ORALLY ACTIVE 1-(CYCLOHEXYLOXYCARBONYLOXY)ALKYL ESTER PRODRUGS OF CEFOTIAM

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Orally active 1-(alkyl substituted cyclohexyloxycarbonyloxy)alkyl ester prodrugs (**9b**~**h**) 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, CTM) have been studied as well as the thia (**9i**) and aza (**9j**) analogs. These represent derivatives of the 1-(cyclohexylacetoxy)ethyl ester (**2**) of CTM. The syntheses and oral bioavailability (BA) in mice are described. Among them, the 1-(cyclohexyloxycarbonyloxy)butyl ester (**9h**) gave the highest BA, 93.5%; the esters having a cyclohexyloxy group in the ester moiety gave BAs of more than 75%, although the BA of the 1-(ethoxycarbonyloxy)ethyl ester (**9a**) was only 23.9%. The thia analog showed a moderate BA, 46%, but the aza analog, **9j**, did not show a BA of CTM. These results indicate that the 1-(substituted cyclohexyloxycarbonyloxy)alkyl group was the suitable promoiety to improve the oral BA of CTM. Chiral 1-(alkoxycarbonyloxy)alkyl groups used as the ester moiety, gave an almost 1:1 mixture of diastereoisomeric esters. These were tested as such. However, an experiment in which the separated isomers of the 1-(cyclohexyloxycarbonyloxy)ethyl ester (**9d**) were administered orally confirmed that both diastereoisomers gave identical BAs.

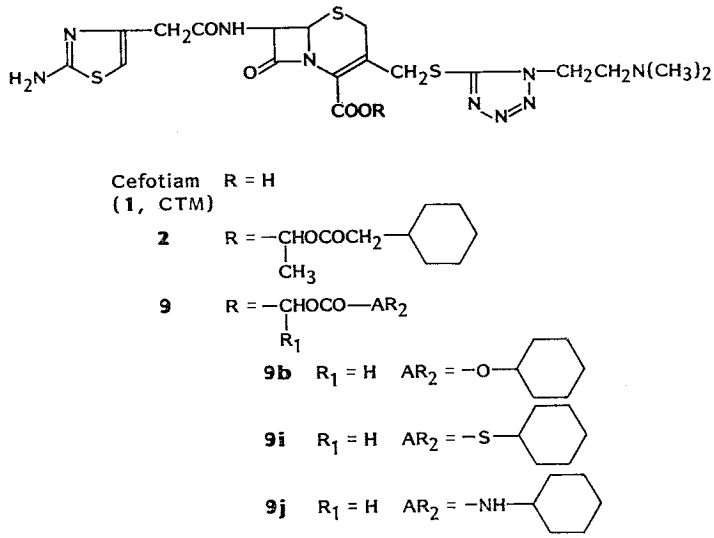
In a series of papers from these laboratories, we have reported that ester prodrugs of parenteral cephalosporins having both good water solubility[1] and lipophilicity[2~4] are absorbed well from the gastrointestinal (GI) tract and proved that among the cephalosporins studied, 1-acyloxyalkyl esters of 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, CTM) showed rather high oral bioavailability (BA(s)) in terms of CTM. Optimization of the 1-acyloxyalkyl promoiety of the CTM esters was then tried,[†,3,4] and we found that the esters having a methyl, an ethyl or a propyl group as the alkyl moiety and a cycloalkyl or a branched alkyl group of 5~7 carbon atoms in acyloxy residue showed oral BAs as high as those of the orally active cephalosporins, *e.g.*, cephalexin or cephradine in mice.

On the other hand, although the 1-(ethoxycarbonyloxy)ethyl ester group, an oxa analog of the 1-acyloxyalkyl group, has been used successfully to improve the oral BA of ampicillin[5], there has been no report of it being successfully applied to improve the BA of cephalosporins probably because the esters involved have a low solubility in the GI fluid.[6]

Having been encouraged by finding that the 1-(cyclohexylacetoxy)ethyl ester (**2**) of CTM, having an ester moiety with adequate lipophilicity and steric hindrance at AR_2, showed a good water solubility in the vicinity of the physiological pH and a good oral BA, 107.8%,[4] we became interested in applying the 1-(cyclohexyloxycarbonyloxy)alkyl, cyclohexylthiocarbonyloxymethyl and *N*-cyclohexylcarbamoyloxymethyl groups as oxa, thia and aza analogs of the promoiety of **2** to CTM to enhance

† YOSHIMURA, Y.: Unpublish results.

Fig. 1.



the oral BA of the latter (Fig. 1).

