SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A NEW ORAL CEPHALOSPORIN, BMY-28100 AND RELATED COMPOUNDS

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(Received for publication January 5, 1987)

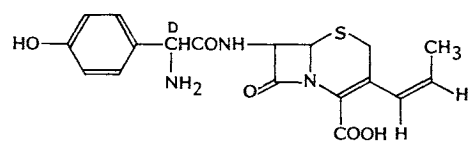
The synthesis and structure-activity relationships of 7-[D-α-amino-α-(4-hydroxyphenyl)-acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic acid (BMY-28100) and its analogs in the 3- and 7-side chains are described. The 3-(substituted-propenyl) groups were introduced by the Wittig reaction of the 3-phosphoniomethyl cephems which were derived from the 3-chloromethyl derivatives. The reaction gave predominantly the *cis* isomer regarding the 3-side chain. The *cis* and *trans* isomers showed characteristic UV and ^{1}H NMR spectra. Most of cephems of this series were well-absorbed orally and more active both *in vitro* and *in vivo* than cephalexin and cefaclor against Gram-positive organisms. Their Gram-negative activity varied depending on the 3- and 7-substituents. Compounds with a *cis*-propenyl group showed the best Gram-negative activity among the 3-alkenyl analogs prepared, whereas the D-4-hydroxyphenylglycyl and D-4-hydroxy-3-methoxyphenylglycyl substitutions in the 7-side chain were found suitable to improve the Gram-negative activity of 3-*cis*-propenyl series of cephalosporins to the level favorably compared with that of cefaclor. The 3,4-dihydroxyphenyl analog was found to be metabolized *in vivo* to the 4-hydroxy-3-methoxyphenyl derivative and, therefore, showed nearly the same *in vivo* activity as that of the latter. BMY-28100 was selected for further evaluation and the results will be reported in the subsequent paper.

Since cephaloglycin[1,2] was launched in 1965, several oral cephalosporins have been developed for clinical use, such as cephalexin[3], cephradine[4], cefatrizine[5], cefaclor[6], cefroxadine[7] and cefadroxil[8], which have a phenylglycyl (or its congener) or 4-hydroxyphenylglycyl group at the 7 position of cephalosporin nucleus. In our search for new orally active cephalosporins, we found that the 3-alkenyl derivatives of 7-phenylglycyl cephalosporins were well absorbed by oral administration. The representative member of this group is 7-[D-α-amino-α-(4-hydroxyphenyl)acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic acid designated as BMY-28100 (Fig. 1). This paper describes the synthesis and the structure-activity relationships of BMY-28100 and analogs†.

Synthesis

BMY-28100 and its analogs were prepared by the synthetic route shown in Scheme 1. The starting material, diphenylmethyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate (**1**)[9], was acylated with *N*-*tert*-butoxycarbonyl (BOC)-protected phenylglycines (**2**) in the presence of dicyclohexylcarbodiimide (DCC) to give the 7-

