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Synthesis and oral absorption of acyloxymethyl esters of 7β-(2-(2-aminothiazol-4-yl)acetamido)-3-(((1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl)thio)-methyl)ceph-3-em-4-carboxylic acid (cefotiam)

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Summary

To obtain quantitative information for the rational design of an orally active prodrug of cefotiam (7β-(2-(2-aminothiazol-4-yl)acetamido)-3-(((1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl)thio)methyl)ceph-3-em-4-carboxylic acid; CTM), 16 acyloxymethyl esters of CTM were prepared and their oral bioavailability (*BA*) in mice was measured as well as the water solubility, lipophilicity, hydrolysis rate to CTM, and isomerization to Δ^2-CTM, which were thought important factors influencing their oral *BA*. The plasma CTM levels after oral administration of the esters were higher than those observed after oral dosing of CTM and the relative bioavailability was improved 2–9-fold. Four esters had *BA* of more than 40% including 2-propylvaleryloxymethyl ester I showing the best *BA*, 53.8%. The water solubility of these esters at pH 4.5 were between 1.31 and 3.46 mg/ml. The lipophilicity was closely related to the Hansch's substituent lipophilic constant (π value) of R. In a homogenate of mice small intestine, the esters were hydrolyzed to CTM and Δ^2-CTM. The esters were also unstable and converted to Δ^2-CTM rapidly in a buffer of pH 7.4. The hydrolysis and isomerization of the CTM ester could be parallel reactions. Analysis of the quantitative structure–absorption correlation revealed a good linear relation between the Taft's steric constant (E_s value) of the ester moiety R and the hydrolysis rate of the ester to CTM in a 1% homogenate of mice small intestine at 37 °C. Also, a close correlation was observed among the E_s value, π value of R, and the *BA* or peak plasma level of CTM. Significant contribution of the steric hindrance of the ester moiety to the *BA* or C_{max} was recognized for the first time in designing an orally active prodrug of a cephalosporin. An optimization of the promoiety (R) of acyloxymethyl ester of CTM revealed that an R with E_s value near −2 and π value between 2 and 4, i.e. alkyl group having carbon atoms between 4 and 8, are necessary to give a good *BA* after oral administration to mice.

Introduction

Parenteral cephalosporins are generally poorly absorbable from the gastrointestinal (GI) tract owing to their high hydrophilicity in the pH partition hypothesis (Tsuji and Yamana, 1981). One of the methods to improve the oral bioavailability (*BA*) of such parenteral cephalosporins without reducing the original antimicrobial activity, is to make a lipophilic prodrug.

The concept of a prodrug was successfully applied to develop an orally active penicillin from a parenterally applicable form. However, there have been only few reports on enhancing the oral *BA*

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of parenteral cephalosporins (Ferres, 1983; Harding et al., 1984; Yoshimura et al., 1985).

Thus, we tried to develop an orally active ester of cefotiam (7β-(2-(2-aminothiazol-4-yl)acetamido)-3-(((1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl)thio)methyl)ceph-3-em-4-carboxylic acid; CTM), a cephalosporin with broad antimicrobial spectrum (Tsuchiya et al., 1978). As a step to design the optimal ester rationally, we synthesized acyloxymethyl esters of CTM and investigated on the structural requirements for the good oral *BA*.

Yalkowsky and Morozowich (1980) pointed out that the consideration of dissolution in the GI fluid, transportation across the GI membrane, and hydrolysis into the parent drug were necessary for a prodrug to be absorbed through the GI tract.

In the previous study (Yoshimura et al., 1985), we found a linear correlation between water solubility of the pivaloyloxymethyl esters of 10 parenteral cephalosporins and the oral *BA* in mice, probably because all of them have enough lipophilicity. Also, Ferres (1983) assumed that one of the causes of the low *BA* of a cephalosporin prodrug was due to the Δ^2-isomerization.

However, no quantitative relations among lipophilicity, hydrolysis rate, Δ^2-isomerization and the *BA* have been established yet. Thus, on the 16 acyloxymethyl esters of CTM, we measured the lipophilicity, hydrolysis rate to CTM in vitro, and oral *BA* in mice for their correlation.

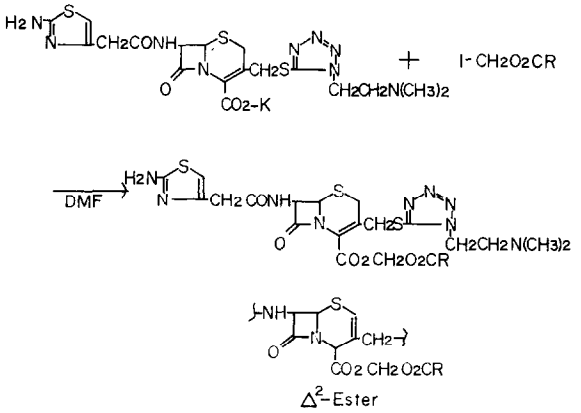
Materials and Methods

Apparatus

Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer. Nuclear magnetic resonance (NMR) spectra were measured with a Varian EM 390 spectrometer using tetramethylsilane as an internal reference. High-performance liquid chromatography (HPLC) was done using a Shimadzu LC-5A instrument equipped with a column (300 × 4 mm i.d.) of Nucleosil C_{18}(10 μm particle size) or a column (300 × 4 mm i.d.) of μBondapak C_{18}(10 μm particle size) and a variable wavelength UV detector (at 254 nm).

Materials

7β-(2-(2-aminothiazol-4-yl)acetamido)-3-(((1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl)thio)-methyl)ceph-3-em-4-carboxylic acid dihydrochloride (cefotiam · 2 HCl; CTM) and its Δ^2-isomer were prepared in the Central Research Division, Takeda Chemical Industries, activity of CTM was 0.842 mg/mg.



Scheme 1

Preparation of the acyloxymethyl esters of cefotiam

The esters of cefotiam were prepared as shown in Scheme 1.

(1) Preparation of iodomethyl acylate; general procedure. Boiling points were uncorrected. Chloromethyl acylate was prepared from acyl chloride and paraformaldehyde in the presence of anhydrous $ZnCl_2$ as a catalyst according to the method of Ulich and Adams (1921).