In this report, we describe the preparation of the 1-(substituted cyclohexyloxycarbonyloxy)alkyl (**9b**~**h**), the cyclohexylthiocarbonyloxymethyl (**9i**), and the *N*-cyclohexylcarbamoyloxymethyl esters (**9j**) of CTM and the results of evaluating their oral BAs in mice.

Chemistry

The processes for the preparation of the esters (**9a**~**j**) listed in Table 1, are outlined in Scheme 1.

Alkyl 1-chloroalkyl carbonates (**7a**~**h**), chloromethyl (cyclohexylthio)formate (**7i**), and chloromethyl (*N*-cyclohexyl)carbamate (**7j**) were prepared from 1-chloroalkyl chloroformates (**3**~**6**)[7,8] on treatment with the corresponding cyclohexyl alcohols, cyclohexanethiol and cyclohexylamine in dichloromethane in the presence of pyridine. The chlorides (**7**) were then converted to the corresponding alkyl 1-iodoalkyl carbonates (**8a**~**h**), iodomethyl (cyclohexylthio)formate (**8i**), and iodomethyl (*N*-cyclohexyl)carbamate (**8j**) on reaction with NaI in acetonitrile.

The treatments of the potassium salt of cefotiam with the iodides (**8a**~**j**) in a short period (*ca.* 10 minutes) below 5°C afforded the corresponding esters (**9a**~**j**), which were purified by column chromatography and isolated as dihydrochlorides.

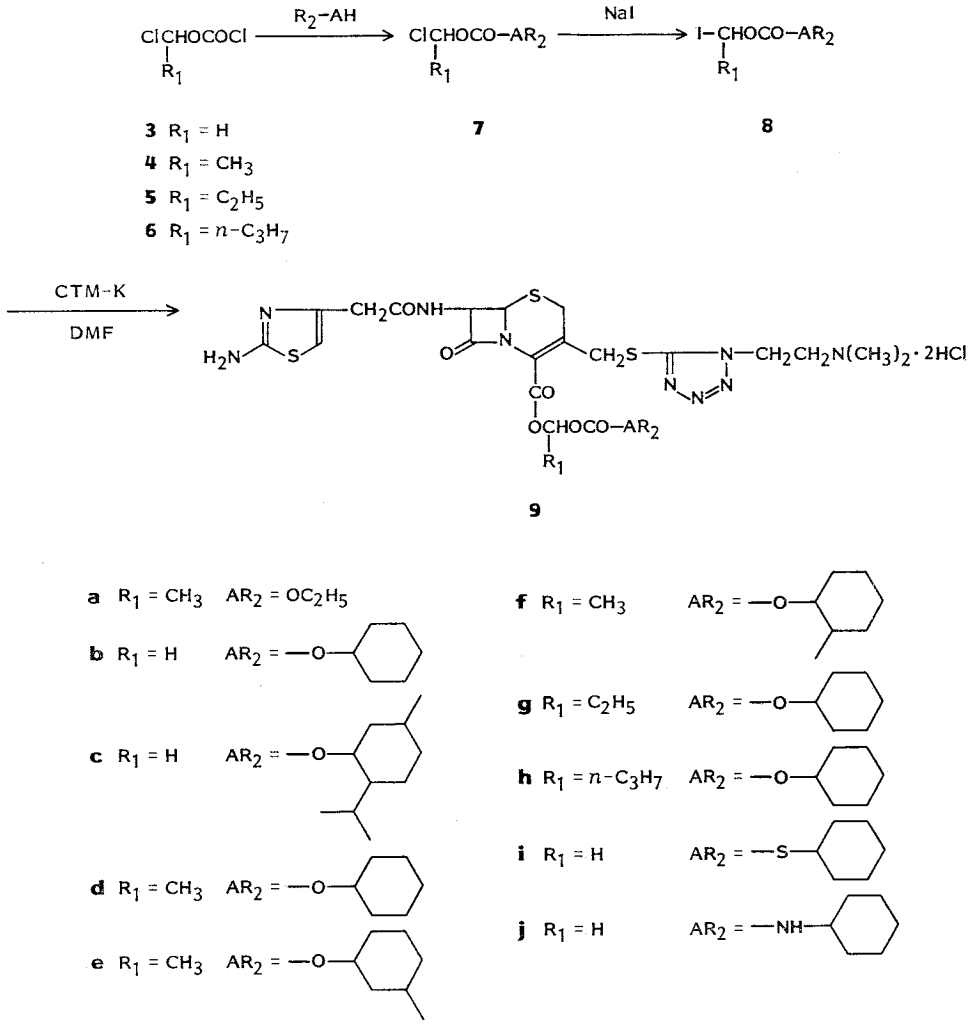
When the C(1) position of the ester moieties is chiral, the esters (**9a**, **9d**~**h**) thus formed were an almost equal mixture of diastereoisomers as judged by signals due to C(1)-H in their ^{1}H NMR spectra (Table 6). To examine the relationship between a diastereoisomer and BA in a typical example, the diastereoisomers of the 1-(cyclohexyloxycarbonyloxy)ethyl ester (α-**9d** and β-**9d**) were separated by chromatography using MCI GEL and their BAs were measured (Table 2).