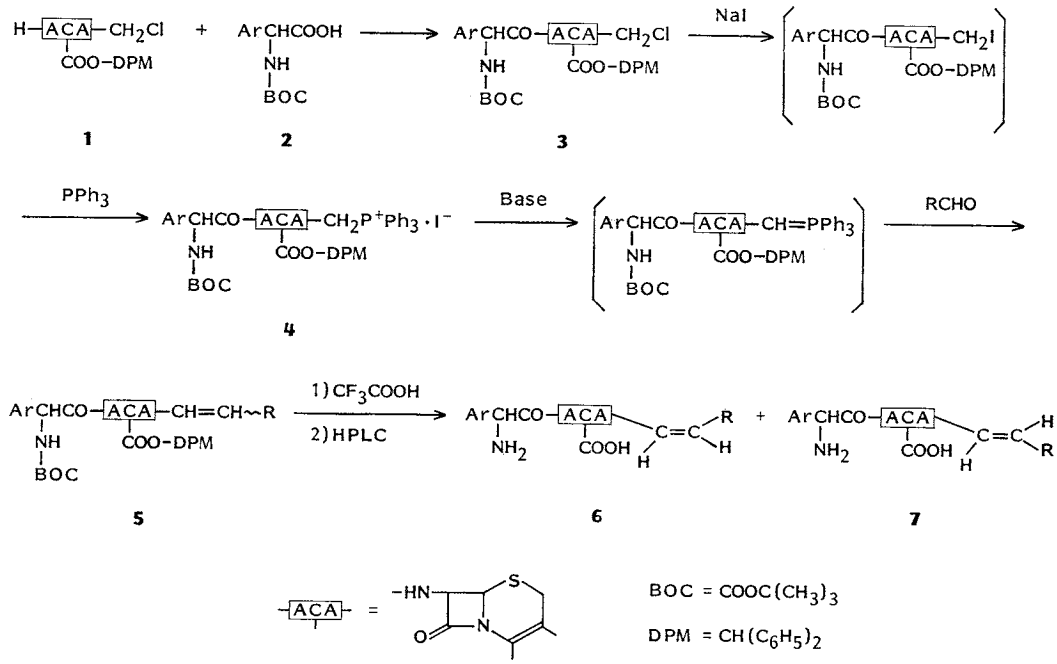
Fig. 1.



BMY-28100

† A part of this paper has been presented at the 14th Int. Congress of Chemother., Kyoto, 1985.

Scheme 1.



(*N*-BOC-phenylglycyl)-3-chloromethylcephalosporanates (**3**) in almost quantitative yield. The chlorides (**3**) were treated with NaI to afford the iodides, which were converted to the triphenylphosphonium iodides (**4**) by treating with triphenylphosphine. In most cases compounds **4** were prepared in one pot from **1** without isolation of the chlorides and iodides in about 60 to 90% overall yield (Table 1).

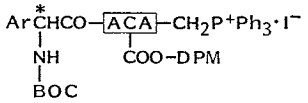
The Wittig reaction of **4** was performed with an appropriate aldehyde in dichloromethane or chloroform at room temperature in the presence of a base, such as sodium hydroxide, sodium carbonate or sodium bicarbonate. The reaction mixture was purified with a silica gel column to give the 3-alkenyl derivatives (**5**) in 20 to 80% yield (Table 2). As shown in the table, most of **5** showed a doublet vinyl proton signal with J=11 Hz around 6.1 ppm in their ^{1}H NMR spectra. This means that the Wittig reaction in this series of the compounds gave predominantly *cis* isomer regarding the 3-side chain. Although HPLC study indicated that **5** contained 15~20% of the *trans* isomer as a minor component, they were used for the next step without separation of the isomers.

Deblocking of **5** with trifluoroacetic acid (TFA) - anisole at room temperature gave crude TFA salts of the final products which were purified by reverse phase column chromatography to give a majority of the *cis* isomer (**6**) and the *trans* isomer (**7**) as a minor product.

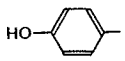
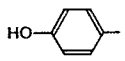
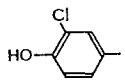
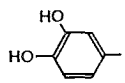
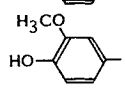
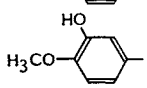
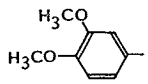
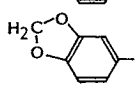
When racemic phenylglycine derivatives were used in the 7-*N*-acylation, compounds **3** through **6** were obtained as a mixture of the corresponding diastereomers. In the final stage, the reaction products were subjected to reverse phase column chromatography to separate the D and L isomers regarding the α-carbon in the 7-side chain, both of which having a *cis* configuration in the 3-side chain. In these cases the corresponding *trans* isomer could not be isolated because of a small amount of the isomer contained.

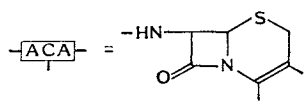
Table 3 summarizes the physico-chemical data of **6** and **7**. The *cis* isomers (**6**) had an absorption maximum at *ca.* 280 nm, whereas the *trans* isomers (**7**) exhibited the maximum at *ca.* 290 nm with greater

Table 1. 3-Triphenylphosphoniomethylcephems (4).