Chloromethyl isobutyrate b.p. 55–60 °C/18–20 mm Hg, IR ν_{max}^{film} cm^{-1}: 1755, 700;

Chloromethyl isovalerate b.p. 94 °C/14 mm Hg, IR ν_{max}^{film} cm^{-1}: 1770, 720;

Chloromethyl 2-cyclohexylacetate b.p. 80–82 °C/7–8 mm Hg, IR ν_{max}^{film} cm^{-1}: 1770, 710;

Chloromethyl 2-ethylbutyrate b.p. 70–72 °C/10–13 mm Hg, IR ν_{max}^{film} cm^{-1}: 1760, 710;

Chloromethyl 2-*n*-propylbutyrate b.p. 78–82 °C/3–5 mm Hg, IR ν_{max}^{film} cm^{-1}: 1760, 710;

Chloromethyl 3,3-dimethylbutyrate b.p. 42–45 °C/32 mm Hg, IR ν_{max}^{film} cm^{-1}: 1770, 715;

Chloromethyl 2,2,3,3-tetramethylbutyrate b.p. 43–45 °C/2–4 mm Hg, IR ν_{max}^{film} cm^{-1}: 1770, 710;

Chloromethyl 2,2-diethylbutyrate b.p. 67–70 °C/

2–4 mm Hg, IR ν_{max}^{film} cm^{-1}: 1770, 710.

Chloromethyl acylate was stirred with NaI (4–5 equiv. mol) in acetone at room temperature for 3–4 h. The undissolved materials were filtered off and the filtrate was concentrated in vacuo. A mixture of *n*-hexane (150 ml) and 5% aqueous $Na_2S_2O_3$ solution (100 ml) was added to the residue. The organic layer was separated and washed with 5% aqueous $Na_2S_2O_3$ solution, and dried over anhydrous Na_2SO_4. The solvent was concentrated in vacuo to give iodomethyl acylate as a yellow oil which was used for the esterification without further purification.

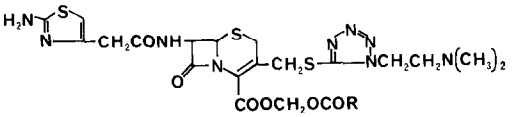
(2) Preparation of acyloxymethyl esters of 7β-(2-(2-aminothiazol-4-yl)acetamido)-3-(((1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl)thio)methyl)ceph-3-em-4-carboxylic acid. The potassium salt of cefotiam (5 mmol) was dissolved in dimethylformamide (20 ml), and cooled to −10 to −20°C. A DMF solution (5 ml) of iodomethyl acylate (5.5 mmol) was added to this solution over a 5-min period with stirring, and the mixture was stirred for a further 5–10 min. This mixture was poured into a mixture of ethyl acetate (150 ml) and ice-water (70 ml), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (100 ml × 2). The combined organic layer was washed with chilled water (100 ml × 3) and saturated brine, then dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was triturated with isopropylether. The crude ester was recrystallized or reprecipitated from acetone–isopropylether. The corresponding ester of Δ^2-CTM was not detected by thin-layer chromatography and NMR spectra. The structures and analytical results of the esters are shown in Tables 1, 2 and 3.

*Dihydrochloride salt of **h** and **j***

An ester, **h** or **j** (free base), was dissolved in a small volume of 0.5 N HCl, adsorbed on a column of Amberlite XAD-II (100–200 mesh), and eluted successively with 0.01 N HCl and 5% acetonitrile in 0.01 N HCl. The fraction containing the ester was concentrated in vacuo and sodium chloride was added to this residual solution. After the mixture being cooled, the crystalline precipitate was collected and recrystallized from 0.5 N HCl.

TABLE 1

The structure and IR data of the acyloxymethyl esters of cefotiam



Ester no.	R	Yield %	IR ν_{max}^{KBr} cm^{-1}
a	CH_3	36.4	1785, 1760, 1670
b	C_2H_5	43.0	1780, 1765, 1740, 1680
c	C_3H_7-*n*	54.3	1790, 1750, 1740, 1680
d	C_3H_7-*i*	42.0	1785, 1740, 1675
e	C_4H_9-*n*	41.4	1785, 1775, 1740, 1680
f	C_4H_9-*i*	50.9	1780, 1740, 1680
g	C_4H_9-*s*	41.1	1785, 1740, 1675
h·2HCl	C_4H_9-*t*	50.0	1775, 1730, 1680
i	C_5H_{11}-*n*	47.8	1780, 1760, 1735, 1680
j·2HCl	$CH(C_2H_5)_2$	46.0	1780, 1740, 1680
k	C_7H_{15}-*n*	56.2	1785, 1760, 1740, 1685
l	$CH(C_3H_7$-$n)_2$	57.8	1785, 1745, 1675
m	$CH_2C_6H_{11}$-*c*	51.5	1790, 1745, 1665
n·2HCl	$CH_2C_4H_9$-*t*	34.5	1780, 1740, 1680
o·2HCl	$C(CH_3)_2C_4H_9$-*t*	36.3	1780, 1745, 1680
p·2HCl	$C(C_2H_5)_3$	55.8	1780, 1745, 1680

*Dihydrochloride salts of **n**, **o** and **p***

A solution of 4.2 N HCl in ethylether was added to the dichloromethane solution of the ester **n**, **o** or **p** (free base). The precipitate was collected, washed with ethylether, and dried.

Absorption studies

Male SLC-ICR mice, weighing about 15 g (4 weeks old), were starved but had free access to water, for 16–18 h before the experiment. The esters were administered orally to a group of 4 mice by intubation either as an aqueous solution (in the case of dihydrochloride salt) or an aqueous solution with tartaric acid (2 mole equiv., in the case of free base) at a dose of 100 mg/kg equiv. to CTM. Blood was taken from the inferior vena cava at 0.25, 0.5, 1, 2 and 3 h after dosing. Also, CTM was administered subcutaneously or orally as the 1% aqueous solution at the same dose. The relative bioavailability (*BA*) was calculated from the ratio of the area under the plasma CTM level–time curve after oral (AUC_{oral}) or subcuta-

