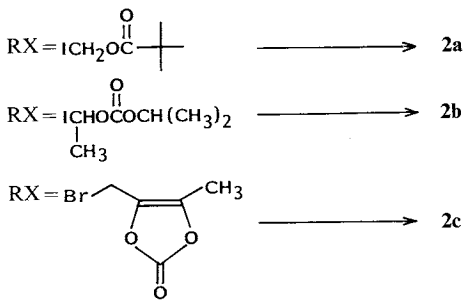


1a R=CH_3
1b R=CH_2COOH
1c R=$C(CH_3)_2COOH$

Scheme 1.



1a **2a**~**2c**



2a

2b

2c

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In the field of cephalosporins similar prodrug concepts have been applied in order to increase oral absorption.[3] Esterification of the cephem carboxylic acid group increases the lipid solubility, thus facilitating the diffusion of the prodrugs across the membranes of the gastrointestinal tract. As ester groups, the pivaloyloxymethyl ester, 1-acetoxyethyl ester, 1-(ethoxycarbonyloxy)ethyl ester, [(1-methyl)ethoxycarbonyloxy]ethyl ester, or (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester have been used.[4] Therefore, we decided to prepare prodrug esters of cephem **1a** and determine their pharmacokinetics.

Chemistry

The preparation of three prodrug esters (**2a**~**2c**) from the parent cephem **1a** is shown in Scheme 1. The pivaloyloxymethyl ester (**2a**), [(1-methyl)ethoxycarbonyloxy]ethyl ester (**2b**) and (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester (**2c**) were prepared in 60% yield by the esterification of **1a** with iodomethyl pivalate, 1-iodoethyl isopropyl carbonate, 4-bromomethyl-5-methyl-2-oxo-1,3-dioxolene[5] in the presence of base, respectively. The ester **2b** was obtained as an 1 : 1 mixture of two diastereoisomers due to the use of racemic halide, as evidenced by a ^{1}H NMR spectrum.

Biological Results and Discussion

In order to determine the oral absorbability of esters **2a**~**2c**, pharmacokinetic parameters were measured in mice and rats after oral administration of these esters. The results are summarized in Tables 1 and 2, respectively. Also, profiles of the mean blood concentrations in mice and rats given single oral dosages of acid **1a**, esters **2a**~**2c**, and cefixime are illustrated in Figs. 1 and 2.

As can be seen in Fig. 1, the blood levels of cephem **1a** after oral administration to mice are much lower than those of cefixime, even though the relative oral bioavailability (23%) of **1a**, calculated on the

Table 1. Pharmacokinetic parameters in mice (n=4).

Compound		Cmax (mg/liter)	Tmax (hours)	$AUC_{0\sim4\ hours}$ (mg·hour/liter)	Ratio po/sc (%)	$UR_{19\ hours}$[a] (%)	Ratio po/sc (%)
1a	sc	49.2±10.9	0.27±0.07	42.9±8.5		46	
1a	po	3.8±1.5	1.0±0.3	10.1±3.7	23	5	11
2a	po	5.0±0.5	0.39±0.15	8.9±2.2	21	10	22
2b	po	14.9±1.4	0.14±0.03	19.7±2.9	46	22	48
2c	po	14.1±2.6	0.30±0.09	23.6±4.3	55	20	43
Cefixime (FK027)	sc	109.9±29.6	0.22±0.00	86.5±19.4		100	
	po	9.3±3.6	0.97±0.24	22.4±11.1	26	19	19

Dose: 40 mg/kg body weight.
[a] $UR_{19\ hours}$ is the urinary recovery during the 19 hours.

Table 2. Pharmacokinetic parameters in rats (n=4).

Compound		Cmax (mg/liter)	Tmax (hours)	$AUC_{0\sim4\ hours}$ (mg·hour/liter)	Ratio po/sc (%)	$UR_{19\ hours}$[a] (%)	Ratio po/sc (%)
1a	sc	25.8±10.6	0.43±0.26	46.0±12.1		78	
1a	po	0.9	—	—	—	3	4
2a	po	2.3±0.8	1.40±0.17	4.2±1.3	9	10	13
2b	po	4.9±1.4	1.10±0.03	10.9±2.9	24	24	30
2c	po	4.6±1.1	1.20±0.30	10.6±2.5	23	15	19
Cefixime (FK027)	sc	48.4±5.2	0.64±0.03	121.4±25.6	92	92	
	po	20.6±3.3	1.20±0.33	61.9±18.3	51	29	32

Dose: 20 mg/kg body weight.
[a] $UR_{19\ hours}$ is the urinary during the 19 hours.

Fig. 1. Mouse pharmacokinetics, 40 mg/kg (po).
□ Cefixime, ● **1a**, ■ **2a**, ○ **2b**, ▲ **2c**.