4

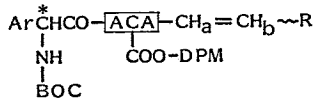
Compound	Ar	*	Yield from 1 (%)	MP (°C, dec)	UV λ_{max}^{MeOH} nm (ε)
4a		D	85	160~165	269 (10,000), 276 (9,600)
4b		D	88	170~180	269 (10,600), 277 (10,000)
4c		DL	83	160~170	269 (11,000), 275 (10,000)
4d		D	58	*ca.* 165	269 (11,000), 276 (12,000)
4e		D	85	*ca.* 170	269 (12,000), 276 (12,000)
4f		D	86	*ca.* 165	269 (12,000), 277 (12,000)
4g		DL	58	160~165	269 (12,000), 276 (13,000)
4h		DL	82	165~170	269 (11,000), 277 (11,000)
4i		DL	85	160~165	269 (10,000), 277 (11,000)



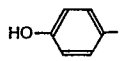
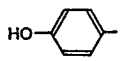
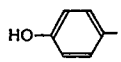
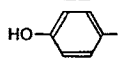
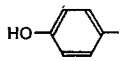
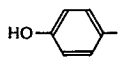
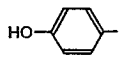
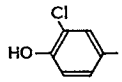
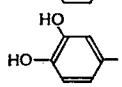
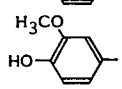
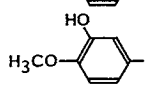
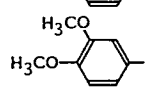
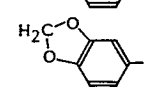
intensity in UV spectra than the corresponding *cis* isomers. Distinct differences between *cis* and *trans* isomers were also observed in their ^{1}H NMR spectra. As shown in Table 3, a vinyl proton closer to the cephem nucleus (H_a in Table 3) of the *cis* isomers appeared at *ca.* 6.0 ppm as a one-proton doublet with a coupling constant of 11 Hz, whereas that of the *trans* isomers resonated at lower field (*ca.* 6.5 ppm) with a 16 Hz coupling constant. Another vinyl proton (H_b) was obscure because of multiplicity due to coupling with neighboring aliphatic protons. The vinylic signal was confirmed by the decoupling study on the ^{1}H NMR of BMY-28100 (**6d**). When the allylic methyl proton at 1.71 ppm was irradiated, a multiplet around 5.8 ppm changed to a doublet with a coupling constant of 11 Hz. On the other hand, the methyl signal was converted from a doublet to a singlet by irradiation at *ca.* 5.8 ppm. Change of multiplicity on the H_a signal at 6.02 ppm, however, could not be detected because its chemical shift was close to the irradiated signal at 5.8 ppm.

Another difference between both isomers was observed in the signal of the 2-H protons of the cephem nucleus. The resonance of the *trans* isomers (**7**) was observed at *ca.* 3.6 ppm as a two-proton

Table 2. The Wittig reaction products (**5**).



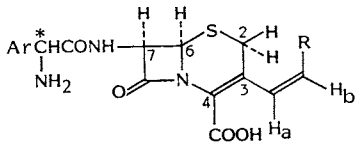
5

Compound	Ar	R	*	Yield (%)	MP (°C, dec)	UV λ_{max}^{MeOH} nm (ε)	^{1}H NMR[a] Vinyl-H$_a$ (δ, ppm)
5a[14]		H	D	79	*ca.* 210	295 (14,000)	6.90[b]
5b		H	D	45	*ca.* 135	286 (14,000)	*ca.* 7.0[c]
5c		CH_3	D	34.5	*ca.* 145	289 (7,000)	6.05
5d		CH_3	D	31	120~130	283 (8,300)	6.07
5e		CH_3	DL	29	*ca.* 120	ND	6.08
5f		C_2H_5	D	55	*ca.* 115	277 (10,000)	6.06
5g			D	21	ND	ND	ND
5h		CH_2OCH_3	D	38	*ca.* 115	278 (8,000)	6.18
5i		CH_2Ph	D	37	*ca.* 120	277 (8,600)	6.15
5j		CH_3	D	21	120~125	287 (8,300)	6.04
5k		CH_3	D	40	120~125	282 (9,800)	6.04
5l		CH_3	D	24.5	100~105	282 (9,900)	6.08
5m		CH_3	DL	20	ND	282 (8,500)	6.10
5n		CH_3	DL	40	ND	280 (9,800)	6.08
5o		CH_3	DL	37	ND	287 (12,700)	6.07