TABLE 2

NMR data of the acyloxymethyl esters of cefotiam

Ester no.	δ ppm
a	2.29 (s, 3H), 3.26 (s, 6H), 3.95 (s, 2H), 4.01 (s, 2H), 4.07 (t, J = 6Hz, 2H), 4.30 and 4.57 (ABq, J = 13.2Hz, 2H), 5.06 (t, J = 6Hz, 2H), 5.40 (d, J = 4.5Hz, 1H), 5.90 (d, J = 4.5Hz, 1H), 6.03 and 6.13 (ABq, J = 6Hz, 2H), 6.90 (s, 1H)
b	1.28 (t, J = 7Hz, 3H), 2.67 (q, J = 7Hz, 2H), 3.27 (s, 6H), 3.94 (s, 2H), 4.01 (s, 2H), 4.07 (t, J = 6Hz, 2H), 4.51 (b-s, 2H), 5.16 (t, J = 6Hz, 2H), 5.37 (d, J = 4.5Hz, 1H), 5.86 (d, J = 4.5Hz, 1H), 5.98 and 6.13 (ABq, J = 6Hz, 2H), 6.87 (s, 1H)
c	1.10 (t, J = 7Hz, 3H), 1.54–2.07(m, 2H), 2.63 (t, J = 7Hz, 2H), 3.27 (s, 6H), 4.00 (s, 2H), 4.01 (s, 2H), 4.07 (t, J = 6 Hz, 2H), 4.43 and 4.60 (ABq, J = 12Hz, 2H), 5.17 (t, J = 6Hz, 2H), 5.39 (d, J = 4.5Hz, 1H), 5.89 (d, J = 4.5Hz, 1H), 5.99 and 6.13 (ABq, J = 6Hz, 2H), 6.97 (s, 1H)
d	1.37 (d, J = 7Hz, 6H), 2.6–3.2 (m, 1H), 3.28 (s, 6H), 4.01 (s, 2H), 4.03 (s, 2H), 4.09 (t, J = 6Hz, 2H), 4.43 and 4.60 (ABq, J = 13.5Hz, 2H), 5.17 (t, J = 6Hz, 2H), 5.38 (d, J = 4.5Hz, 1H), 5.90 (d, J = 4.5Hz, 1H), 5.98 and 6.13 (ABq, J = 6 Hz, 2H), 6.93 (s, 1H)
e	1.07 (t, J = 6Hz, 3H), 1.2–2.0 (m, 4H), 2.57 (t, J = 6Hz, 2H), 3.24 (s, 6H), 3.96 (s, 2H), 4.01 (s, 2H), 4.08 (t, J = 6Hz, 2H), 4.48 (b-s, 2H), 5.03 (t, J = 6Hz, 2H), 5.50 (d, J = 4.5Hz, 1H), 5.89 (d, J = 4.5 Hz, 1H), 6.00 and 6.21(ABq, J = 6Hz, 2H), 6.90 (s, 1H)
f	1.10 (d, J = 6Hz, 6H), 2.0–2.5 (m, 1H), 2.53 (d, J = 6Hz, 2H), 3.26 (s, 6H), 3.92 (b-s, 2H), 4.00 (s, 2H), 4.07 (t, J = 6Hz, 2H), 4.53 (b-s, 2H), 5.14 (t, J = 6Hz, 2H), 5.39 (d, J = 4.5Hz, 1H), 5.88 (d, J = 4.5Hz, 1H), 6.00 and 6.14 (ABq, J = 6Hz, 2H), 6.83 (s, 1H)
g	1.05 (t, J = 6Hz, 3H), 1.32 (d, J = 6Hz, 3H), 1.5-2.0 (m, 2H), 2.4–2.9 (m, 1H), 3.27 (s, 6H), 4.01 (s, 2H), 4.03 (s, 2H), 4.08 (t, J = 6Hz, 2H), 4.43 and 4.60 (ABq, J = 13.5Hz, 2H), 5.16 (t, J = 6Hz, 2H), 5.39 (d, J = 4.5Hz, 1H), 5.89 (d, J = 4.5Hz, 1H), 6.00 and 6.13 (ABq, J = 6Hz, 2H), 6.93 (s, 1H)
h	1.26 (s, 6H), 3.26 (s, 6H), 3.7–4.1 (m, 4H), 3.93 (t, J = 6Hz, 2H), 4.26 and 4.44 (ABq, J = 13.5Hz, 2H), 5.00 (t, J = 6Hz, 2H), 5.23 (d, J = 4.5Hz, 1H), 5.74 (d, J = 4.5Hz, 1H), 5.81 and 6.00 (ABq, J = 6Hz, 2H), 6.77 (s, 1H)
i	1.06(t, J = 7Hz, 3H), 1.2–2.0 (m, 6H), 2.66 (q, J = 7Hz, 2H), 3.27 (s, 6H), 3.94 (s, 2H), 4.01 (s, 2H), 4.08 (t, J = 6Hz, 2H), 4.50 (b-s, 2H), 5.07 (t, J = 6Hz, 2H), 5.38 (d, J = 4.5Hz, 1H), 5.90 (d, J = 4.5Hz, 1H), 6.00 and 6.15 (ABq, J = 6Hz, 2H), 6.90 (s, 1H)
j	0.88 (t, J = 7Hz, 6H), 1.4–1.8 (m, 4H), 2.1-2.6 (m, 1H), 3.14 (s, 6H), 3.6-4.1 (m, 4H), 3.85 (s, 2H), 4.38 (b-s, 2H), 4.95 (t, J = 6Hz), 5.22 (d, J = 4.5Hz, 1H), 5.71 (d, J = 4.5Hz, 1H), 5.80 and 5.98 (ABq, J = 6Hz, 2H), 6.76 (s, 1H)
k	1.07 (t, J = 6Hz, 3H), 1.49 (b-s, 8H), 2.61 (t, J = 6Hz, 2H), 3.28 (s, 6H), 3.98 (s, 2H), 4.00 (s, 2H), 4.07 (t, J = 6Hz, 2H), 4.50 (b-s, 2H), 5.09 (t, J = 6Hz, 2H), 5.30 (d, J = 4.5Hz, 1H), 5.88 (d, J = 4.5Hz, 1H), 6.13 (b-s, 2H), 6.89 (s, 1H)
l	0.89 (t, J = 7Hz, 6H), 1.21–1.87 (m, 8H), 4.03 (s, 2H), 4.06 (t, J = 6Hz, 2H), 4.37 and 4.75 (ABq, J = 13.5Hz, 2H), 5.02 (t, J = 6Hz, 2H), 5.22 (d, J = 4.5Hz, 1H), 5.77 (d, J = 4.5Hz, 1H), 5.82 and 6.01 (ABq, J = 6Hz, 2H), 6.79 (s, 1H)
m	0.8–2.1 (m, 11H), 2.50 (d, J = 6Hz, 2H), 3.28 (s, 6H), 3.97 (s, 2H), 4.01 (s, 2H), 4.07 (t, J = 6Hz, 2H), 4.61 (b-s, 2H), 5.13 (t, J = 6Hz, 2H), 5.34 (d, J = 4.5Hz, 1H), 5.89 (d, J = 4.5Hz, 1H), 5.9–6.3 (m, 2H), 6.94 (s, 1H)
n	1.11 (s, 9H), 2.44 (s, 2H), 3.20 (s, 6H), 3.92 (b-s, 4H), 4.45 (b-s, 2H), 5.06 (t, J = 6Hz, 2H), 5.28 (d, J = 5Hz, 1H), 5.77 (d, J = 5Hz, 1H), 5.91 and 6.02 (ABq, J = 6Hz, 2H), 6.82 (s, 1H)
o	1.02 (s, 9H), 1.24 (s, 6H), 3.17 (s, 6H), 3.90 (s, 4H), 3.97 (t, J = 6Hz, 2H), 4.28 and 4.50 (ABq, J = 14Hz, 2H), 5.27 (d, J = 5Hz, 1H), 5.77 (d, J = 5Hz, 1H), 5.88 and 5.99 (ABq, J = 6Hz, 2H), 6.80 (s, 1H)
p	0.75 (t, J = 7Hz, 9H), 1.60 (q, J = 7Hz, 6H), 3.09 (s, 6H), 3.7–4.6 (m, 8H), 3.93 (t, J = 6Hz, 2H), 4.93 (t, J = 6Hz, 2H), 5.13 (d, J = 5Hz, 1H), 5.69 (d, J = 5Hz, 1H), 5.91 (b-s, 2H), 6.71 (s, 1H)