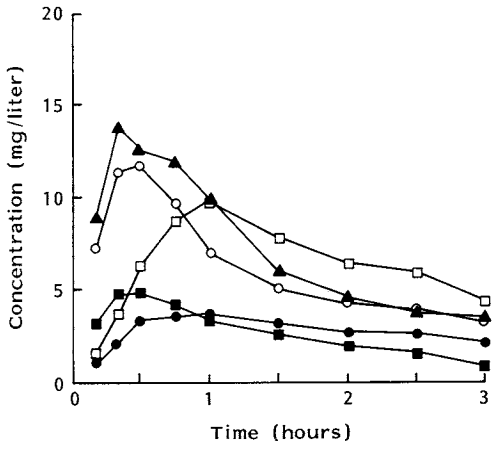
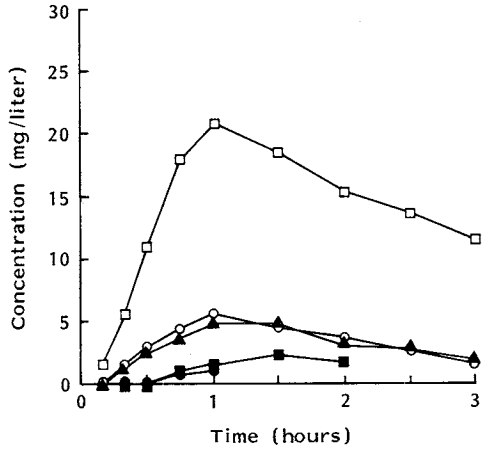


Fig. 2. Rat pharmacokinetics, 20 mg/kg (po).
□ Cefixime, ● **1a**, ■ **2a**, ○ **2b**, ▲ **2c**.



basis of the area under the drug concentration-time curves (AUCs) is similar to that of cefixime (26%).

While pivaloyloxymethyl ester (**2a**) did not show an improved oral absorption in mice, esters **2b** and **2c** showed significant improvement in oral absorption, as one can see in terms of oral bioavailability (43, 48%) and urinary recoveries.

The blood levels of **1a** in rats after oral administration are just above the detection limit of 1 mg/liter, as can be seen in Fig. 2. Prodrugs **2b** and **2c** were much more absorbed orally than pivaloyloxymethyl ester (**2a**), as in the case of mice.

In summary, prodrugs **2b** and **2c** showed improved oral absorption of the parent cephem **1a** by 4~5 times in mice and rats. In comparison to cefixime, the absorption rates were superior in mice and inferior in rats.

Experimental

Pharmacokinetic studies were performed using mice and rats as described before.[1)]

Bioassay

Concentrations of the antibiotics in the blood and urine samples were determined using the agar-diffusion test using Mueller-Hinton agar.

For the determination of cefixime the agar was seeded with *Proteus mirabilis* 112/3 as the indicating organism. In the case of prodrug esters (**2a**~**2c**), the agar was supplemented with 10% sheep's blood and seeded with *Streptococcus pyogenes* A77. The detection limit was about 1 mg/liter. Various pharmacokinetic parameters were determined using method described before.[1)]

Iodomethyl Pivalate

A solution of 30 g (0.20 mol) of chloromethyl pivalate in 350 ml of acetone was allowed to react with 120 g (0.8 mol) of NaI 0°C for 3 hours. Then, the mixture was filtered and the filtrate was concentrated. The residue was extracted with 200 ml of petroleum ether and the extract was washed with 5% $Na_2S_2O_3$ solution, dried and concentrated to give 39 g (81%) of the product as an oil after vacuum distillation (71~72°C/12 mmHg).

Pivaloyloxymethyl 7-[(*Z*)-2-(2-Amino-4-thiazole)-2-methoxyiminoacetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**2a**)

A solution of 0.21 g (0.50 mmol) of 7-[(*Z*)-2-(2-amino-4-thiazole)-2-methoxyiminoacetamido]-3-(2-propenyl)-3-cephem-4-carboxylic acid (**1a**) in 10 ml of *N*,*N*-dimethylacetamide was treated with 0.1 g (0.55 mmol) of dicyclohexylamine followed by 0.16 g (0.66 mmol) of iodomethyl pivalate at 0°C for 30 minutes. Then, the reaction mixture was diluted with 50 ml of ethyl acetate and washed with dil HCl, $NaHCO_3$ solution and brine. The organic solution was dried and concentrated to give 0.16 g (66%) of the product, mp 150~153°C; ^{1}H NMR ($CDCl_3$) δ 8.35 (1H, d, J=9 Hz, NH), 6.67 (1H, s), 6.10 (1H, dd, J=9 and 5 Hz, C-7), 5.80 (2H, br s, NH_2), 5.87, 5.81 (2H, ABq, J=5.5 Hz, $COOCH_2$), 5.13 (1H, d, J=5 Hz, C-6), 4.96, 4.81 (2H, s, $=CH_2$), 4.05 (3H, s), 3.47 (2H, s, C-2), 1.94 (3H, s), 1.21 (9H, s, *tert*-Bu).

