PREPARATION OF 1-ACYLOXYETHYL ESTERS OF 7-[2-(2-AMINOTHIAZOL-4-YL)ACETAMIDO]-3-[[[1-(2-DIMETHYLAMINOETHYL)-1*H*-TETRAZOL-5-YL]THIO]-METHYL]CEPH-3-EM-4-CARBOXYLIC ACID (CEFOTIAM) AND THEIR ORAL ABSORPTION IN MICE

YOSHINOBU YOSHIMURA,* NAORU HAMAGUCHI and TAKATSUKA YASHIKI

Central Research Division, Takeda Chemical Industries, Ltd.,
2-17-85 Juso-honmachi, Yodogawa-ku, Osaka 532, Japan

(Received for publication April 21, 1986)

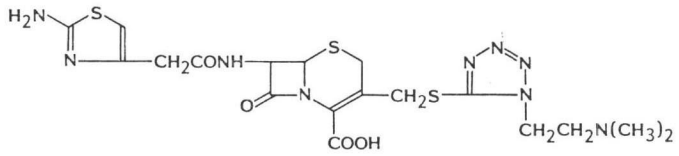
In a separate study on the orally active acyloxymethyl esters (**1**) of 7-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid (cefotiam, CTM), we have shown, by quantitative structure-oral bioavailability (BA) relation analysis, that the R_2 group in the acyl group R_2CO must have both an adequate lipophilicity (HANSCH's lipophilic parameter, π) and steric hindrance (TAFT's Es value). However, to satisfy these requirements, a complex alkyl group R_2 must be employed, the ester of which is difficult to synthesize and has unique metabolic fate. In this study, we selected and prepared the 1-acyloxyethyl esters (**2**) of CTM instead of **1** to avoid R_2 groups that are too complicated. We found that the esters (**2**) gave improved oral BAs over **1**: the 1-(3-methylvaleryloxy)ethyl ester (**2h**) showed the highest peak plasma CTM level (C_{max}) comparable to that obtained after subcutaneous injection of CTM. The 1-(cyclohexylacetoxy)ethyl ester (**2t**), the 1-(2-ethylbutyryloxy)ethyl ester (**2j**), and **2h** showed BAs near 100%. For these esters (**2**), good correlations were also observed among the π, the Es values of R_2, and the log C_{max} and log BA in the analysis of the quantitative structure-oral bioavailability relation: an ester having an alkyl group as R_2 with a π value of 3.07 or 3.08 and a Es value of -1.04 or -1.29 gave the highest C_{max} or BA, respectively. As expected, the optimal π values are almost the same as those obtained with **1** but the optimal Es values are larger (Es$=-2.07$). Thus, it has been confirmed by preparing 1-acyloxyethyl esters (**2**) of CTM that the oral bioavailability of CTM can further be improved without preparing acyloxymethyl esters (**1**) with a complicated acyl group.

Cefotiam (CTM), 7-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid,[1] have excellent *in vitro* and *in vivo* antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria and is effective in treating serious infections caused by various microorganisms.[2] However, CTM after it is administered orally is poorly absorbed from the gastrointestinal (GI) tract[3] owing probably to its high hydrophilicity. Consequently, its clinical use has been limited to the parenteral route.

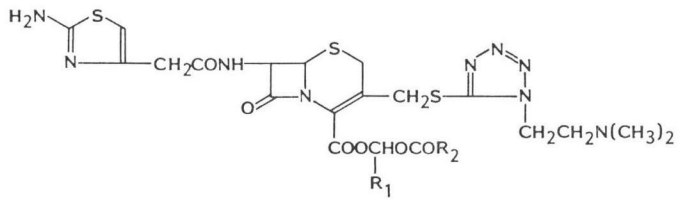
Considerable effort has been made to improve the oral bioavailability (BA) of many parenteral cephalosporins such as, CTM,[3,4] AL-226,[5] cefamandole,[6,7] cefuroxime,[8] and KY-087,[9] by preparing their ester prodrugs. Although the oral BA of these esters are considerably improved over those of the parent cephalosporins, they are still lower than those of the orally active cephalosprorins, *e.g.*, cephalexin (CEX), cefatrizine (CFT) and cefadroxil (CDX).

In a separate study on the acyloxymethyl ($R_2CO_2CH_2$) esters of cefotiam, we found that the 2-propylvaleryloxymethyl ester (**1n**) showed the best BA, 53.8% after being orally administered to mice[4] and proposed that the R_2 group in the ester moiety should have the HANSCH's lipophilic parameter (π value[10])

Fig. 1.



Cefotiam (CTM)

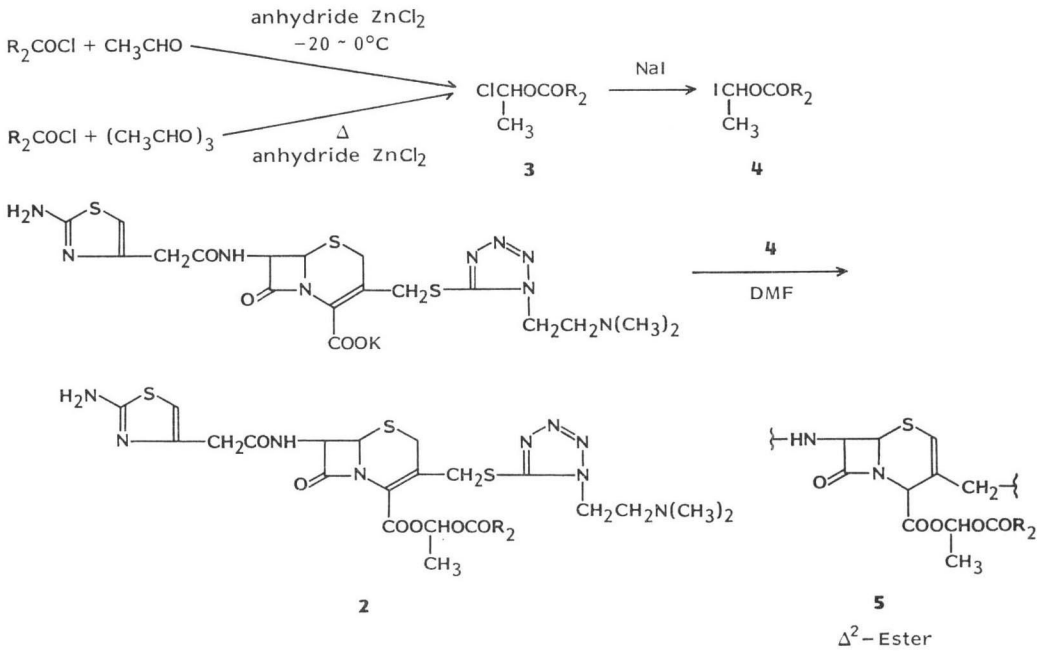


1 R_1 = H R_2 = alkyl

1n R_1 = H R_2 = CH(n-C_3H_7)$_2$ BA = 53.8 %

2 R_1 = CH_3 R_2 = alkyl

Scheme 1.



between 2 and 4 (alkyl group with carbon number between 4 and 8) and the TAFT's Es value (a steric parameter[11]) of −2.06, for good oral bioavailability.

A branched alkyl group as R_2 seemed to meet these requirements. However, it was suspected that esters with such a branched alkyl (R_2) group might raise synthetic and toxic problems. If, however, a methyl group is introduced to the methylene group adjacent to the carboxyl oxygen, an acyl group R_2CO, in which the R_2 group is of the kind which has smaller values of π (0.3 unit less) and Es, may be accepted. In other words, a simpler alkyl, R_2, might be used in case of the 1-acyloxyethyl esters (**2**)

Table 1. Plasma levels, area under plasma level-time curve and relative bioavailability (BA) after oral administration of the 1-acyloxyethyl esters (**2**) of CTM in mice at a dose of 100 mg/kg equiv to CTM.