h, **j**, **n**, **o**, and **p** were measured in D_2O and the other esters were measured in DCl.

neous administration ($AUC_{s.c.}$). As CTM was not detected at 3 h after dosing, *BA* was calculated from AUC_{0-2h}. The *BA*s were covered more than 95% of those calculated from AUC_{0-3h}.

TABLE 3

Elemental analyses of the acyloxymethyl esters of cefotiam

Ester no.	R	Formula	Analyses %					
			Calcd.			Found		
			C	H	N	C	H	N
a	CH_3	$C_{21}H_{27}N_9O_6S_3 \cdot H_2O$	40.97	4.75	20.48	40.86	4.68	20.50
b	C_2H_5	$C_{22}H_{29}N_9O_6S_3 \cdot 1/2H_2O$	42.57	4.87	20.31	42.42	4.79	20.41
c	C_3H_7-*n*	$C_{23}H_{31}N_9O_6S_3 \cdot 1/2H_2O$	43.52	5.08	19.86	43.68	5.17	19.48
d	C_3H_7-*i*	$C_{23}H_{31}N_9O_6S_3 \cdot 3/4H_2O$	43.21	5.12	19.72	43.47	5.02	19.37
e	C_4H_9-*n*	$C_{24}H_{33}N_9O_6S_3 \cdot H_2O$	43.82	5.32	19.17	44.06	5.34	19.18
f	C_4H_9-*i*	$C_{24}H_{33}N_9O_6S_3 \cdot 1/4H_2O$	44.74	5.24	19.57	44.87	5.11	19.28
g	C_4H_9-*s*	$C_{24}H_{33}N_9O_6S_3 \cdot H_2O$	43.82	5.36	19.17	43.98	5.11	18.88
h · 2HCl	C_4H_9-*t*	$C_{24}H_{33}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	38.50	5.25	16.84	38.36	5.19	16.85
i	C_5H_{11}-*n*	$C_{25}H_{35}N_9O_6S_3 \cdot H_2O$	44.70	5.55	18.77	44.90	5.33	18.12
j · 2HCl	$CH(C_2H_5)_2$	$C_{25}H_{35}N_9O_6S_3 \cdot 2HCl \cdot 3/2H_2O$ [a]	39.12	5.25	16.42	38.98	5.05	16.70
k	C_7H_{15}-*n*	$C_{27}H_{39}N_9O_6S_3$	47.56	5.76	18.49	47.63	5.94	18.37
l	$CH(C_3H_7$-*n*$)_2$	$C_{27}H_{39}N_9O_6S_3 \cdot 1/2H_2O$	46.94	5.84	18.25	46.90	5.74	18.38
m	$CH_2C_6H_{11}$-*c*	$C_{27}H_{37}N_9O_6S_3$	47.70	5.49	18.54	47.97	5.67	17.99
n · 2HCl	$CH_2C_4H_9$-*t*	$C_{25}H_{35}N_9O_6S_3 \cdot 2HCl \cdot 3/2H_2O$	39.98	5.35	16.72	39.86	5.42	16.59
o · 2HCl	$C(CH_3)_2C_4H_9$-*t*	$C_{27}H_{39}N_9O_6S_3 \cdot 2HCl \cdot 3/2H_2O$	41.48	5.67	16.13	41.67	5.94	16.15
p · 2HCl	$C(C_2H_5)_3$	$C_{27}H_{39}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	41.01	5.74	15.94	41.14	5.72	15.70

[a] Containing 1.8% of sodium chloride.

Water solubility and lipophilicity

An ester (10 mg) was added to 1/15 M phosphate buffer of pH 4.5 and shaken vigorously for 30 min at room temperature. After the mixture was filtered, the concentration of the ester in the filtrate was measured by HPLC.

HPLC analysis: column, Nucleosil C_{18}; mobile phase, (0.05 M $(NH_4)_2SO_4$/MeCN/AcOH (400 : 200 : 1); flow rate, 1.5 ml/min.

Hydrolysis of the ester to CTM in vitro

A 1% small intestine homogenate of mouse was prepared according to the method previously reported (Yoshimura et al., 1985).

An ester (10 mg equiv. of CTM), was dissolved in a mixture of 1 N HCl (2 drops) and dioxane (1.0 ml), then diluted to the concentration 200 μg/ml with saline. The solution (1.0 ml) was added to the 1% small intestine homogenate (19.0 ml) preheated at 37 °C rapidly so that the final concentration of the ester was equivalent to 10 μg/ml of CTM. Sampling was carried out at 2, 5, 10, 15, 30 and 60 min after the incubation at 37 °C. The sample (1.0 ml) was added to a mixture of 1/15 M phosphate buffer, pH 7.4 (2.0 ml) and dichloromethane (5.0 ml) and shaken vigorously for 1 min. The concentration of CTM in the aqueous layer was measured by bioassay.

Hydrolysis of pivaloyloxymethyl ester ***h*** *in 10% tissue homogenate*

The small intestine or liver removed from mice was homogenized in an ice-cooled saline (1 part tissue: 9 parts saline) and centrifuged at 3000 rpm for 10 min; the supernatant was used as a 10% homogenate of small intestine or liver. The plasma was diluted with 9 times the volume of saline. The hydrolysis of pivaloyloxymethyl ester **h** to CTM was measured according to the above-mentioned procedure.

Δ^2-isomerization of ***h***

The ester **h** (5.0 mg) was dissolved in 0.01 N HCl (10.0 ml). This solution (1.0 ml) was added into an isotonic phosphate buffer of pH 7.4 (9.0 ml) or into a mixture of 10% homogenate of mouse small intestine (1.0 ml) and an isotonic phosphate buffer of pH 7.4 (8.0 ml) and incubated at 37 °C. Sampling was carried out immediately after mixing, then after incubation for 2, 5, 10, 15

and 20 min. Each sample (0.6 ml) was diluted with 0.1 N HCl (0.4 ml) and a definite amount was injected into HPLC through a filter (Cathivex-HA, 0.45 μm, Millipore Corp.).