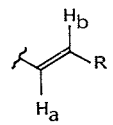
[a] Determined in $CDCl_3$; 1H, d, J=11 Hz.
[b] Determined in $CDCl_3$ - DMSO-d_6; 1H, dd, J=17 and 11 Hz.
[c] 1H, m.
ND: Not determined.

singlet. This was also the case in 3-vinyl derivatives, **6a** and **6b**. On the contrary, that of the *cis* isomers appeared at 3.1 to 3.6 ppm as an AB quartet with an 18 Hz coupling constant. This observation may reflect that the 2-H protons of the *trans* isomers are magnetically equivalent, while those of the *cis* isomers are not equivalent because of the steric effect caused by the *cis*-substituent on the double bond of

Table 3. BMY-28100 derivatives (**6** and **7**).



6



7

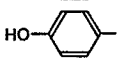
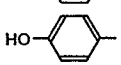
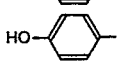
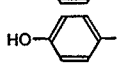
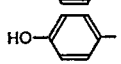
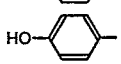
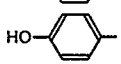
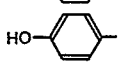
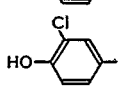
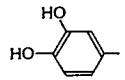
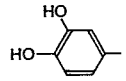
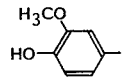
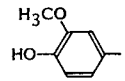
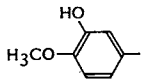
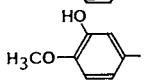
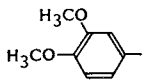
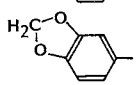
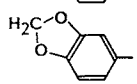
Compound	Ar	R	*	Yield (%)	MP (°C, dec)	UV λ_{max} nm (ε)[a]	^{1}H NMR δ_{ppm} in D_2O (+$NaHCO_3$)			
							2-H[b]	6-H[c]	7-H[c]	Vinyl-H_a[d]
6a[14]		H	D	46	*ca.* 190	287 (13,000)	3.62 (s)	5.20	5.75	7.3[e]
6b		H	D	31	*ca.* 190	283 (14,000)	3.60 (s)	5.33	5.73	6.77[f]
6c		CH_3	D	60	*ca.* 200	282 (8,800)	3.12, 3.48	5.03	5.63	5.92
6d		CH_3	D	40	218~220	279 (9,800)	3.27, 3.59	5.18	5.73	6.02
7d		CH_3	D	2	*ca.* 230	292 (16,900)	3.60 (s)	5.13	5.68	6.54
6e		CH_3	L	9	*ca.* 200	279 (9,900)	3.36, 3.67	5.21	5.59	6.02
6f		C_2H_5	D	9	*ca.* 180	278 (7,200)	3.12, 3.38	5.01	5.58	5.78
6g			D	23	*ca.* 195	281 (7,700), 287 (7,600)	3.29, 3.59	5.07	5.62	5.83
6h		CH_2OCH_3	D	75	*ca.* 160	279 (9,400)	3.24, 3.57	5.19	5.77	6.20
6i		CH_2Ph	D	31	*ca.* 175	280 (8,900)	—	5.07	5.74	6.20
6j		CH_3	D	48	180~185	280 (10,500)	3.25, 3.57	5.18	5.72	5.97

Table 3. (Continued)