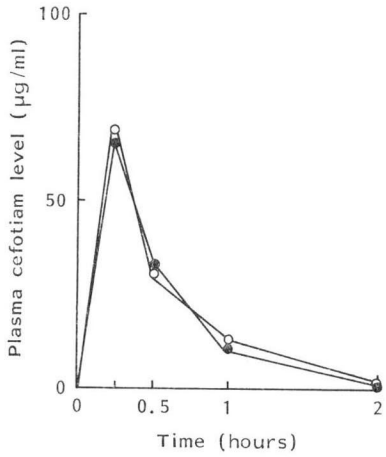
Ester No.	R_2	Plasma level of CTM (μg/ml) Time (hour(s)) 0.25	0.5	1	2	AUC (μg·hours/ml)	BA (%)
2a	CH_3	7.27 (0.97)*	4.84 (0.44)	1.46 (0.05)	0.24 (0.08)	4.85	12.5
2b	*i*-C_3H_7	27.7 (5.1)	12.0 (1.6)	4.57 (1.23)	0.90 (0.39)	15.3	39.5
2c	*n*-C_4H_9	32.5 (3.6)	12.6 (3.5)	6.06 (1.15)	1.81 (0.44)	18.3	47.2
2d	*i*-C_4H_9	35.5 (7.0)	28.4 (3.6)	6.97 (1.50)	1.73 (0.38)	25.6	66.0
2e	*t*-C_4H_9	32.0 (7.5)	23.5 (5.0)	4.52 (0.62)	0.56 (0.13)	20.5	52.8
2f	*n*-C_5H_{11}	44.6 (4.0)	24.2 (3.4)	7.46 (0.94)	0.72 (0.09)	26.2	67.5
2g	*i*-$C_4H_9CH_2$	36.0 (3.3)	20.8 (3.5)	4.37 (0.55)	0.77 (0.08)	20.5	52.8
2h	*s*-$C_4H_9CH_2$	64.4 (7.5)	33.5 (1.8)	9.43 (1.20)	0.98 (0.09)	36.6	93.2
2i	*t*-$C_4H_9CH_2$	23.8 (3.1)	20.0 (0.4)	5.45 (0.87)	1.22 (0.09)	18.2	46.8
2j	$(C_2H_5)_2CH$	21.6 (10.0)	30.0 (9.2)	27.1 (4.3)	3.65 (0.51)	38.8	100.0
2k	*n*-C_6H_{13}	33.5 (2.8)	14.4 (0.8)	4.09 (1.05)	0.62 (0.01)	17.2	44.2
2l	$(C_2H_5)_2CHCH_2$	37.1 (4.9)	36.0 (8.1)	6.84 (2.06)	4.93 (0.86)	30.4	78.2
2m	*n*-C_7H_{15}	36.6 (8.2)	24.1 (6.4)	6.55 (0.77)	0.86 (0.32)	23.5	60.6
2n	$(n\text{-}C_3H_7)_2CH$	18.7 (3.4)	30.5 (1.0)	11.3 (1.1)	2.20 (0.42)	25.7	66.2
2o	*n*-C_9H_{19}	28.9 (2.0)	15.2 (3.1)	6.05 (1.42)	0.24 (0.05)	17.6	45.3
2p	*c*-C_4H_7	36.5 (1.4)	16.5 (1.5)	3.82 (0.40)	0.75 (0.18)	18.6	47.8
2q	*c*-C_5H_9	42.5 (8.1)	29.9 (4.0)	10.5 (0.5)	1.35 (0.45)	30.4	78.3
2r	*c*-$C_5H_9CH_2$	23.2 (3.2)	27.2 (5.7)	16.9 (3.2)	6.46 (1.69)	31.9	82.2
2s	*c*-C_6H_{11}	43.6 (5.7)	31.7 (2.3)	8.38 (2.36)	1.01 (0.43)	29.6	76.3
2t	*c*-$C_6H_{11}CH_2$	49.7 (3.9)	46.8 (3.7)	13.4 (1.7)	3.59 (0.90)	41.8	107.8
2u	$C_6H_5CH_2$	21.3 (3.5)	20.0 (1.1)	6.03 (0.80)	0.0	17.3	44.7
CTM (sc)[b]		69.2 (6.1)	29.0 (1.3)	13.2 (1.5)	1.50 (0.60)	38.8	100.0

( )*: SE (n=4).

[b] Subcutaneous administration of CTM at the same dose.

c; Cyclic.

Fig. 2. Plasma levels of CTM after **2h** was administered at 100 mg/kg equivalent to CTM to mice.
○: **2h** po, ●: CTM sc.



than in case of the acyloxymethyl ester (**1**) (Fig. 1).

To examine this hypothesis, we synthesized the 1-acyloxyethyl esters (**2**) of CTM, the acyl group of which contains an alkyl group, R_2, of 4 to 8 carbon atoms, and evaluated their oral BAs in mice.

Chemistry

The 1-acyloxyethyl esters (**2**) were prepared as shown in Scheme 1. As an acyloxyethyl esterification of CTM should be carried out quickly to avoid the by-production of the corresponding esters (**5**) of the inactive Δ^2-isomer of CTM, it is preferable to use an esterification reagent as reactive as possible. An 1-iodoethyl acylate (**4**) was prepared from NaI and a 1-chloroethyl acylate (**3**). The latter compound **3** was prepared from the corresponding acid chloride and paraaldehyde in the presence of a Lewis acid, *e.g.*, anhydrous $ZnCl_2$ by heating (Method B).[12] However, by this method, an acid chloride having a straight or branched alkyl group of more than five carbon atoms gave **3** only a very low yield. We found that when an acid chloride was reacted with acetaldehyde in the presence of anhydrous $ZnCl_2$ at low temperature ($-20\sim0$°C) and the reaction mixture was chromatographed on silica gel with petroleum ether as the eluent, **3** was obtained in good yield as shown in Table 5. Also, **3k** and **3m**, which were not obtained by Method B, were isolated in good yield by this method.

The potassium salt of CTM was then treated with **4** in *N*,*N*-dimethylformamide (DMF) at $-10\sim0$°C for $5\sim10$ minutes to afford 1-acyloxyethyl esters (**2**) in $16\sim60\%$ yield. The ester was isolated as the free base or the dihydrochloride of a mixture of diastereoisomers (almost 1:1) and no Δ^2-esters (**5**) were detected by high performance liquid chromatography (HPLC) or NMR.

Results and Discussion

Oral Absorption Study

The esters (**2**) were administered orally to mice at a dose of 100 mg/kg equivalent to CTM and the plasma levels of CTM were measured and compared with those after CTM was administered subcutaneously at the same dose. The results are shown in Table 1.

With most of the esters (**2**), the plasma CTM levels peaked at 0.25 hour, and then decreased to below 1 μg/ml 2 hours after dosing. Among the esters, the 1-(3-methylvaleryloxy)ethyl ester (**2h**) showed the highest peak plasma CTM level (C_{max}), 64.4 μg/ml, comparable to that observed after the CTM administered subcutaneously at the same dose (Fig. 2), and was followed by the 1-(cyclohexylacetoxy)-ethyl ester (**2t**) (49.7 μg/ml), the 1-(hexanoyloxy)ethyl ester (**2f**) (44.6 μg/ml), the 1-(cyclohexanecarbonyloxy)ethyl ester (**2s**) (43.6 μg/ml), and the 1-(cyclopentanecarboxy)ethyl ester (**2q**) (42.5 μg/ml).