HPLC analysis: column, μBondapak C_{18}; solvent to detect CTM and Δ^2-CTM, 0.03 M $(NH_4)_2SO_4$/AcOH/MeOH (100 : 3 : 2); flow rate, 0.8 ml/min; retention time CTM, 9.12 min; retention time Δ^2-CTM, 7.95 min.

Bioassay

The plasma concentration of CTM was measured by a cylinder-plate method using *Proteus mirabillis* ATCC 21100 as the assay organism according to the method of Fugono and Maeda (1979).

Results and Discussion

New 16 acyloxymethyl esters of CTM were synthesized to improve the oral bioavailability (*BA*) of CTM as shown in Table 1. On the esters, *BA* and physicochemical properties were measured for the correlation between them.

Oral absorption study

The plasma CTM levels and the area under the plasma CTM level–time curves (*AUC*) for 0–2 h were measured after oral administration of CTM or the esters of CTM in mice at a dose of 100 mg/kg equiv. to CTM; CTM administered s.c. served as a reference. The results are shown in Table 4.

After oral administration of the esters, the plasma CTM levels were higher than those observed after oral dosing with CTM; most of the esters showed the peaks at 0.25 h and the plasma levels below the detection limit at 3 h after dosing. The 2-ethylbutyryloxymethyl ester **j** showed the highest peak plasma CTM level (C_{max}), 27 μg/ml, followed by the 2-propylvaleryloxymethyl ester **l**, 24.2 μg/ml, the pivaloyloxymethyl ester **h** and the 3,3-dimethylbutyryloxymethyl ester **n**, more than 20 μg/ml.

The relative *BA* of CTM after oral administra-

TABLE 4

Plasma levels of cefotiam (CTM), area under plasma CTM levels–time curve and relative bioavailability after oral administration of the acyloxymethyl esters of CTM in mice

Ester no.	Dosing route	Plasma levels of CTM (μg/ml)				AUC_0^{2h} (μg · h/ml)	Bioavailability (%)
		0.25 h	0.5 h	1 h	2 h		
CTM	s.c.	69.2 ± 6.1	29.0 ± 1.6	13.2 ± 1.8	1.5 ± 0.7	38.82	100.0
CTM	p.o.	2.3 ± 0.4	2.8 ± 0.1	1.1 ± 0.1	0.0	2.45	6.3
a	p.o.	4.1 ± 0.2	3.6 ± 0.5	1.7 ± 0.6	0.0	3.65	9.4
b	p.o.	9.0 ± 0.3	5.2 ± 0.7	3.4 ± 0.1	2.1 ± 0.1	7.80	20.1
c	p.o.	11.3 ± 0.2	4.0 ± 1.4	4.0 ± 0.5	0.5 ± 0.0	6.34	16.3
d	p.o.	14.6 ± 3.0	11.8 ± 3.9	2.0 ± 0.5	1.2 ± 0.2	10.18	26.2
e	p.o.	20.0 ± 0.0	6.0 ± 0.4	1.6 ± 0.1	0.8 ± 0.1	8.8	22.7
f	p.o.	17.7 ± 0.6	13.5 ± 0.8	4.6 ± 0.6	0.6 ± 0.0	13.23	34.1
g	p.o.	14.6 ± 0.4	8.8 ± 0.8	2.9 ± 0.2	0.8 ± 0.5	9.53	24.5
h	p.o.	21.0 ± 1.3	16.2 ± 3.1	6.1 ± 0.5	0.6 ± 0.1	16.20	41.8
i	p.o.	16.8 ± 0.5	8.3 ± 0.7	2.1 ± 0.7	0.6 ± 0.0	9.20	23.7
j	p.o.	27.0 ± 0.5	11.4 ± 1.8	7.1 ± 0.3	3.0 ± 1.8	17.85	46.0
k	p.o.	15.4 ± 2.4	13.9 ± 1.8	4.0 ± 0.2	0.6 ± 0.1	13.30	34.3
l	p.o.	24.2 ± 3.1	18.8 ± 2.7	9.0 ± 2.2	2.1 ± 0.3	20.90	53.8
m	p.o.	15.8 ± 2.4	13.4 ± 1.6	4.1 ± 0.6	1.2 ± 0.4	12.65	32.5
n	p.o.	23.5 ± 2.1	10.1 ± 2.2	7.7 ± 2.5	1.3 ± 0.5	16.09	41.4
o	p.o.	10.9 ± 0.7	6.9 ± 0.8	3.2 ± 0.9	0.3 ± 0.3	7.86	20.2
p	p.o.	11.5 ± 1.4	9.5 ± 0.9	7.0 ± 0.7	2.1 ± 0.6	12.74	32.8

Values are mean ± S.E. $n = 4$. Dose = 100 mg/kg equiv. to CTM.

tion was only 6.3%, whereas, the *BA* of most esters, except that of the acyloxymethyl ester **a**, were improved 2.5 to 8.5 fold. Among the esters, the ester **l** showed the highest *BA*, 53.8%, followed by the ester **j**, 46%; the esters **h** and **n**, more than 40%.

We found the *BA* of **l** was 1.3-fold improved from that (41.8%) of **h**, which showed the highest *BA* in the previous study (Yoshimura et al., 1985).

In vitro study

Although, we found an ester of CTM which showed the oral CTM *BA* 53.8%, the *BA*s were much different among 16 esters of CTM probably due to the structural difference in the ester moiety. Thus, we carried out an investigation on the physicochemical factors of the ester of CTM influencing the *BA* of CTM, e.g. water solubility, lipophilicity and hydrolysis of the ester to CTM and Δ^2-isomerization. Optimization of the factors are necessary for the rational design of the best ester of CTM.

Water solubility

As the water solubility of a pivaloyloxymethyl ester of a parenteral cephalosporin at pH 4.5 was closely related to the oral *BA* (Yoshimura et al., 1985), we determined the water solubility of the acyloxymethyl esters of CTM (**a–j**, **l–n**, and **p**) in this study under the same conditions (Table 5); the solubilities were between 1.31 mg/ml and 3.46 mg/ml. At pH 4.5, dimethylaminoethyl group having pK_a 7.0 is protonated, and this dissociation contributes to the water solubility of the esters.

Although the correlation between water solubility and C_{max} or *BA* was examined by least-squares analysis, no significant correlation was observed. As they have adequate water solubility, the dissolution in the GI tract seems not to be the critical factor for the oral absorption of an ester of CTM.