Results and Discussion

Oral Absorption Study in Mice

The esters (**9a**~**j**) were administered orally to mice at a dose of 100 mg/kg equivalent to CTM. The plasma CTM levels are shown in Table 1 with the relative bioavailability (BA) calculated from

Scheme 1.

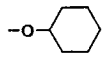
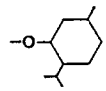
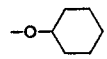
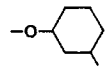
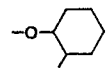
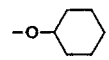
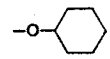
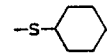
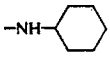
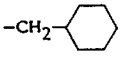


the ratio of the area under the plasma CTM levels-time curve (AUC) for 0~2 hour(s) after oral dosing of the esters to AUC after subcutaneous administration of CTM at the same dose.

The 1-(cyclohexyloxycarbonyloxy)alkyl esters (**9b~h**) and cyclohexylthiocarbonyloxymethyl ester (**9i**) showed higher plasma CTM levels than those observed after the oral administration of CTM or the 1-(ethoxycarbonyloxy)ethyl ester (**9a**) as a reference ester, whereas the *N*-cyclohexylcarbamoyloxymethyl ester (**9j**) did not show plasma CTM levels.

The BA observed after oral dosing with CTM was only 6.3% and that of ester **9a** was 23.9%, whereas the BAs of the 1-(substituted cyclohexyloxycarbonyloxy)alkyl esters were improved largely. Among the esters, the 1-(cyclohexyloxycarbonyloxy)butyl ester (**9h**) showed the highest BA (93.5%), and was followed by the 1-(2-methylcyclohexyloxycarbonyloxy)ethyl ester (**9f**, 91.2%), the 1-(cyclohexyloxycarbonyloxy)propyl ester (**9g**, 80.7%), the 1-(3-methylcyclohexyloxycarbonyloxy)ethyl ester (**9e**, 77.4%), the 1-(cyclohexyloxycarbonyloxy)ethyl ester (**9d**, 77.2%), the cyclohexyloxycarbonyloxymethyl ester (**9b**, 77.2%), and the menthyloxycarbonyloxymethyl ester (**9c**, 75.9%).

Table 1. Plasma cefotiam levels after oral administration of the 1-(alkoxycarbonyloxy)alkyl, the *S*-cyclohexylthiocarbonyloxymethyl and the *N*-cyclohexylcarbamoyloxymethyl esters of cefotiam to mice at a dose of 100 mg/kg equivalent to cefotiam.

Ester No.	R_1	AR_2	Plasma CTM levels (μg/ml)				AUC (μg·hours/ml)	BA[a] (%)
			0.25 hour	0.5 hour	1 hour	2 hours		
9a	CH_3	OC_2H_5	14.7 (3.0)[b]	9.20 (0.22)	2.41 (0.39)	0.70 (0.0)	9.28	23.9
9b	H		28.2 (5.6)	26.0 (6.7)	16.4 (1.3)	1.70 (0.44)	30.0	77.2
9c	H		25.5 (4.2)	34.3 (1.3)	12.6 (1.6)	1.50 (0.25)	29.4	75.9
9d	CH_3		39.7 (1.5)	34.5 (1.6)	8.86 (1.60)	0.92 (0.12)	30.0	77.2
9e	CH_3		34.2 (4.2)	32.5 (6.8)	11.3 (0.4)	1.69 (0.20)	30.1	77.4
9f	CH_3		35.3 (2.9)	29.1 (1.3)	14.9 (1.3)	1.47 (0.55)	35.4	91.2
9g	C_2H_5		49.7 (3.4)	38.0 (1.6)	5.84 (0.24)	0.56 (0.0)	31.3	80.7
9h	n-C_3H_7		51.7 (2.6)	44.9 (1.9)	7.90 (1.12)	1.14 (0.28)	36.3	93.5
9i	H		21.2 (1.9)	17.1 (3.8)	7.46 (1.36)	1.08 (0.24)	17.4	46.0
9j	H		—[c]	—	—	—	—	—
2[d]	CH_3		49.7 (3.9)	46.8 (3.7)	13.4 (1.7)	3.59 (0.90)	41.8	107.8
CTM (po)			2.3 (0.4)	2.8 (0.1)	1.1 (0.1)	—	2.45	6.3
CTM (sc)			69.2 (6.1)	29.0 (1.6)	13.2 (1.8)	1.5 (0.7)	38.8	100.0

[a] Relative bioavailability.
[b] SE in parentheses.
[c] Not detected.
[d] In ref 4.