Compound	Ar	R	*	Yield (%)	MP (°C, dec)	UV λ_{max} nm (ε)[a]	^{1}H NMR δ_{ppm} in D_2O (+$NaHCO_3$)			
							2-H[b]	6-H[c]	7-H[c]	Vinyl-H_a[d]
6k		CH_3	D	54	*ca.* 200	281 (11,000)	3.26, 3.58	5.22	5.77	5.97
7k		CH_3	D	2	*ca.* 180	287 (16,000)	3.59 (s)	5.17	5.74	6.54
6l		CH_3	D	50.5	175~180	280 (11,000)	3.12, 3.50	5.08	5.68	5.92
7l		CH_3	D	3	*ca.* 200	286 (16,500)	3.58 (s)	5.12	5.70	6.55
6m		CH_3	D	22	175~185	281 (12,000)	3.25, 3.58	5.23	5.78	5.98
6m(L)		CH_3	L	20	170~180	281 (12,000)	3.40, 3.69	5.26	5.57	6.04
6n		CH_3	D	21	*ca.* 170	278 (11,000)	3.28, 3.62	5.26	5.77	6.03
6o		CH_3	D	27	181~185	284 (15,000)	3.34, 3.66	5.24	5.78	6.07
6o(L)		CH_3	L	17	165~175	284 (13,000)	2.96, 3.24	5.30	5.67	6.10

[a] Determined in pH 7 phosphate buffer.
[b] d, J=18 Hz.
[c] d, J=4.5 Hz.
[d] **6**: d, J=11 Hz. **7**: d, J=16 Hz.
[e] Determined in TFA; 1H, dd, J=17 and 11 Hz.
[f] 1H, dd, J=17 and 11 Hz.

the 3-side chain which might be sterically close to the 2-H protons.

Antimicrobacterial Acitivity

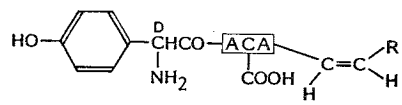
Minimum inhibitory concentrations (MICs) of cephalosporins in the present study were determined by 2-fold serial agar dilution method in Mueller-Hinton agar against 15 test organisms, which consist of five strains each of penicillin-sensitive *Staphylococcus aureus* (designated GP-S group), penicillinase-proudcing *S. aureus* (GP-R group) and cephalothin-sensitive Gram-negative bacteria (GN group). The *in vitro* activity of the derivatives was primarily assessed by the geometric mean of MICs for each of the 3 groups of test organisms.

Table 4 shows the *in vitro* antibacterial activity of BMY-28100 (**6d**) and its analogs having a variety of alkenyl groups at the 3-side chain. Most of the 3-alkenylcephems in Table 4 were more active than cephalexin and cefaclor against both Gram-positive test organism groups, GP-S and GP-R, whereas they, except BMY-28100 and **6i**, were between cephalexin and cefaclor in the activity against Gram-negative organisms (the GN group). BMY-28100 (**6d**) was significantly more acitive than cephalexin against all of the three groups of test organisms. As compared to cefaclor, BMY-28100 was more active against both GP-S and GP-R groups, and slightly more active against the GN group. Compounds **6b** and **6f**, the lower and higher homologs of **6d**, were comparable to **6d** against both GP groups, but 3~4 times less active against the GN group. The phenylpropenyl derivative (**6i**) is the most active against both GP-S and GP-R groups, but almost inactive against the GN group.

Table 5 shows *in vitro* antibacterial activity of a variety of substituted phenyl derivatives in the 7-side chain. All compounds except **6k** were more active than cephalexin and cefaclor against GP-S and GP-R. Against the GN group, unsubstituted phenyl (**6c**) and 4-hydroxy-substituted phenyl derivatives (**6d**, **6j** and **6l**, except **6k**) were as active as or more active than cefaclor. Only compound **6k** was weakly active against all test organism groups.

Compounds having a 4-hydroxy substitution in the phenyl ring, **6d**, **6j**, **6k** and **6l**, were examined

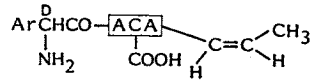
Table 4. *In vitro* activity of BMY-28100 and analogs (1), (Mueller-Hinton agar, 10^6 cfu/ml, 37°C, 18 hours).