TABLE 5

Water solubility, retention time and capacity factor of the acyloxymethyl esters of cefotiam and Hansch's lipophilic constant and Taft's steric constant of R

Ester no.	Water solubility S (mg/ml) [a]	Retention time/ t_r (min) [b]	Capacity factor k' [c]	π [d]	E_s [e]
a	4.35	1.93	0.229	0.5	0.00
b	2.72	2.13	0.365	1.0	−0.07
c	3.28	2.31	0.480	1.50	−0.36
d	3.46	2.34	0.500	1.37	−0.47
e	2.52	2.96	0.897	2.00	−0.39
f	2.81	2.80	0.795	1.87	−0.93
g	2.38	2.75	0.763	1.87	−1.13
h · 2HCl	2.71	2.80	0.795	1.68	−1.54
i	1.82	4.14	1.65	2.50	−0.40
j · 2HCl	3.14	3.56	1.28	2.37	−1.98
k	N.D.	N.D. [f]		3.50	−0.40
l	1.88	7.88	4.05	3.37	−2.11
m	1.65	5.92	2.80	2.89	−0.98
n · 2HCl	1.31	3.60	1.31	2.18 [g]	−1.74
o · 2HCl	N.D.	N.D.		2.89	−3.9
p · 2HCl	1.77	6.39	3.10	3.18	−3.8

[a] At pH 4.5.
[b] See Materials and Methods.
[c] $k' = (t_r - t_0)/t_0$, where t_0 is the retention time of potassium iodide used as an unretained compound.
[d] Hansch's lipophilic constant of R (Craig, 1971).
[e] Taft's steric constant of R (Craig, 1971).
[f] N.D. not done.
[g] Estimated from (t-$C_4H_9CH_2$) = 1.68 (t-C_4H_9) + 0.50 (CH_2).

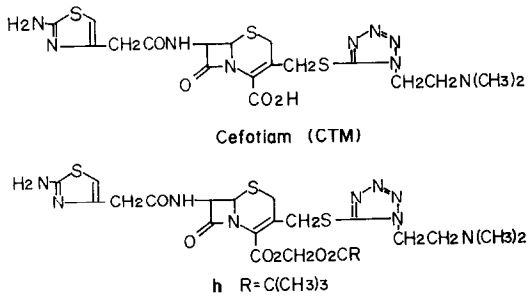


Fig. 1. Structure of cefotiam and its pivaloyloxymethyl ester (**h**).

Lipophilicity

The transportation of a drug across the GI membrane is markedly influenced by its partition coefficient (Lien, 1975). However, there have been few reports on the quantitative structure–GI absorption relationship of cephalosporins (Austel and Kutter, 1983; Yoshimura et al., 1985). As the structural difference among the esters is only in the R of the ester moiety, the log P value of the undissociated form of an ester can be calculated from the substituent lipophilic constant (π value) of R according to the Hansch's method (Leo et al., 1971; Tute, 1971). The log P value of the esters calculated from that (1.57) of the undissociated form of the pivaloyloxymethyl ester (**h**) as a standard were between 0.39 and 3.07.

However, to design an ester moiety, it is more convenient to use π value than log P value as lipophilic parameter. Also the lipophilicity of a compound is closely related to the capacity factor, k', obtained by reverse-phase high-performance liquid chromatography (HPLC), and HPLC method has been proposed as one of the useful methods to determine log P (Mirrlees et al., 1976; Yamana et al., 1977; Miyake and Terada, 1978; Caron and Shroot, 1984). To confirm the Hansch's additivity of the log P on the esters of CTM, we measured the retention time of the esters by reverse-phase HPLC (column: Nucleosil C_{18}, 300 × 4 mm i.d.; solvent: 0.05 M $(NH_4)_2SO_4$/MeCN/AcOH (400 : 200 : 1). Table 5 shows the retention time (t_r) and capacity factor (k') of the esters. The t_r was between 2.13 and 7.88 min. A good linear correlation between π and log k' was observed following Eqn. 1 derived by least-squares analysis.

$$\log k' = \log(t_r - t_0)/t_0 = -0.905 + 0.445\pi \quad (1)$$

$$n = 14,\ r = 0.993,\ s = 0.147,$$

$$F_{1,11} = 848.2\ (F_{1,11;\alpha=0.005} = 12.2)$$

where t_0 is the retention time of potassium iodide used as an unretained solute, n number of studies, r the correlation coefficient, s the standard deviation, and F the F-statistic.

The result shows that log P value of the ester can be calculated from the π value of R and that π value or log k' can be used as the lipophilic parameter of an ester of CTM.

When the π values of R of the esters were plotted against log C_{max} and log BA of CTM (Fig. 2A and B), good parabolic correlations following Eqns. 2 and 3 derived through least-squares analysis were observed.

$$\log C_{max}(\mu g/ml) = 0.300 + 0.0807\pi - 0.163(\pi)^2 \quad (2)$$

$$n = 16,\ r = 0.817,\ s = 0.126,\ (\pi)_0 = 2.48,$$

$$F_{2,13} = 13.04\ (F_{2,13;\alpha=0.005} = 8.19)$$

$$\log BA(\%) = 0.831 + 0.485\pi - 0.082(\pi)^2 \quad (3)$$

$$n = 16,\ r = 0.714,\ s = 0.144,\ (\pi)_0 = 2.97,$$

$$F_{2,13} = 6.76\ (F_{2,13;\alpha=0.01} = 6.70)$$

where $(\pi)_0$ is the optimal π value.

These results show that C_{max} and BA observed after oral administration of the ester are closely correlated to the lipophilicity of R, suggesting the esters are absorbed from the GI tract by the passive transport.

From Eqn. 3, the π value of R resulting in a BA of more than 30% is between 2.03 and 3.88 and the optimal π values, $(\pi)_0$, obtained from Eqns. 2 and 3 were 2.48 and 2.97, respectively.