On the other hand, although the ester **9i** showed moderate BA (46%), the ester (**9j**) did not show any BA.

These results show that the 1-(cyclohexyloxycarbonyloxy)alkyl group is a more suitable promoiety to improve the oral BA of CTM compared to its thia or aza analog. Among the 1-(substituted cyclohexyloxycarbonyloxy)ethyl esters, **9f** bearing a methyl group at position 2 of the cyclohexane ring showed higher BA than those of **9d** and **9g**, and among the esters bearing cyclohexyl ring (**9b, d, g** and **h**) as R_2, the ester having propyl group (**9h**) as R_1 showed the highest BA. These results suggest that an adequate steric hindrance of the ester moiety is required to give good BA.

The ester **2**, **9d**, **9i** and **9j** had water solubility more than 1 mg/ml at pH 4.5. The CHARTON's steric constant (ν value[9]) of $CH_2C_6H_{11}$, OC_6H_{11} and NHC_6H_{11} is 0.97, 0.81 and 0.92, respectively. That of the thia analog was not described but it was estimated higher than that of oxa analog. There-

Table 2. Plasma cefotiam levels after oral administration of the isomers of the 1-(cyclohexyloxycarbonyloxy)ethyl ester (**9d**) to mice at a dose of 100 mg/kg equivalent of cefotiam.

Compound	Plasma CTM levels (μg/ml)				AUC (μg·hours/ml)	BA[a] (%)
	0.25 hour	0.5 hour	1 hour	2 hours		
α-Isomer	37.4	28.5	6.05	0.39	24.8	63.9
	(3.7)[b]	(14.5)	(0.30)	(0.04)		
β-Isomer	42.2	19.6	7.41	0.57	23.7	61.1
	(7.5)	(2.0)	(3.76)	(0.13)		

[a] Relative bioavailability.
[b] SE in parentheses.

Table 3. Half-life of the 1-(cyclohexyloxycarbonyloxy)ethyl ester (**9d**) of cefotiam and formation of cefotiam, Δ^2-**9d**, and Δ^2-cefotiam in 5% homogenate of mice small intestine and liver, and 50% plasma at 37°C.

Tissue	$t_{1/2}$ (minutes)		Percent of formation at 30 minutes		
	α	β	Δ^2-**9d**	CTM	Δ^2-CTM
Intestine	2.03	0.24	0.0	87.6	10.6
Liver	4.25	1.03	0.0	76.7	26.3
Plasma	1.02	0.49	0.0	60.5	29.6

fore, we think the water solubility and steric hindrance are not critical factors for the low BA of **9i** and **9j**.

In a 1% homogenate of mice small intestine at 37°C, **2** and **9d** were hydrolyzed to CTM with a half-life (12.5 and 33.5 minutes, respectively) but **9j** did not produce CTM. As an ester of CTM was isomerized to the corresponding ester of Δ^2-CTM under neutral or alkaline medium to give Δ^2-CTM,[3] **9j** must be converted to Δ^2-CTM. FERRES[10] described that the acyloxymethyl ester and its oxa analog were hydrolyzed rapidly but the aza analog very slowly. These results suggest that the low BA of aza analog is caused by the slow hydrolysis rate, *i.e.*, inactivation.

As many of the esters tested, *e.g.*, **9a** and **9d**~**h**, were used as mixtures of their diastereoisomers, it was necessary to examine the difference in BA or hydrolysis rates between the two isomers. Each isomer of **9d** was separated by chromatography and, administered to mice at the same dose and subjected to hydrolysis experiments *in vitro* in 5% homogenates of mice small intestine and liver and 50% mice plasma at 37°C.

The results of these experiments are summarized in Tables 2 and 3.

Table 2 shows that there was no significant difference between BAs of the two isomers.

Table 3 shows that the elimination of both isomers followed pseudo-first order kinetics but the rates were different; however, the difference in the half-lives of both isomers, was less than 5 minutes. The ester **9d** in the small intestine homogenate was mainly hydrolyzed to CTM but 30% of the ester **9d** in the homogenate of liver or plasma was converted to Δ^2-CTM.