The 1-acetoxyethyl ester (**2a**) (7.27 μg/ml) showed a relatively low C_{max}.

The relative bioavailability (BA) of an ester was calculated from the AUC value on the basis of the

Table 2. Water solubility and retention time of the 1-acyloxyethyl esters of CTM.

Ester No.	R_2	Water solubility S (mg/ml)[a]	Retention time	
			t_{r_1} minutes	t_{r_2} minutes
2a	CH_3	3.93	2.14[b]	
2b	*i*-C_3H_7	4.73	2.74	3.09
2c	*n*-C_4H_9	3.63	3.62	3.85
2d	*i*-C_4H_9	3.37	3.48	3.65
2e·2HCl	*t*-C_4H_9	2.33	3.33	3.55
2f·2HCl	*n*-C_5H_{11}	3.61	5.38	5.84
2g·2HCl	*i*-$C_4H_9CH_2$	2.20	4.99	5.42
2h·2HCl	*s*-$C_4H_9CH_2$	3.50	4.85	5.18
2i	*t*-$C_4H_9CH_2$	4.50	4.58	4.76
2j·2HCl	$(C_2H_5)_2CH$	3.52	4.41	4.79
2k	*n*-C_6H_{13}	3.09	8.79	9.69
2l·2HCl	$(C_2H_5)_2CHCH_2$	3.50	6.93	7.50
2m	*n*-C_7H_{15}	1.78	15.34	17.2
2n·2HCl	$(n\text{-}C_3H_7)_2CH$	1.35	10.42	11.92
2o	*n*-C_9H_{19}	NT		
2p·2HCl	*c*-C_4H_7	NT		
2q·2HCl	*c*-C_5H_9	3.54	3.71	4.04
2r·2HCl	*c*-$C_5H_9CH_2$	2.49	5.45	5.88
2s·2HCl	*c*-C_6H_{11}	1.95	5.06	5.62
2t·2HCl	*c*-$C_6H_{11}CH_2$	1.30	7.83	8.53

[a] At pH 4.5.
[b] Each isomer was not separated under the conditions.
t_{r_1}, t_{r_2}: Retention times of each isomer.
NT: Not tested.

AUC after CTM was administered subcutaneously at the same dose (Table 1). The 1-(cyclohexylacetoxy)ethyl ester (**2t**), the 1-(2-ethylbutyryloxy)ethyl ester (**2j**), and the 1-(3-methylvaleryloxy)ethyl ester (**2h**) showed the highest BAs 107.8, 100.0, and 93.2%, respectively. These BAs were comparable to those of the orally active cephalosporins, *e.g.*, CEX and cephradine. The 1-acetoxyethyl ester (**2a**) showed a low BA, 12.5%.

In Vitro Study

To have good oral BA, an ester prodrug must be dissolved in the GI fluid, transported across the GI membrane, and hydrolyzed to the parent drug. Thus, physico-chemical properties such as water solubility, lipophilicity and hydrolysis rate, affect the absorption efficiency of a prodrug from the GI tract.[3~5]

The water solubility of esters (**2**) at pH 4.5 (near the virtual pH) was measured by HPLC and was shown to be between 1.30 and 4.73 mg/ml. All the solubilities were adequate.

In the previous report,[4] we found that lipophilicity of the ester was related to the capacity constant (k′) calculated from the retention time (t_r) in HPLC.† As the esters (**2**) exhibited a larger t_r compared

† High linear correlations were observed between log k'_1 or log k'_2 of the esters (**2**) and HANSCH's lipophilic parameter π value of R_2 as shown in Table 4.

$$\log k'_1 = \log (t_{r_1} - t_0)/t_0 = -0.727 + 0.452\pi$$
$$n=16,\ r=0.991$$
$$\log k'_2 = \log (t_{r_2} - t_0)/t_0 = -0.693 + 0.479\pi$$
$$n=16,\ r=0.992$$

t_0: Retention time of potassium iodide using as an unretained solute: 1.56 minutes.

Table 3. Time to release 50% of CTM from the 1-acyloxyethyl esters of CTM in 1% homogenate of small intestine of mice at 37°C.

Ester No.	R_2	Time[a] (minutes)	Release of CTM[b] (%)
2e	t-C_4H_9		12.2
2d	i-C_4H_9	18.4	
2f	n-C_5H_{11}	1.1	
2h	s-$C_4H_9CH_2$	12.3	
2i	t-$C_4H_9CH_2$		8.4
2j	$(C_2H_5)_2CH$	66.4	
2k	n-C_6H_{13}	2.5	
2l	$(C_2H_5)_2CHCH_2$	35.9	
2q	c-C_5H_9	25.5	
2r	c-$C_5H_9CH_2$	2.5	
2s	c-C_6H_{13}	10.7	
2t	c-$C_6H_{11}CH_2$	12.2	

[a] Time to release 50% of CTM.
[b] At 30 minutes after incubation.

Table 4. π and Es values of R_2 of the 1-acyloxyethyl esters (**2**).

Ester No.	R_2	π[a]	Es[b]
2a	CH_3	0.5	0.00
2b	i-C_3H_7	1.37	−0.47
2c	n-C_4H_9	2.0	−0.39
2d	i-C_4H_9	1.87	−0.93
2e	t-C_4H_9	1.68	−1.54
2f	n-C_5H_{11}	2.5	−0.40
2g	i-$C_4H_9CH_2$	2.37	−0.35
2h	s-$C_4H_9CH_2$	2.37	−1.07[c]
2i	t-$C_4H_9CH_2$	2.18[c]	−1.74
2j	$(C_2H_5)_2CH$	2.37	−1.98
2k	n-C_6H_{13}	3.0	−0.39
2m	n-C_7H_{15}	3.5	−0.40
2n	$(C_3H_7)_2CH$	3.37	−2.11
2o	n-C_9H_{19}	4.50	−0.40
2q	c-C_5H_9	1.97	−0.51
2s	c-C_6H_{11}	2.39	−0.79
2t	c-$C_6H_{11}CH_2$	2.89	−0.98

[a] HANSCH's lipophilic parameter of R_2 (CRAIG, 1971)[11].
[b] TAFT's steric parameter (CRAIG, 1971)[11].
[c] Estimated value.

with that (2.80 minutes) of the pivaloyloxymethyl ester of CTM, which showed a good BA in mice,[4] compounds as **2** must have enough lipophilicity to be absorbed from the GI tract (Tables 2 and 4).

Also, as an orally active ester prodrug is hydrolyzed to CTM during or after transportation through the intestinal mucosa at first, we were interested in measuring its hydrolysis rate by esterases in the GI wall. From the results of absorption studies, these esters must be hydrolyzed to CTM rapidly *in vivo*. To determine differences among the hydrolysis rates of the esters more distinctly, we selected the conditions for an *in vitro* experiment, *i.e.*, 0.01 mg/ml of an ester as CTM in a 1% homogenate of mouse small intestine (pH 6.4) at 37°C.

The time-period (T_{50}) values, which represent the time by which 50% of the CTM content is released, were taken as an index of hydrolysis of the initial esters. The observed T_{50} values are summarized in Table 3. As some of the esters (**2** (**2d**, **2l** and **2t**)) were stable in 0.01 N HCl at 37°C for 2 hours, the esters (**2**) must be stable in the gastric juice.