Hydrolysis to the parent drug in vitro

A prodrug must be hydrolyzed to the parent

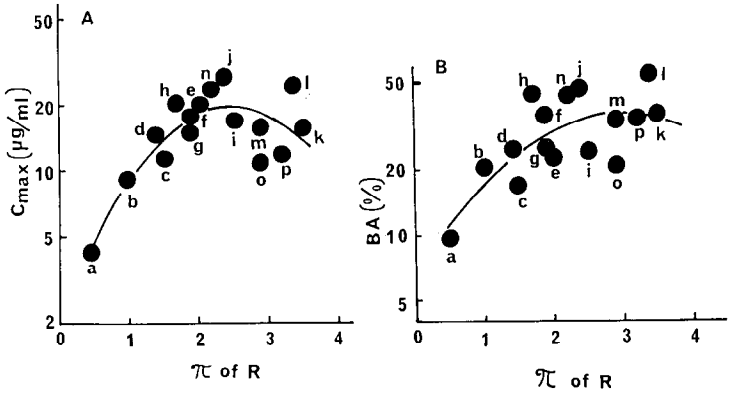


Fig. 2. Relation between Hansch's substituent lipophilic constant (π value) of R and the peak plasma CTM level (C_{max}) (A) and relation between Hansch's substituent lipophilic constant (π value) of R and relative bioavailability (BA) (B) of cefotiam after oral administration of the acyloxymethyl ester of CTM at a dose of 100 mg/kg equiv. to CTM.

drug during or after absorption into the body. Consequently, rate of hydrolysis of the starting ester to CTM is one of the important factors affecting GI absorption of CTM and it is desirable for an ester to be hydrolyzed in the GI wall during absorption from the safety point of view (Wright and Line, 1976).

From the results of absorption study, the esters must be hydrolyzed to CTM rapidly in vivo. However, to determine the difference of the hydrolysis rates of the esters more clearly, we measured the hydrolysis rates of the esters (**b**–**j**, **m**, **o** and **p**) to CTM in a 1% homogenate of mouse small intestine at 37°C; the results are shown in Table 6. The hydrolysis took place following pseudo-first-order kinetics. The esters **b**, **c**, **d**, **e** and **i**, having Rs of straight alkyl chains, were rapidly hydrolyzed to CTM with a half-life ($t_{1/2}$) of less than 10 min and the esters, **g**, **h**, **j** and **m**, having Rs of branched alkyl chains, were hydrolyzed to CTM with a $t_{1/2}$ between 10 and 30 min. The hydrolysis of **o** or **p** were very slow with a $t_{1/2}$ of more than 100 min.

TABLE 6

Hydrolysis rate constant (k) and half-life ($t_{1/2}$) to CTM from its acyloxymethyl esters in 1% homogenate of mouse small intestine at 37°C [a]

Ester no.	k (min^{-1}) [b]	$t_{1/2}$ (min)
b	1.05×10^{-1}	6.6
c	2.17×10^{-1}	3.2
d	2.10×10^{-1}	3.3
e	1.98×10^{-1}	3.5
f	1.12×10^{-1}	6.2
g	5.02×10^{-2}	13.8
h	3.07×10^{-2}	22.6
i	2.77×10^{-1}	2.5
j	2.49×10^{-2}	27.8
m	5.78×10^{-2}	12.0
o	5.54×10^{-3}	125.0
p	6.54×10^{-3}	106.0

[a] The initial concentration of an ester was 10 μg/ml as CTM.
[b] At pH 4.5.

The steric hindrance of a substrate affects the enzymatic or non-enzymatic hydrolysis of an ester (Morozowich et al., 1977). In Fig. 3, the Taft's steric constant (E_s value) of R are plotted against the log $t_{1/2}$. A good linear correlation following Eqn. 4 derived by least-squares analysis was observed.

$$\log t_{1/2}(\text{min}) = 0.496 - 0.424E_s \quad (4)$$

$$n = 12,\ r = 0.951,\ s = 0.187,$$

$$F_{1,10} = 94.0\ (F_{1,10;\alpha=0.005} = 12.2)$$

This result indicates that an enzymatic hydrolysis of the acyloxymethyl ester is closely related to the steric hindrance of R in the ester moiety and that it is reasonable to use the E_s value as a parameter of the hydrolysis rate.

We also measured the hydrolysis rate of the

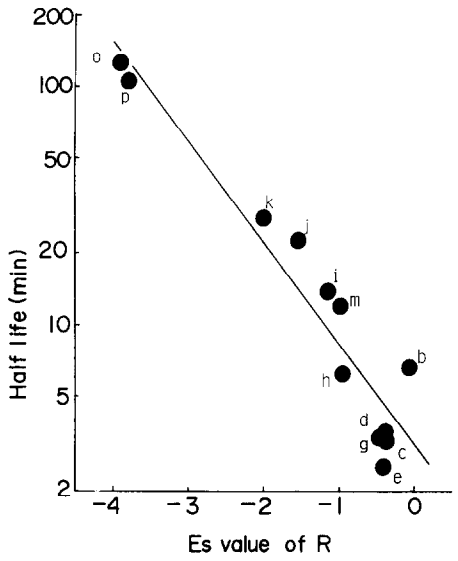


Fig. 3. Relation between Taft's steric constant (E_s value) and the half-life ($t_{1/2}$) of the hydrolysis of acyloxymethyl ester to CTM in 1% homogenate of small intestine of mice at 37 °C.

ester **h** to CTM in the 10% homogenates of mouse small intestine, liver, and 10% plasma at 37 °C. The ester **h** was hydrolyzed rapidly to CTM in the homogenates of small intestine, liver and plasma with a $t_{1/2}$ of 0.90 min, 2.3 min and 2.4 min, respectively.

As the E_s value of R significantly affected to the hydrolysis rates in vitro, we tried to correlate the E_s value with C_{max} or BA. In Fig. 4A and B, the Taft's E_s values of R were plotted against log C_{max} or log BA. Improved parabolic correlations following Eqns. 5 and 6 derived through least-squares analysis were observed.

$$\log C_{max}(\mu g/ml) = 0.914 - 0.466E_s - 0.112E_s^2 \quad (5)$$

$n = 16,\ r = 0.818,\ s = 0.124,\ (E_s)_0 = -2.07,$

$F_{2,13} = 13.14\ (F_{2,13;\alpha=0.005} = 8.19)$

$$\log BA(\%) = 1.171 - 0.452E_s - 0.101E_s^2 \quad (6)$$

$n = 16,\ r = 0.858,\ s = 0.109,\ (E_s)_0 = -2.24,$

$F_{2,13} = 18.1\ (F_{2,13;\alpha=0.005} = 8.19)$

where $(E_s)_0$ is the optimal E_s value.

These results suggest that oral BA or C_{max} is also related to the hydrolysis rates in vitro as in Eqn. 4, and that consideration of the steric hindrance of an ester moiety is important to design the orally active ester of cephalosporin, although much attention has not been paid to it.

The E_s values of R giving a C_{max} of more than 20 μg/ml and a BA of more than 40% is between -1.15 and -3.01 and between -1.38 and -3.10, respectively.