1-Iodoethyl Isopropyl Carbonate

A mixture of 28 ml (0.29 mol) of ethyl chloroformate and 26 ml (0.32 mol) of sulfuryl chloride was refluxed for 7.5 hours in the presence of 0.1 g of benzoyl peroxide. Then, the mixture was distilled at 120~140°C to give 21 g (51%) of 1-chloroethyl chloroformate. A solution of 20 g (0.14 mol) of 1-chloroethyl chloroformate in 140 ml of CH_2Cl_2 was treated with 27 ml (0.35 mol) of 2-propanol followed by 15 ml (0.19 mol) of pyridine under ice cooling. The mixture was washed with water, brine and 5% $KHSO_4$, and dried. Concentration gave crude 1-chloroethyl isopropyl carbonate, ^{1}H NMR ($CDCl_3$) δ 6.40 (1H, q, J=6 Hz), 5.00~4.80 (1H, m,), 1.85 (3H, d, J=6 Hz), 1.30, 1.31 (6H, d, J=6 Hz). A solution of 10.2 g (61 mmol) of 1-chloroethyl isopropyl carbonate and 20 g (133 mmol) of NaI in 100 ml of benzene was refluxed for 10 hours in the presence of 0.5 g of 18-crown-6. The mixture was washed with water and 5% $Na_2S_2O_3$, dried and concentrated to give 14 g (89%) of the product as an oil. This oil was used for the next step without further purification, ^{1}H NMR ($CDCl_3$) δ 6.70 (1H, q, J=6 Hz, CHI), 4.85 (1H, septet, J=6 Hz), 2.20 (3H, d, J=6 Hz), 1.30 (6H, d, J=6 Hz).

[(1-Methyl)ethoxycarbonyloxy]-1-ethyl 7-[(*Z*)-2-(2-Amino-4-thiazole)-2-methoxyiminoacetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**2b**)

A solution of 0.40 g (0.95 mmol) of cephem **1a** in 3 ml of *N*,*N*-dimethylacetamide was treated with 0.2 g (1.1 mmol) of dicyclohexylamine followed by 0.37 g (1.43 mmol) of 1-iodoethyl isopropyl carbonate at 0°C for 30 minutes. Then, after workup as before 0.3 g (60%) of the product was obtained as an 1 : 1 diastereomeric mixture; ^{1}H NMR ($CDCl_3$) δ 8.26~8.16 (1H, m, NH), 6.72 (1H, s), 6.95, 6.86 (1H, q, J=6 Hz, COOCH), 6.13~6.05 (1H, m, C-7), 5.78 (2H, br s, NH_2), 5.03~4.84 (2H+1H, m, $=CH_2$ and $OCH(CH_3)_2$), 5.15~5.12 (1H, m, C-6), 4.06 (3H, s), 3.49 (2H, s, C-2), 1.54 (3H, d, J=6 Hz), 1.99, 1.95 (3H, s), 1.34~1.25 (6H, m).

4-Bromomethyl-5-methyl-2-oxo-1,3-dioxolene

A solution of 3.42 g (30 mmol) of 4,5-dimethyl-2-oxo-1,3-dioxolene and 5.34 g (45 mmol) of *N*-bromosuccinimide in 150 ml of CCl_4 was refluxed for 4 hours in the presence of a catalytic amount of 2,2′-azobisisobutyronitrile. The mixture was concentrated to half the volume and filtered. The filtrate was concentrated to give 4.20 g (73%) of the product after vacuum distillation (95~100°C/2 mmHg); ^{1}H NMR ($CDCl_3$) δ 4.20 (2H, s), 2.10 (3H, s).

(5-Methyl-2-oxo-1,3-dioxolen-4-yl)methyl 7-[(*Z*)-2-(2-Amino-4-thiazole)-2-methoxyiminoacetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**2c**)

A solution of 0.10 g (0.24 mmol) of cephem **1a** in 5 ml of *N*,*N*-dimethylacetamide was treated with 0.05 ml (0.25 mmol) of dicyclohexylamine followed by 60 mg (0.31 mmol) of 4-bromomethyl-5-methyl-2-oxo-1,3-dioxolene at 0°C for 30 minutes. After workup as before 80 mg (64%) of the product was obtained; ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, d, J=8 Hz, NH), 6.70 (1H, s), 6.00 (1H, dd, J=8 and 4 Hz, C-7), 5.50 (2H, br s, NH_2), 5.15 (1H, d, J=4 Hz, C-6), 5.00, 4.85 (2H, s, $=CH_2$), 4.95, 4.75 (2H, ABq, J=14 Hz, $COOCH_2$), 4.05 (3H, s), 3.45 (2H, s, C-2), 2.15 (3H, s), 1.95 (3H, s).

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