Those esters, *e.g.*, 1-(hexanoyloxy)ethyl ester (**2f**) and 1-(heptanoyloxy)ethyl ester (**2k**), with an R_2 comprising a straight alkyl chain, were hydrolyzed to CTM rapidly, whereas the 1-(isovaleryloxy)-ethyl ester (**2d**) and 1-pivaloyloxyethyl ester (**2e**), with an R_2 comprising an alkyl group with a high steric hindrance were hydrolyzed to CTM slowly. The release of CTM from the ester (**2i**) or (**2e**) was very slow: 8.4 and 12.2%, respectively after 30 minutes of incubation.

No significant relationship was observed between the Es value (TAFT's steric parameter) of R_2 and T_{50}.

Correlation of the Physico-chemical Properties with Peak Plasma Levels and Bioavailability

In a separate study,[4] we found good correlations between the physico-chemical properties of the acyloxymethyl esters (**1**) of CTM and oral bioavailability in mice. As the esters (**2**) used in the present

Fig. 3.

(A) Relation between the π value of R_2 and peak plasma levels (C_{max}) of CTM.

(B) Relation between the π value of R_2 and BA after the 1-acyloxyethyl esters (**2**) were orally administered to mice at a dose of 100 mg/kg equiv to CTM.

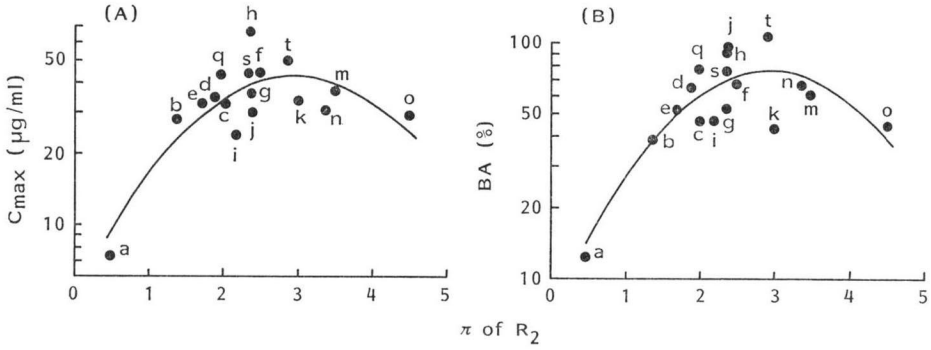
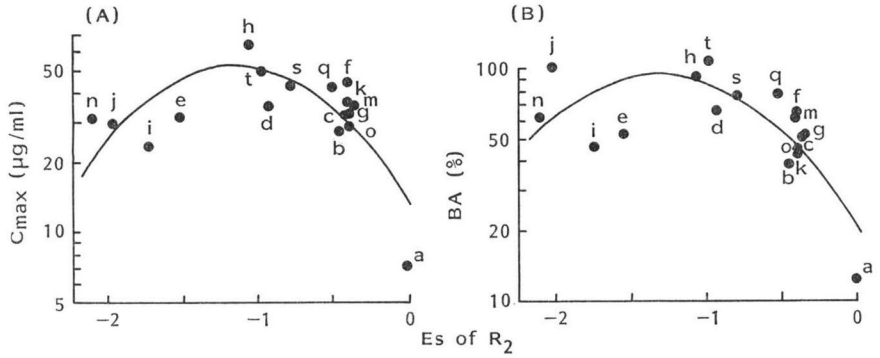


Fig. 4.

(A) Relation between TAFT's steric parameter (Es) of R_2 and peak plasma levels (C_{max}) of CTM.

(B) Relation between TAFT's steric parameter (Es) of R_2 and the relative BA after the 1-acyloxyethyl esters (**2**) were orally administered to mice at a dose of 100 mg/kg equiv to CTM.



study differ from each other only in the structure of R_2 in the ester moiety, their oral bioavailability was expected also to depend on the physico-chemical parameters, *e.g.*, π and Es values, of R_2. Hence, the relation between the π or Es value of R_2 and the C_{max} or BA were analyzed for the 17 esters shown in Table 4.

As the transport of an ester prodrug of a cephalosporin across the GI membrane is affected by lipophilicity,[4,5] the log C_{max} or log BA values were plotted against HANSCH's lipophilic parameter (π values) of R_2 of the esters (**2**) (Figs. 3A and 3B).

Good parabolic correlations following the equations 1 and 2 derived by least square analysis were obtained.

$$\log C_{max}\ (\mu g/ml) = 0.652 + 0.663\pi - 0.0112\pi^2 \quad (1)$$

$n=17,\ r=0.847,\ s=0.112$

$F_{2,14}=17.8\ (F_{2,14;\,\alpha=0.005}=7.92),\ (\pi)_0=2.97$

$$\log BA\ (\%) = 0.822 + 0.722\pi - 0.123\pi^2 \quad (2)$$

$n=17,\ r=0.849,\ s=0.120$

$F_{2,14}=18.1,\ (\pi)_0=2.94,$

where n is the numbers of studies, r the correlation coefficient, s the standard deviation, and F an F statistic.

The optimal π value, $(\pi)_0$, giving the highest C_{max} and BA are 2.97 and 2.94, respectively. The π value giving a C_{max} of more than 40 μg/ml is found between 2.42 and 3.51 and that giving a BA of more than 70% is between 2.38 and 3.50. Hence, an alkyl group consisting of carbon atoms between 5 and 7 is suitable.

As good correlations between the Es value of R_2 and C_{max} or BA of the acyloxymethyl esters (**1**) were observed in the separate study,[4] the log C_{max} or log BA values of **2** were plotted against the Es values (Figs. 4A and 4B). By least square-analysis, good parabolic correlations following the equations 3 and 4 were obtained.

$$\log C_{max}\ (\mu g/ml) = 1.141 - 1.00Es - 0.434Es^2 \quad (3)$$
$$n=17,\ r=0.755,\ s=0.139$$
$$F_{2,14}=9.18\ (F_{2,14;\,\alpha=0.005}=7.92),\ (Es)_0=-1.15$$
$$\log BA\ (\%) = 1.338 - 0.974Es - 0.373Es^2 \quad (4)$$
$$n=17,\ r=0.751,\ s=0.151$$
$$F_{2,14}=8.98,\ (Es)_0=-1.31$$

The optimal Es values, $(Es)_0$, giving the highest C_{max} and BA are -1.15 and -1.31, respectively. Es value giving a C_{max} of more than 40 μg/ml is observed between -0.64 and -1.67 and that giving a BA of more than 70% is between -0.72 and -1.89. Hence, a 5 or 6 membered cycloalkyl group or an alkyl group branched at α or β position is suitable as an R_2 group.

Also, among π, Es and C_{max} or BA, good correlations following the equations 5 and 6 derived through least-square analysis were obtained.

$$\log C_{max}\ (\mu g/ml) = 0.646 + 0.539Es - 0.259Es^2 + 0.519\pi - 0.084\pi^2 \quad (5)$$
$$n=17,\ r=0.942,\ s=0.077$$
$$F_{4,12}=23.73\ (F_{4,12;\,\alpha=0.005}=6.52)$$
$$(Es)_0=-1.04,\ (\pi)_0=3.07$$
$$\log BA\ (\%) = 0.861 - 0.528Es - 0.204Es^2 + 0.500\pi - 0.081\pi^2 \quad (6)$$
$$n=17,\ r=0.905,\ s=0.105$$
$$F_{4,12}=13.49,\ (Es)_0=-1.29,\ (\pi)_0=3.08$$

From equations 5 and 6, the π value of R_2 giving the highest C_{max} or BA is 3.07 or 3.08, respectively and the Es value of R_2 giving the highest C_{max} or BA is -1.04 or -1.29, respectively. The π and Es values of the R_2 of **2t**, **2j**, or **2h** which gave the highest C_{max} or BA were near the optimal values.