Δ^2-isomerization

Isomerization of an ester of a cephalosporin to the corresponding ester of Δ^2-cephalosporin in a

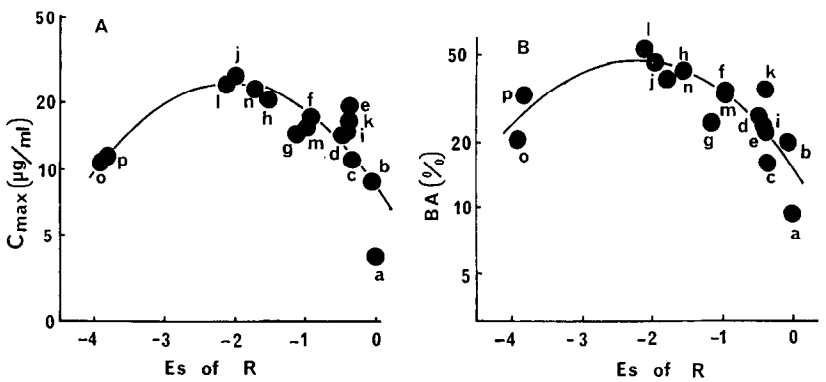


Fig. 4. Relation between Taft's steric constant (E_s value) and peak plasma CTM level (A) and relation between Taft's steric constant (E_s value) and relative bioavailability (BA) (B) of cefotiam after oral administration of acyloxymethyl ester of CTM in mice at a dose of 100 mg/kg equivalent to CTM.

neutral or alkaline medium was reported (Ferres, 1983).

Therefore, we investigated on the Δ^2-isomerization of the ester of CTM (**h**) in a buffer of pH 7.4 at 37 °C by HPLC.

In the buffer solution, the ester **h** was unstable to decompose following pseudo-first-order kinetics with a half-life of 12.3 min. The produced compound was not CTM but Δ^2-CTM. As CTM was not isomerized to Δ^2-CTM under the conditions, the ester of CTM must be transformed Δ^2-CTM via the corresponding ester of Δ^2-CTM (Scheme 2). This instability of the CTM ester was ascribed to the dimethylaminoethyl group having pK_a of 7.0 as an intramolecular catalyst.

On the other hand, by adding the homogenate of mouse small intestine into the buffer of pH 7.4, the ester **h** was enzymatically hydrolyzed to CTM but Δ^2-CTM was also detected (CTM, 39.5% and Δ^2-CTM, 13.1% in 5 min). As CTM was not isomerized to Δ^2-CTM under these conditions, Δ^2-CTM must be derived from the ester via the corresponding ester of Δ^2-CTM. These results suggest that the hydrolysis and isomerization of the CTM ester may be parallel reactions in vitro and the ratio of the products would change depending on the enzyme activity or the susceptibility of an ester toward hydrolysis.

From Eqns. 5 and 6, it is suggested that an ester having a low sterically hindered R is easily hydrolyzed to CTM before absorption to give a low C_{max} or *BA* and that an ester having a high sterically hindered R is hydrolyzed slowly showing a low *BA* owing probably to the inactivation through Δ^2-isomerization.

Also, correlations among π, E_s, and C_{max} or *BA* through least-squares analysis were attempted to give the Eqns. 7 and 8. As the contribution of $(\pi)^2$-term to *BA* was very little in Eqn. 8, it is modified as Eqn. 9.

$$\log C_{max}(\mu g/ml) = 0.420 + 0.556\pi - 0.105(\pi)^2 - 0.260E_s - 0.073E_s^2 \quad (7)$$

$n = 16,\ r = 0.941,\ s = 0.080,$

$F_{4,11} = 21.15\ (F_{4,11;\alpha=0.005} = 6.88)$

$$\log BA(\%) = 1.033 + 0.100\pi - 0.002(\pi)^2 - 0.372E_s - 0.090E_s^2 \quad (8)$$

$n = 16,\ r = 0.910,\ s = 0.093,$

$F_{4,11} = 13.21\ (F_{4,11;\alpha=0.005} = 6.88)$

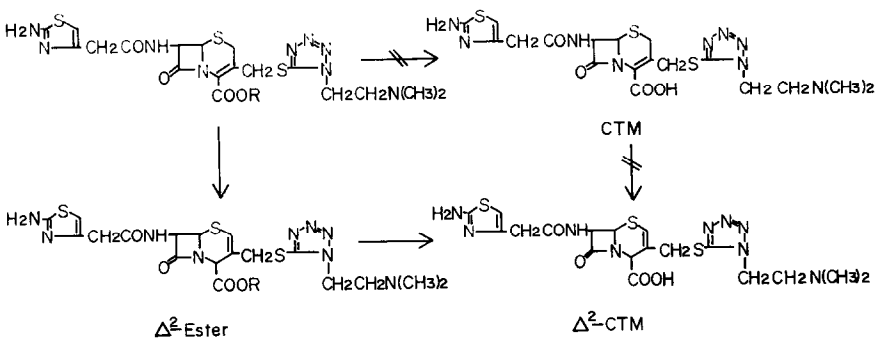
$$\log BA(\%) = 1.039 + 0.092\pi - 0.375E_s - 0.091E_s^2 \quad (9)$$

$n = 16,\ r = 0.911,\ s = 0.089,$

$F_{3,12} = 19.41\ (F_{3,12;\alpha=0.005} = 7.23)$

The optimal $(\pi)_0$ and $(E_s)_0$ are 2.65 and −1.80, respectively in Eqn. 7 and $(E_s)_0$ is −2.06 in the Eqn. 9. Although the $(\pi)_0$ value is large value from Eqn. 8, it seems that $(\pi)_0$ is between 2 and 4 from Eqns. 2, 3 and 7.

The analysis promises that an acyloxymethyl



Scheme 2

ester of CTM having R with the E_s value of -2.06 and the π value between 2 and 4 results in a good oral CTM *BA*.

Conclusion

Newly synthesized acyloxymethyl esters of CTM showed more enhanced plasma levels and *BA* of CTM after oral administration than CTM per se and among the esters, the 2-propylvaleryloxymethyl ester (**I**) showed the best *BA* of 53.8%.

Although water solubility, lipophilicity, hydrolysis rate to the parent drug and Δ^2-isomerization of an ester are well known as important factors influencing the oral *BA* of an ester prodrug of a parenteral cephalosporin, we characterized these factors for designing a prodrug of CTM to show more improved oral *BA*. Further, we found one more important factor, steric hindrance of the ester moiety, affecting oral *BA* of the CTM prodrug.

The results of the present study indicate that an acyloxymethyl ester of CTM should be designed to have R group in the promoiety with a π value between 2 and 4 and an E_s value near -2 for the GI absorption if an ester has adequate water solubility.

A successful application of these results to the prodrug of CTM will be reported elsewhere.

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