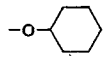
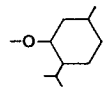
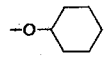
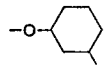
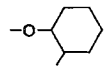
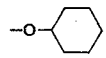
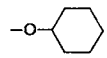
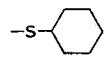
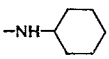
As no difference was observed in the BA after oral dosing of the two isomers, the difference in the hydrolysis rates of the ester to CTM *in vivo* would be smaller compared with the rates observed *in vitro*.

Conclusion

Among the oxa, thia and aza analogs of the 1-(cyclohexylacetoxy)alkyl group studied as a pro-

Table 4. Alkyl chloroalkyl carbonates (**7b~h**), *S*-cyclohexyl chloromethyl thiocarbonate (**7i**) and chloromethyl *N*-cyclohexylcarbamate (**7j**).

$ClCH(R_1)OCOAR_2$

No.	R_1	AR_2	Yield (%)	Formula	Analysis (%) Calcd/Found C	Analysis (%) Calcd/Found H	IR[a] (cm^{-1}) C=O	^1H NMR ($CDCl_3$) δ $ClCH_2$ or ClCH
7b	H		77	$C_8H_{13}ClO_3$	49.88 49.54	6.80 6.70	1760	5.70 (s)
7c	H		75	$C_{12}H_{21}ClO_3$	57.94 57.83	8.51 8.35	1760	5.71 (s)
7d	CH_3		88	$C_9H_{15}ClO_3$	52.30 52.26	7.32 7.32	1760	6.40 (q)
7e	CH_3		71	$C_{10}H_{17}ClO_3$	54.42 54.18	7.77 7.48	1765	6.40 (q)
7f	CH_3		94	$C_{10}H_{17}ClO_3$	54.42 54.19	7.77 7.76	1760	6.40 (q)
7g	C_2H_5		95	$C_{10}H_{17}ClO_3$	54.42 54.41	7.77 7.70	1765	6.23 (t)
7h	n-C_3H_7		92	$C_{11}H_{19}ClO_3$	56.29 56.02	8.16 8.01	1760	6.27 (t)
7i	H		70	$C_8H_{13}ClO_2S$	NT	NT	1730	5.75 (s)
7j	H		54	$C_8H_{14}ClNO_2$	50.13 51.03 (N, 7.31/7.22)	7.36 7.07	1720	5.77 (s)

[a] Liquid film.

s, Singlet; t, triplet J=5 Hz; q, quartet J=6 Hz.

NT: Not tested.

moiety, the oxa analog, 1-(substituted cyclohexyloxycarbonyloxy)alkyl group, was more suitable promoiety than its thia or aza analog to improve the oral BA of CTM.

As previously reported, to show good oral BA of CTM, the ester moiety is required to have adequate lipophilicity and steric hindrance in addition to have both good water solubility and adequate hydrolysis rate of the ester to CTM. Among the 1-(substituted cyclohexyloxycarbonyloxy)alkyl esters studied, those having a methyl group in the cyclohexane ring or a propyl group as R_1 showed a BA of more than 90%. These results indicate that these esters (**9b~h**) must satisfy these requirements.

Among the esters studied, the 1-(cyclohexyloxycarbonyloxy)ethyl ester of CTM (**9d**, SCE-2174) was selected as a candidate for further testing.

Experimental

All boiling points are uncorrected. IR spectra were measured on a Hitachi 215 spectrophotometer and ^1H NMR spectra were recorded on Varian XL 100A (100 MHz) and EM 360 (60 MHz) spectrometers using tetramethylsilane as an internal reference. High performance liquid chromatography (HPLC) was done using a Shimadzu LC 3A instrument equipped with a column (300×4 mm i.d.) of μBondapak C_{18} and a Shimadzu LC 5A instrument equipped with a column (300×4 mm i.d.) of Nucleosil C_{18} and a variable wave length UV detector. No attempt was made to optimize the yield.

1-Chloroalkyl Chloroformates

1-Chloroalkyl chloroformates (**3~6**) were prepared according to MÜLLER's[7] or CAGNON's methods[8]:

Chloromethyl chloroformate (**3**); yield 63%; bp 102~110°C (literature[7] 106°C); ^{1}H NMR ($CDCl_3$) δ 5.71 (2H, s).