Conclusion

In this study designed to improve the oral bioavailability of CTM, it was found that some of the 1-acyloxyethyl esters (**2**) of CTM with an alkyl-acyl or a cycloalkyl-acyl group, which is simpler and sterically less hindered than that which is optimal in the case of acyloxymethyl esters (**1**) give the highest bioavailability. These esters (**2**) have an R_2 group comprising an α or β-branched alkyl or a cycloalkyl group of 5 to 7 carbon atoms.

The esters (**2**) were found to be sufficiently water soluble near the virtual pH in the gastrointestinal tract, an improved lipophilicity (π, 2.4~3.5), and adequate hydrolysis rates (T_{50}, 10~66 minutes) in a 1% homogenate of mouse small intestine.

Bioavailability of CTM as high as those of the orally active cephalosporins, such as CEX, were reached by preparing the optimal 1-acyloxyethyl esters (**2**). Further evaluation of the 1-acyloxyalkyl

Table 5. Physico-chemical data of 1-chloroethyl acylates (3).

Compound No.	R_2	Method[a]	Yield (%)	BP (°C/mmHg)	IR[b] cm^{-1}	NMR[c] δ (OCHO)
3a	CH_3	A	78		1775, 1760	6.58 (q, J=6 Hz)
3b	i-C_3H_7	A	74		1760	
3c	n-C_4H_9	B	53	76~80/27	1760	6.59 (q, J=6 Hz)
3d	i-C_4H_9	A (B)	79 (43)	83~85/41~43	1760	6.55 (q, J=6 Hz)
3e	t-C_4H_9	A	83		1755	
3f	n-C_5H_{11}	A	100		1760	6.55 (q, J=6 Hz)
3g	i-$C_4H_9CH_2$	B	64		1760	6.57 (q, J=6 Hz)
3h	s-$C_4H_9CH_2$	A	74	92~94/37	1765, 1750	6.56 (q, J=6 Hz)
3i	t-$C_4H_9CH_2$	A	72		1765, 1755	6.55 (q, J=6 Hz)
3j	$(C_2H_5)_2CH$	A	70	83~85/35	1760	6.53 (q, J=6 Hz)
3k	n-C_6H_{13}	A (B)	87 (—)		1760	
3l	$(C_2H_5)_2CHCH_2$	A	59	100~102/45	1760	6.55 (q, J=6 Hz)
3m	n-C_7H_{15}	A (B)	69 (—)		1760	
3n	$(n$-$C_3H_7)_2CH$	A	65		1760	
3o	n-C_9H_{19}	A	72		1760	
3p	c-C_4H_7	A	53	90~92/40~42	1770, 1755	6.54 (q, J=6 Hz)
3q	c-C_5H_9	A	65	70~72/4~5	1760, 1740	6.55 (q, J=6 Hz)
3r	c-$C_5H_9CH_2$	A	73	64~65/3~4	1765, 1755	6.55 (q, J=6 Hz)
3s	c-C_6H_{11}	A	68	70~72/4	1765, 1755	6.54 (q, J=6 Hz)
3t	c-$C_6H_{11}CH_2$	A	74	77~79/4	1765, 1755	6.54 (q, J=6 Hz)

[a] See text.
[b] Liquid film.
[c] In $CDCl_3$.

Table 6. Structure and IR spectra of the l-acyloxyethyl esters (2) of CTM.

Ester No.	R_2	Yield (%)	IR ν_{max}^{KBr} cm^{-1}	Ester No.	R_2	Yield (%)	IR ν_{max}^{KBr} cm^{-1}
2a	CH_3	24	1780,1760,1680	**2k**	n-C_6H_{13}	16	1780,1750,1670
2b	i-C_3H_7	17	1780,1750,1660	**2l**·2HCl	$(C_2H_5)_2CHCH_2$	41	1785,1755,1685
2c	n-C_4H_9	20	1780,1750,1665	**2m**	n-C_7H_{15}	16	1780,1750,1670
2d	i-C_4H_9	36	1775,1750,1670	**2n**·2HCl	$(n$-$C_3H_7)_2CH$	18	1780,1750,1670
2e·2HCl	t-C_4H_9	27	1785,1755,1665	**2o**	n-C_9H_{19}	23	1780,1750,1660
2f·2HCl	n-C_5H_{11}	30	1780,1750,1670	**2p**·2HCl	c-C_4H_7	24	1775,1740,1650
2g·2HCl	i-$C_4H_9CH_2$	27	1785,1750,1670	**2q**·2HCl	c-C_5H_9	33	1775,1740,1640
2h·2HCl	s-$C_4H_9CH_2$	36	1785,1750,1660	**2r**·2HCl	c-$C_5H_9CH_2$	29	1775,1750,1640
2i	t-$C_4H_9CH_2$	26	1780,1755,1660	**2s**·2HCl	c-C_6H_{11}	60	1775,1750,1670
2j·2HCl	$(C_2H_5)_2CH$	31	1785,1755,1675	**2t**·2HCl	c-$C_6H_{11}CH_2$	43	1775,1750,1670

and 1-(alkoxycarbonyloxy)alkyl esters of CTM are in progress.

Materials and Methods

All boiling points are uncorrected. IR spectra were measured on a Hitachi 215 spectrometer. NMR spectra were recorded on a Varian EM 390 spectrometer with tetramethylsilane as an internal reference. HPLC was done using a Shimadzu LC-5A instrument equipped with a column (300 mm ×4 mm) of Nucleosil C_{18} (10 μm particle size) and a variable wavelength UV detector.

Materials

7-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]-ceph-3-em-4-carboxylic acid dihydrochloride (CTM·2HCl) was prepared in the Central Research

Table 7. NMR spectra of 1-acyloxyethyl esters (**2**) of CTM at 90 MHz.

Ester No.	R_2	δ ppm in DMSO-d_6* or D_2O
2a*	CH_3	1.47 and 1.52 (3H, 2×d, J=6 Hz), 2.01 and 2.07 (3H, 2×s), 2.21 (6H, s), 2.78 (2H, t, J=6 Hz), 3.37 (2H, s), 3.60 and 3.83 (2H, ABq, J=18 Hz), 4.13 and 4.33 (2H, ABq, J=13 Hz), 4.39 (2H, t, J=6 Hz), 5.06~5.20 (1H, m), 5.3~5.58 (1H, m), 5.6~5.9 (1H, m), 6.23 (1H, s), 6.80 (2H, br s), 8.83 (1H, d, J=9 Hz)
2b*	i-C_3H_7	0.90 (6H, d, J=6 Hz), 1.48 and 1.51 (3H, 2×d, J=6 Hz), 2.24 (6H, s), 2.76 (2H, t, J=6 Hz), 3.37 (2H, s), 3.76 (2H, br s), 4.17 and 4.27 (2H, ABq, J=13.5 Hz), 4.42 (2H, t, J=6 Hz), 5.0~5.2 (1H, m), 5.6~5.9 (1H, m), 6.23 (1H, s), 6.72~7.18 (3H, m), 8.86 (1H, d, J=9 Hz)
2c*	n-C_4H_9	0.87 (3H, t, J=7 Hz), 1.1~1.7 (7H, m), 2.18 (6H, s), 2.36 (2H, t, J=7 Hz), 2.69 (2H, t, J=6 Hz), 3.37 (2H, s), 3.73 (2H, br s), 4.13 and 4.40 (2H, ABq, J=13.5 Hz), 4.37 (2H, t, J=6 Hz), 5.0~5.2 (1H, m), 5.6~5.9 (1H, m), 6.23 (1H, s), 6.55~7.15 (3H, m), 8.85 (1H, d, J=9 Hz)
2d*	i-C_4H_9	0.92 (6H, d, J=6 Hz), 1.49 and 1.54 (3H, 2×d, J=6 Hz), 2.27 (6H, s), 2.28 (2H, t, J=6 Hz), 2.80 (2H, t, J=6 Hz), 3.37 (2H, s), 3.73 (2H, br s), 4.17 and 4.31 (2H, ABq, J=13.5 Hz), 4.43 (2H, t, J=6 Hz), 5.0~5.2 (1H, m), 5.58~5.92 (1H, m), 6.23 (1H, s), 6.68~7.18 (3H, m), 8.86 (1H, d, J=9 Hz)
2e·2HCl	t-C_4H_9	1.50 (9H, s), 1.84 (3H, d, J=6 Hz), 3.37 (6H, s), 4.02~4.30 (2H, m), 4.32 (2H, s), 4.51 (2H, t, J=6 Hz), 4.51 and 4.73 (2H, ABq, J=18 Hz), 5.26 (2H, t, J=6 Hz), 5.50 (1H, d, J=4.5 Hz), 7.00 (1H, s), 7.21 (1H, q, J=6 Hz)
2f·2HCl	n-C_5H_{11}	1.3~2.1 (12H, m), 2.70 (2H, t, J=6 Hz), 3.38 (6H, s), 4.12 and 4.33 (2H, ABq, J=16 Hz), 4.13 (2H, s), 4.20 (2H, t, J=6 Hz), 4.63 (2H, br s), 5.32 (2H, t, J=6 Hz), 5.45 (1H, d, J=4.5 Hz), 5.99 (1H, d, J=4.5 Hz), 7.02 (1H, s), 7.23 (1H, q, J=6 Hz)
2g·2HCl	i-$C_4H_9CH_2$	1.17 (6H, d, J=6 Hz), 1.3~1.97 (4H, m), 1.82 (3H, d, J=6 Hz), 2.76 (2H, t, J=6 Hz), 3.37 (6H, s), 4.11 (2H, s), 4.29 (2H, br s), 4.68 (2H, br s), 5.23 (2H, t, J=6 Hz), 5.41 (1H, d, J=4.5 Hz), 5.98 (1H, d, J=4.5 Hz), 7.01 (1H, s), 7.23 (1H, q, J=6 Hz)
2h·2HCl	s-$C_4H_9CH_2$	1.17 (3H, t, J=6 Hz), 1.20 (3H, d, J=6 Hz), 1.85 (3H, d, J=6 Hz), 1.97~2.41 (1H, m), 2.58 (2H, t, J=6 Hz), 3.38 (6H, s), 4.12 (2H, br s), 4.70 (2H, br s), 5.24 (2H, t, J=6 Hz), 5.47 (1H, d, J=4.5 Hz), 5.98 (1H, d, J=4.5 Hz), 7.02 (1H, s), 7.21 (1H, q, J=6 Hz)
2i*	t-$C_4H_9CH_2$	0.97 (9H, s), 1.47 and 1.51 (3H, 2×d, J=6 Hz), 2.25 (6H, s), 2.78 (2H, t, J=7 Hz), 3.35 (2H, s), 3.47 (2H, s), 3.46 and 3.59 (2H, ABq, J=18 Hz), 4.00 and 4.33 (2H, ABq, J=13.5 Hz), 4.40 (2H, t, J=6 Hz), 5.0~5.18 (1H, m), 5.6~5.85 (1H, m), 6.22 (1H, s), 6.6~6.88 (3H, m), 8.68 (1H, d, J=9 Hz)
2j·2HCl	$(C_2H_5)_2CH$	1.05 (6H, t, J=7 Hz), 1.55~2.02 (7H, m), 2.55 (2H, t, J=6 Hz), 3.27 (6H, s), 3.85~4.25 (5H, m), 4.52 (2H, br s), 5.15 (2H, t, J=6 Hz), 5.38 (1H, d, J=4.5 Hz), 5.88 (1H, d, J=4.5 Hz), 6.93 (1H, s), 7.17 (1H, q, J=6 Hz)
2k*	n-C_6H_{13}	0.82 (3H, t, J=6 Hz), 1.24 (8H, br s), 1.46 and 1.52 (3H, 2×d, J=6 Hz), 2.17 (6H, s), 2.18 (2H, t, J=6 Hz), 2.69 (2H, t, J=6 Hz), 3.37 (2H, s), 3.59 and 3.84 (2H, ABq, J=18 Hz), 4.14 and 4.35 (2H, ABq, J=13.5 Hz), 4.39 (2H, t, J=6 Hz), 5.03~5.19 (1H, m), 5.6~5.9 (1H, m), 6.23 (1H, s), 6.68~7.18 (3H, m), 8.86 (1H, d, J=9 Hz)
2l·2HCl	$(C_2H_5)_2CHCH_2$	1.07 (6H, t, J=7 Hz), 1.45~2.17 (11H, m), 2.60 (2H, t, J=6 Hz), 3.30 (6H, s), 3.85~4.25 (5H, m), 4.65 (2H, br s), 5.20 (2H, t, J=6 Hz), 5.45 (1H, d, J=4.5 Hz), 5.92 (1H, d, J=4.5 Hz), 7.00 (1H, s), 7.20 (1H, q, J=6 Hz)

Table 7. (Continued)