1-Chloroethyl chloroformate (**4**); yield 37%; bp 118~120°C (literature[7] 115~116°C); ^{1}H NMR ($CDCl_3$) δ 1.85 (3H, d, J=6 Hz), 6.41 (1H, q, J=6 Hz).

1-Chloropropyl chloroformate (**5**); bp 55.5~56.5°C/55 mmHg; IR (film) cm^{-1} 1780, 1470, 1390; ^{1}H NMR ($CDCl_3$) δ 1.09 (3H, t, J=7 Hz), 2.11 (2H, quintet, J=6 Hz), 6.28 (1H, t, J=5 Hz).

1-Chlorobutyl chloroformate (**6**); bp 58~60°C/22 mmHg; IR (film) cm^{-1} 1780, 1690, 1470, 1350; ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, J=7 Hz), 1.1~2.7 (4H, m), 6.28 (1H, t, J=5 Hz).

Preparation of Alkyl 1-Chloroalkyl Carbonates (**7a~h**): General Method

1-Chloroethyl chloroformate (2 ml) was added dropwise to a solution of cyclohexanol (1.83 g) and pyridine (1.45 g) in CH_2Cl_2 (30 ml) under dry-ice cooling with stirring. The reaction mixture was stirred at room temperature for 16 hours, then washed with a saturated aqueous sodium chloride solution and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was distilled under reduced pressure to afford 1-chloroethyl cyclohexyl carbonate (**7d**) as a colorless oil in 88% yield; bp 100~103°C/5 mmHg; IR (film) cm^{-1} 1760, 1455, 1390; ^{1}H NMR ($CDCl_3$) δ 1.0~2.3 (10H, m), 1.83 (3H, d, J=6 Hz), 4.68 (1H, m), 6.40 (1H, q, J=6 Hz).

Other 1-chloroalkyl carbonates (**7**), the *S*-cyclohexyl chloromethyl thiocarbonate (**7i**), and the chloromethyl *N*-cyclohexylcarbamate (**7j**) were obtained by the similar procedure. The physico-chemical data are summarized in Table 4.

Preparation of 1-(Cyclohexyloxycarbonyloxy)ethyl 7β-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylate Dihydrochloride (**9d**)

(1) Cyclohexyl 1-Iodoethyl Carbonate (**8d**): 1-Chloroethyl cyclohexyl carbonate (**7d**, 1.56 g) and NaI (5 g) were stirred in acetonitrile (50 ml) at 60°C for 70 minutes and the mixture was concentrated *in vacuo*. The residue was extracted with ethyl ether. The combined ethereal solution was concentrated *in vacuo* to give cyclohexyl 1-iodoethyl carbonate (**8d**) as an oil. ^{1}H NMR ($CDCl_3$) δ 0.7~2.3 (10H, m), 2.18 (3H, d, J=6 Hz), 4.1~4.9 (1H, m), 6.67 (1H, q, J=6 Hz).

(2): A dimethylformamide solution of **8d** was added to a solution of the potassium salt of cefotiam (3.6 g) in DMF (30 ml) under ice-cooling and stirring. The mixture was stirred for 5 minutes and then was poured into a mixture of ice-cooled 20% brine (150 ml) and ethyl acetate (150 ml). The organic layer was separated, washed with a saturated brine and then extracted with 1 N HCl (40 ml). The aqueous extract was chromatographed on MCI GEL CHP 20P (75~150 μm, Mitsubishi Kasei) column, with 0.01 N HCl and acetonitrile - 0.01 N HCl (1 : 4) as successive eluents. The fractions containing the ester were combined and concentrated *in vacuo*, then the residue was lyophilized to afford 1-(cyclohexyloxycarbonyloxy)ethyl 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylate dihydrochloride (**9d**) in 20% yield.

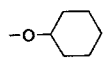
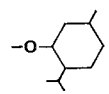
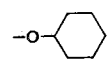
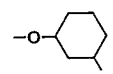
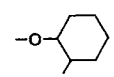
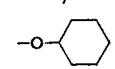
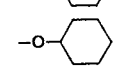
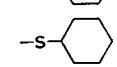
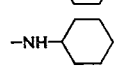
IR (KBr) cm^{-1} 1780, 1750, 1680, 1540; ^{1}H NMR (DMSO-d_6) δ 1.0~2.2 (10H, m), 1.52 and 1.55 (2H, two d, J=6 Hz), 2.86 (6H, s), 3.66 (2H, s), 3.73 and 3.96 (2H, ABq, J=18 Hz), 4.29 and 4.56 (2H, two ABq, J=13 Hz), 4.2~4.9 (1H, m), 4.82 (2H, t, J=6 Hz), 5.14 and 5.18 (1H, two d, J=5 Hz), 5.70 and 5.75 (1H, two dd, J=5 and 8 Hz), 6.68 (1H, s), 6.81 and 6.89 (1H, two q, J=6 Hz), 9.27 and 9.31 (1H, two d, J=8 Hz).

Other 1-(alkoxycarbonyloxy)alkyl esters (**9a~c** and **9e~h**), the cyclohexylthiocarbonyloxymethyl ester (**9i**) and the *N*-cyclohexylcarbamoyloxymethyl ester (**9j**) were prepared by a procedure similar to that described above for the preparation of **9d**. The analytical results are summarized in Table 5.

Separation of Each of the Diastereoisomers of **9d**

The ester **9d** (0.5 g) was dissolved in water (1 ml) and chromatographed on MCI GEL CHP 20P (75~150 μm, 3×60 cm, Mitsubishi Kasei) with acetonitrile - 0.01 N HCl (1 : 4) as eluent and the eluates were fractionated in 20-ml portions. Fractions (No.) 39~45 were combined, concentrated *in vacuo* and the residue was lyophilized to afford one of the isomers (depicted as α-isomer, 0.19 g) as a powder. Fractions (No.) 50~58 were combined, concentrated *in vacuo* and the residue was then lyophilized to give another isomer (β-isomer, 0.07 g) as a powder. The physico-chemical data of the isomers are summarized in Table 6.

Table 5. 1-(Alkoxycarbonyloxy)alkyl, *S*-cyclohexylthiocarbonyloxymethyl and *N*-cyclohexylcarbamoyloxymethyl esters (**9a**~**j**) of cefotiam.

Ester No.	R_1	AR_2	Yield (%)	Formula	Analysis (%) Calcd/Found			IR[a] (cm^{-1})	^1H NMR (DMSO-d_6) δ	
					C	H	N		$R_1(CH_2)$ R_1CH	Th5H[b] (s)
9a	CH_3	OC_2H_5	39	$C_{23}H_{31}N_9O_7S_3$	43.05 42.86	4.87 4.76	19.64 19.32	1780 1760	6.55~ 6.95[d]	6.20
9b	H		29	$C_{26}H_{35}N_9O_7S_3 \cdot 2HCl \cdot 5/2H_2O$	39.05 39.02	5.29 5.06	15.76 16.00	1780 1770	(5.76)[c] (5.90)	6.65
9c	H		43	$C_{30}H_{43}N_9O_7S_3 \cdot 2HCl \cdot 5/2H_2O$	42.10 42.02	5.89 6.04	14.73 14.71	1780 1760	(5.78)[c] (5.91)	6.64
9d	CH_3		20	$C_{27}H_{37}N_9O_7S_3 \cdot 2HCl \cdot 2H_2O$	40.30 40.31	5.39 5.32	15.66 15.82	1780 1750	6.81[e] 6.89[e]	6.68
9e	CH_3		9	$C_{28}H_{39}N_9O_7S_3 \cdot 2HCl \cdot 3H_2O$	40.19 40.41	5.66 5.69	15.06 15.25	1780 1760	6.81[e] 6.89[e]	6.66
9f	CH_3		12	$C_{28}H_{39}N_9O_7S_3 \cdot 2HCl \cdot 3H_2O$	40.19 40.25	5.66 5.56	15.06 15.03	1780 1760	6.81[e] 6.91[e]	6.66
9g	C_2H_5		38	$C_{28}H_{39}N_9O_7S_3 \cdot 2HCl \cdot 5/2H_2O$	40.19 40.11	5.66 5.24	15.06 15.11	1780 1755	6.69[f] 6.76[f]	6.65
9h	*n*-C_3H_7		19	$C_{29}H_{41}N_9O_7S_3 \cdot 2HCl \cdot 3H_2O$	41.38 41.04	5.75 5.53	14.97 14.96	1780 1755	6.76[f] 6.83[f]	6.68
9i	H		29	$C_{26}H_{35}N_9O_6S_4 \cdot 2HCl \cdot 2H_2O$	38.71 38.71	5.12 5.13	15.62 15.55	1780 1730	(5.86)[c] (5.97)[c]	6.65
9j	H		17	$C_{26}H_{36}N_{10}O_6S_3 \cdot 2HCl \cdot 5/2H_2O$	39.10 39.10	5.43 5.85	17.54 17.77	1780 1740	(5.78)[g]	6.66

[a] KBr disk.
[b] Proton at 5-position of thiazole ring.
[c] AB quartet, J=6 Hz.
[d] Multiplet.
[e] Double quartet, J=5 Hz.
[f] Double triplet, J=5 Hz.
[g] Singlet.