Ester No.	R_2	δ ppm in DMSO-d_6* or D_2O
2m*	n-C_7H_{15}	0.84 (3H, t, J=6 Hz), 1.24 (10H, br s), 1.47 and 1.52 (3H, 2×d, J=6 Hz), 2.23 (6H, s), 2.30 (2H, t, J=6 Hz), 3.37 (2H, s), 3.71 (2H, br s), 4.23 and 4.33 (2H, ABq, J=13.5 Hz), 4.40 (2H, t, J=6 Hz), 5.0~5.2 (1H, m), 5.4~5.9 (1H, m), 6.22 (1H, s), 6.7~7.7 (3H, m), 8.84 (1H, d, J=9 Hz)
2n·2HCl	$(C_3H_7)_2CH$	1.07 (6H, t, J=6 Hz), 1.2~2.05 (8H, m), 2.4~2.6 (1H, m), 3.27 (6H, s), 3.85~4.28 (6H, m), 4.57 (2H, br s), 5.09 (2H, t, J=6 Hz), 5.28 (1H, d, J=4.5 Hz), 5.91 (1H, d, J=4.5 Hz), 6.91 (1H, s), 7.14 (1H, q, J=6 Hz)
2o*	n-C_9H_{19}	0.84 (6H, t, J=6 Hz), 1.23 (14H, br s), 1.39~1.50 (3H, 2×d, J=6 Hz), 2.18 (6H, s), 2.33 (2H, t, J=6 Hz), 2.71 (2H, t, J=6 Hz), 3.37 (2H, s), 3.36 and 3.86 (2H, ABq, J=18 Hz), 4.16 and 4.33 (2H, ABq, J=13.5 Hz), 4.39 (2H, t, J=6 Hz), 5.02~5.08 (1H, m), 5.5~5.9 (1H, m), 6.23 (1H, s), 6.85~7.2 (3H, m), 8.95 (1H, d, J=9 Hz)
2p·2HCl	c-C_4H_7	1.73 (3H, d, J=6 Hz), 1.88~2.67 (7H, m), 3.33 (6H, s), 4.02 (2H, s), 3.91~4.22 (4H, m), 4.53 (2H, br s), 5.16 (2H, t, J=6 Hz), 5.39 (1H, d, J=4.5 Hz), 5.92 (1H, d, J=4.5 Hz), 6.94 (1H, s), 7.16 (1H, q, J=6 Hz)
2q·2HCl	c-C_5H_9	1.73 (3H, t, J=6 Hz), 1.51~2.43 (9H, m), 3.28 (6H, s), 4.02 (2H, s), 3.92~4.23 (4H, m), 4.54 (2H, br s), 5.11 (2H, t, J=6 Hz), 5.38 (1H, d, J=4.5 Hz), 5.91 (1H, d, J=4.5 Hz), 6.95 (1H, s), 7.16 (1H, q, J=6 Hz)
2r·2HCl	c-$C_5H_9CH_2$	1.53~2.17 (12H, m), 2.57 (2H, d, J=6 Hz), 3.28 (6H, s), 4.01 (2H, s), 3.92~4.23 (4H, m), 4.55 (2H, br s), 5.14 (2H, t, J=6 Hz), 5.39 (1H, d, J=4.5 Hz), 5.91 (1H, d, J=4.5 Hz), 6.83 (1H, s), 7.17 (1H, q, J=6 Hz)
2s·2HCl	c-C_6H_{11}	1.33~2.7 (11H, m), 1.73 (3H, d, J=6 Hz), 3.28 (6H, s), 3.92~4.23 (4H, m), 4.02 (2H, s), 4.54 (2H, br s), 5.13 (2H, t, J=6 Hz), 5.33 (1H, d, J=4.5 Hz), 5.87 (1H, d, J=4.5 Hz), 6.93 (1H, s), 7.17 (1H, q, J=6 Hz)
2t·2HCl	c-$C_6H_{11}CH_2$	1.56~2.27 (14H, m), 2.44 and 2.47 (2H, 2×d, J=6 Hz), 3.28 (2H, s), 3.86~4.30 (4H, m), 4.04 (2H, s), 4.33~4.87 (2H, m), 5.13 (2H, t, J=6 Hz), 5.33 (1H, d, J=4.5 Hz), 5.87 (1H, d, J=4.5 Hz), 6.96 (1H, s), 7.17 (1H, q, J=6 Hz)

Division, Takeda Chemical Industries, Ltd.; its activity was 842 μg/mg.

Methods

Chemistry

(1) Preparation of 1-Chloroethyl Acylates (**3**): Method A; Acetaldehyde (10 ml) was added dropwise into a mixture of cyclohexanecarbonyl chloride (25 g) and anhydrous $ZnCl_2$ (catalytic amount) with stirring at −20°C. The mixture was then stirred below 0°C for 1 hour then at room temp for 1 hour. The mixture was purified through by silica gel column chromatography with petroleum ether as an eluent. After the solvent was removed *in vacuo*, the residue was distilled by reduced pressure to give 1-chloroethyl cyclohexanecarboxylate (**3s**) as a colorless liquid in 68% yield. BP 70~72°C/4 mmHg.

Anal Calcd for $C_9H_{15}O_2Cl$: C 56.69, H 7.93.
Found: C 56.93, H 7.92.

Method B; n-Valeryl chloride (25 g) was heated with paraaldehyde (9 g) and anhydrous $ZnCl_2$ (catalytic amount) at 130~140°C for 4 hours and the mixture was distilled by reduced pressure to collect the fraction at 64~80°C/32 mmHg. This fraction was distilled by reduced pressure to give 1-chloroethyl valerate (**3c**) as a colorless liquid in 53% yield. BP 76~80°C/27 mmHg.

Table 8. Elemental analyses of the 1-acyloxyethyl esters (**2**) of CTM.

Ester No.	Formula	*Anal*					
		Calcd			Found		
		C	H	N	C	H	N
2a	$C_{22}H_{29}N_9O_6S_3 \cdot \frac{1}{2}H_2O$	42.57,	4.71,	20.31	42.61,	4.96,	20.61
2b	$C_{24}H_{33}N_9O_6S_3$	45.06,	5.20,	19.71	44.96,	5.49,	19.72
2c	$C_{25}H_{35}N_9O_6S_3$	45.93,	5.40,	19.28	45.66,	5.45,	19.16
2d	$C_{25}H_{35}N_9O_6S_3 \cdot \frac{1}{2}H_2O$	45.30,	5.47,	19.01	45.58,	5.65,	18.41
2e·2HCl	$C_{25}H_{35}N_9O_6S_3 \cdot 2HCl \cdot 2\frac{1}{2}H_2O$	38.90,	5.50,	16.34	39.24,	5.78,	16.00
2f·2HCl	$C_{26}H_{37}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	40.25,	5.60,	16.25	40.14,	6.02,	15.99
2g·2HCl	$C_{26}H_{37}N_9O_6S_3 \cdot 2HCl \cdot 2\frac{1}{2}H_2O$	39.79,	5.66,	16.07	39.84,	5.53,	15.73
2h·2HCl	$C_{26}H_{37}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	40.25,	5.60,	16.25	40.31,	5.54,	16.02
2i	$C_{26}H_{37}N_9O_6S_3$	46.75,	5.59,	18.88	46.43,	5.71,	18.50
2j·2HCl	$C_{26}H_{37}N_9O_6S_3 \cdot 2HCl \cdot 1\frac{1}{2}H_2O$	40.67,	5.51,	16.42	40.70,	5.89,	16.17
2k	$C_{27}H_{39}N_9O_6S_3$	46.94,	5.84,	18.24	46.66,	5.82,	18.47
2l·2HCl	$C_{27}H_{39}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	41.07,	5.74,	15.94	40.69,	5.92,	15.78
2m	$C_{28}H_{41}N_9O_6S_3 \cdot \frac{1}{2}H_2O$	47.71,	6.01,	17.88	47.66,	5.92,	17.94
2n·2HCl	$C_{28}H_{41}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	41.78,	5.90,	15.67	41.78,	5.98,	15.68
2o	$C_{30}H_{45}N_9O_6S_3$	49.77,	6.27,	17.42	49.55,	6.27,	17.55
2p·2HCl	$C_{25}H_{33}N_9O_6S_3 \cdot 2HCl \cdot 1\frac{1}{2}H_2O$	40.20,	5.32,	16.31	39.95,	5.10,	16.77
2q·2HCl	$C_{26}H_{35}N_9O_6S_3 \cdot 2HCl \cdot 1\frac{1}{2}H_2O$	41.59,	5.43,	16.17	41.42,	5.43,	15.91
2r·2HCl	$C_{27}H_{37}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	41.11,	5.49,	15.98	40.87,	5.89,	15.69
2s·2HCl	$C_{27}H_{37}N_9O_6S_3 \cdot 2HCl \cdot H_2O$	42.07,	5.36,	16.36	42.39,	5.77,	16.15
2t·2HCl	$C_{28}H_{39}N_9O_6S_3 \cdot 2HCl \cdot H_2O$	42.85,	5.52,	16.06	42.87,	5.91,	15.69
2u·2HCl	$C_{28}H_{33}N_9O_6S_3 \cdot 2HCl \cdot 2H_2O$	44.17,	5.17,	16.56	44.43,	5.53,	16.34

Anal Calcd for $C_7H_{13}O_2Cl$: C 51.07, H 7.96.
Found: C 51.15, H 8.03.

The other 1-chloroethyl acylates (**3**) were prepared following Method A or B (Table 5).

(2) Preparation of 1-Iodoethyl Acylate (**4**): 1-Chloroethyl cyclohexanecarboxylate (**3s**, 8 g) was added to the acetonitrile (100 ml) solution of NaI (16 g) at 40°C and the mixture was stirred for 15 minutes. The undissolved solid was filtered off and the filtrate was concd *in vacuo*. A mixture of petroleum ether (or ethyl ether) and 5% aq $Na_2S_2O_3$ solution was added to the residue; the organic layer was separated, washed with 5% aq $Na_2S_2O_3$ solution, and dried over anhydrous $MgSO_4$. The solvent was concd *in vacuo* to give 1-iodoethyl cyclohexanecarboxylate (**4s**) as yellow-brown liquid in 41% (5.3 g) yield. IR ν_{max}^{film} cm^{-1} 1760, 1750.

Other compounds in **4** were prepared following this procedure and used for the esterification without further purification.

(3) Preparation of 1-Acyloxyethyl 7-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylate (**2**): The potassium salt of cefotiam was dissolved in *N*,*N*-dimethylformamide (50 ml), and cooled to −5°C. 1-Iodoethyl hexanoate (**4f**, 5 g) was added to this solution with stirring, which was continued for another 10 minutes. The mixture was poured into a mixture of EtOAc (300 ml) and ice water (200 ml), and the organic layer was separated. The aqueous layer was exteracted with EtOAc (200 ml). The combined organic layer was washed with ice cooled water (150 ml×3), saturated brine, then dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo*, and the residue was triturated with isopropyl ether. The solid was reprecipitated from Me_2CO - isopropyl ether to give 1-(*n*-hexanoyloxy)ethyl 7-[2-(2-aminothiazol-4-yl)-acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylate (**2f**, 1.8 g) as an amorphous powder.

This free base (1.6 g) was dissolved in dichloromethane (20 ml) and cooled at 0°C; 4.9 N HCl-ethereal solution (2 ml) was added to this solution. The precipitated solid was collected by suction, washed with ether, and dried to give the dihydrochloride salt of **2f** (1.5 g). Other esters (**2**) were pre-

pared following this procedure. The analytical results are shown in Tables 6, 7 and 8.

Water Solubility

An ester (50 mg) was added to 1/15 M phosphate buffer (2 ml) of pH 4.5 and the mixture was adjusted to pH 4.5, then shaken vigorously for 30 minutes at room temp. After the mixture was filtered, the concentration of the ester in the filtrate was measured by HPLC (Nucleosil C_{18}, 300 mm × 4 mm). As the esters were unstable at neutral or alkaline pH, pH 4.5 was selected for measuring the water solubility. In the HPLC analysis; mobile phase (0.05 M $(NH_4)_2SO_4$ - CH_3CN - AcOH, 400 : 200 : 1) was used with a flow rate 1.5 ml/minute.

Oral Absorption Study

Male SLC-ICR mice, weighing about 15 g (4 weeks old), were starved, but given free access to water, for 16~18 hours before the experiment. The esters were administered orally to a group of 4 mice by intubation as an aqueous solution with 2.5 equimolar of tartaric acid at a dose of 100 mg/kg equivalent to CTM. Blood was taken from the inferior vena cava at 0.25, 0.5, 1 and 2 hours after dosing. CTM was administered subcutaneouly as a 1% aqueous solution at a dose of 100 mg/kg. The relative bioavailability was calculated from the area under the plasma CTM level-time curves after oral (AUC oral) and subcutaneous administration (AUC sc). The plasma concentration of CTM was measured by the cylinder-plate method using *Proteus mirabilis* ATCC 21100 as the test organism.[13]

In Vitro Study

A 1% homogenate of mouse small intestine was prepared according to the procedure reported elsewhere.[4] An ester (10 mg equiv to CTM) was dissolved in a mixture of 1 N HCl (two drops) and dioxane (1.0 ml), then diluted to a concentration of 0.20 mg/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 equiv to 10 μg/ml of CTM. Sampling was carried out at 2, 5, 10, 15, 30 and 60 minutes after the incubation. 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 for 1 minute. The concentration of CTM in the aqueous layer was measured by bioassay as described above.

An ester (50 mg) was dissolved in 0.01 N HCl (50 ml) at 37°C, and the concentration of the ester was measured by HPLC.

Acknowledgment

The authors thank Drs. MORITA, FUJINO and SHIMAMOTO of this Division for their encouragement throughout this work.

References

1) NUMATA, M.; I. MINAMIDA, M. YAMAOKA, M. SHIRAISHI, T. MIYAWAKI, H. AKIMOTO, K. NAITO & M. KIDA: A new cephalosporin. SCE-963: 7-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid. Chemistry and structure-activity relationships. J. Antibiotics 31: 1262~1271, 1978
2) TSUCHIYA, K.; M. KIDA, M. KONDO, H. ONO, T. TAKEUCHI & T. NISHI: SCE-963, a new broad-spectrum cephalosporin: In vitro and in vivo antibacterial activities. Antimicrob. Agents. Chemother. 14: 557~568, 1979
3) YOSHIMURA, Y.; N. HAMAGUCHI & T. YASHIKI: Synthesis and relationship between physicochemical properties and oral absorption of pivaloyloxymethyl esters of parenteral cephalosporins. Int. J. Pharm. 23: 117~129, 1985
4) YOSHIMURA, Y.; N. HAMAGUCHI & T. YASHIKI: Synthesis and oral absorption of acyloxymethyl esters of 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid (cefotiam). Int. J. Pharm., to submitted
5) YOSHIMURA, Y.; N. HAMAGUCHI, N. KAKEYA & T. YASHIKI: Synthesis and relationship between physicochemical properties and oral absorption of 7-*O*-acylmandelamido-3-methyl-3-cephem-4-carboxylic acids. Int. J. Pharm. 26: 317~328, 1985
6) WRIGHT, W. E.; W. J. WHEELER, V. D. LINE, J. A. FROGGE' & D. R. FINLEY: Orally active esters of

cephalosporin antibiotics. II. Synthesis and biological properties of the acetoxymethyl ester of cefamandole. J. Antibiotics 32: 1155~1160, 1979

7) WHEELER, W. J.; D. A. PRESTON, W. E. WRIGHT, G. W. HUFFMAN, H. E. OSBORNE & D. P. HOWARD: Orally active esters of cephalosporin antibiotics. 3. Synthesis and biological properties of aminoacyloxymethyl esters of 7-[D-(−)-mandelamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. J. Med. Chem. 22: 657~661, 1979
8) HARDING, S. M.; P. E. O. WILLIAMS & J. AYRTON: Pharmacology of cefuroxime as the 1-acetoxyethyl ester in volunteers. Antimicrob. Agents Chemother. 25: 78~82, 1984
9) KAKEYA, N.; S. NISHIZAWA, K. NISHIMURA, A. YOSHIMI, S. TAMAKI, T. MORI & K. KITAO: KY-109, a new bifunctional pro-drug of a cephalosporin. Chemistry, physico-chemical and biological properties. J. Antibiotics 38: 380~389, 1985
10) LEO, A.; C. HANSCH & K. ELKINS: Partition coefficients and their uses. Chem. Rev. 71: 525~616, 1971
11) CRAIG, P. N.: Interdependence between physical parameters and selection of substituent groups for correlation studies. J. Med. Chem. 14: 680~684, 1971 and references cited therein
12) ULICH, L. H. & R. ADAMS: The reaction between acid halides and aldehydes. J. Am. Chem. Soc. 43: 660~667, 1921
13) FUGONO, T. & K. MAEDA: Microbiological assay method of cefotiam (SCE-963) in biological specimens. Chemotherapy (Tokyo) 27 (Suppl.): 106~111, 1979