Table 6. The physico-chemical properties of two isomers of the 1-(cyclohexyloxycarbonyloxy)ethyl ester (**9d**) of cefotiam.

	α-Isomer	β-Isomer
$[\alpha]_D^{22}$ (H_2O)	+36.7° (*c*, 0.215)	+62.9° (*c*, 0.24)
Rf[a]	0.47	0.56
R_t[b] (minutes)	5.5	6.5
^{1}H NMR (DMSO-d_6) δ	1.22 (3H, d, *J*=6 Hz), 3.69 and 3.91 (2H, ABq, *J*=18 Hz), 4.27 and 4.53 (2H, ABq, *J*=13 Hz), 5.12 (1H, d, *J*=5 Hz), 5.71 (1H, dd, *J*=5 and 8 Hz), 6.90 (1H, q, *J*=6 Hz), 9.29 (1H, d, *J*=8 Hz)	1.51 (3H, d, *J*=6 Hz), 3.72 and 3.92 (2H, ABq, *J*=18 Hz), 4.30 (2H, br s), 5.16 (1H, d, *J*=5 Hz), 5.76 (1H, dd, *J*=5 and 8 Hz), 6.80 (1H, q, *J*=6 Hz), 9.22 (1H, d, *J*=8 Hz)
IR (KBr) cm^{-1}	1790, 1760, 1695, 1680	1780, 1760, 1680, 1625

[a] TLC (Merck 5715, Silica gel), solvent; EtOAc - CH_2Cl_2 - MeOH (2: 2: 1).

[b] Retention time of HPLC. HPLC: column, Nucleosil C_{18} 300×4 mm i.d.; solvent 0.05 M $(NH_4)_2SO_4$ - AcOH - MeCN (180 : 1 : 70); flow rate 1.5 ml/minute.

Oral Absorption Study

Male SLC-ICR mice, weighing about 15 g (4 weeks old), were starved but had free access to water for 16~18 hours before the experiment. The ester was 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 of cefotiam. Blood was taken from the *inferior vena cava* 0.25, 0.5, 1 and 2 hour(s) after dosing. Cefotiam was administered subcutaneously as a 1% aqueous solution at a dose of 100 mg/kg. The relative bioavailability (BA) was calculated from the area under the plasma cefotiam levels-time curve (AUC_{oral}) and that after subcutaneous administration (AUC_{sc}). The plasma concentration of cefotiam was measured by FUGONO and MAEDA's method using *Proteus mirabilis* ATCC 21100 as the test organism.[11]

Water Solubility

The water solubility at pH 4.5 was measured according to the procedure reported previously.[3,4]

Hydrolysis of Ester to CTM in Homogenates of Mouse Tissue

a) In a 1% Homogenate of Mouse Small Intestine: The concentration of CTM released from **9d** and **9i** in 1% homogenate of mouse small intestine at 37°C was measured according to the procedure reported previously.[3,4]

b) In 5% Homogenate of Mouse Small Intestine and Liver and 50% Mouse Plasma: Fresh small intestine or liver of mouse was homogenated with isotonic buffer of pH 7.4 (one part of tissue to nine parts of buffer). After centrifugation at 9,000×*g*, the supernatant was used as a 10% homogenate of small intestine or liver. The ester **9d** (5 mg) was dissolved in 0.005 N HCl (10 ml). This solution (1.0 ml) was added into a mixture of 10% homogenate or fresh plasma (5 ml) and an isotonic buffer of pH 7.4 (4 ml) and the mixture incubated at 37°C. Sampling was carried out at immediately after mixing and then after incubation for 1, 2, 3, 5, 10, 15 and 30 minute(s). Each sample (0.6 ml) was diluted with 0.1 N HCl and a definite amount was injected into the HPLC through a filter (Cathivex-HA, 0.45 μm, Millipore Corp.).

HPLC; column, μBondapak C_{18}.

Solvent; to detect **9d** and Δ^2-**9d**, 0.05 M $(NH_4)_2SO_4$ - MeCN - AcOH (400 : 160 : 1). Flow rate 0.8 ml/minute. Retention time; **9d**, 7.24 minutes, Δ^2-**9d**, 10.84 minutes.

Solvent; to detect CTM and Δ^2-CTM, 0.03 M $(NH_4)_3PO_4$ - AcOH - MeOH (100 : 3 : 2). Flow rate 0.8 ml/minute. Retention time; CTM, 9.12 minutes, Δ^2-CTM, 7.95 minutes.

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