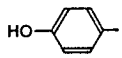
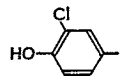
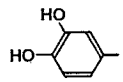
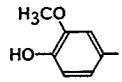
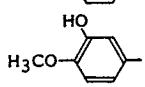
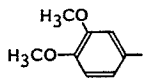
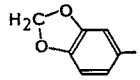


Compound	R	Geometric mean of MIC (μg/ml)		
		GP-S*	GP-R	GN
6b	H	0.40	1.2	3.1
6d (BMY-28100)	CH_3	0.30	0.70	0.92
6f	C_2H_5	0.40	1.4	4.1
6g		0.30	1.1	4.2
6h	CH_2OCH_3	0.70	2.1	3.1
6i	CH_2Ph	0.23	0.70	>50
Cephalexin		1.2	4.1	5.8
Cefaclor		0.61	3.6	1.1

* GP-S: Penicillin (PC)-sensitive *Staphylococcus aureus* (5 strains), GP-R: PC-resistant *S. aureus* (5), GN: Cephalothin-sensitive *Escherichia coli* (2), *Klebsiella pneumoniae* (1) and *Proteus mirabilis* (2).

Table 5. *In vitro* activity of BMY-28100 and analogs (2), (Mueller-Hinton agar, 10^6 cfu/ml, 37°C, 18 hours).



Compound	Ar	Geometric mean of MIC (μg/ml)		
		GP-S	GP-R	GN
6c		0.40	1.2	1.2
6d (BMY-28100)		0.30	0.70	0.92
6j		0.13	0.40	1.2
6k		13	14	14
6l		0.35	1.1	0.92
6m		0.35	1.1	2.1
6n		0.46	1.2	7.2
6o		0.20	0.61	7.2
Cephalexin		1.2	4.1	5.8
Cefaclor		0.61	3.6	1.1

Abbreviations: See footnote in Table 4.

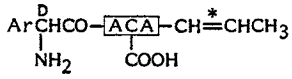
Table 6. Effect of culture medium on *in vitro* activity of BMY-28100 and analogs.

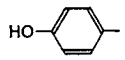
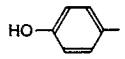
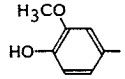
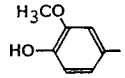
Compound	Medium*	Geometric mean of MIC (μg/ml)		
		GP-S	GP-R	GN
6d	MHA	0.30	0.70	0.92
(BMY-28100)	NA	0.20	0.40	0.80
6j	MHA	0.13	0.40	1.2
	NA	0.11	0.26	0.80
6k	MHA	13	14	14
	NA	0.92	1.4	2.1
6l	MHA	0.35	1.1	0.92
	NA	0.40	0.80	0.70
Cephalexin	MHA	1.2	4.1	5.8
	NA	0.7	1.8	5.5

* MHA: Mueller-Hinton agar, NA: nutrient agar (incubation: 37°C, 18 hours, 10^6 cfu/ml).
Abbreviations: See footnote in Table 4.

for their activity in Mueller-Hinton agar and nutrient agar. As shown in Table 6, **6k** demonstrated much greater activity in nutrient agar than in Mueller-Hinton agar. The MICs of other compounds in this group did not show any significant difference between the two media tested. HPLC study

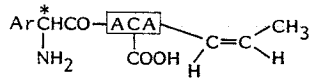
Table 7. Effect of stereochemistry of the 3-propenyl group on *in vitro* activity of BMY-28100 and analogs, (Mueller-Hinton agar, 10^6 cfu/ml, 37°C, 18 hours).

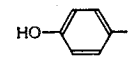
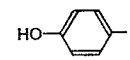
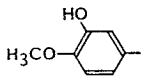
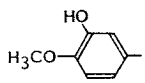
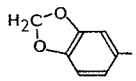
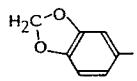


Compound	Ar	*	Geometric mean of MIC (μg/ml)		
			GP-S	GP-R	GN
6d (BMY-28100)		*Z*	0.30	0.70	0.92
7d		*E*	0.36	1.4	6.3
6l		*Z*	0.35	1.1	0.92
7l		*E*	0.46	1.6	14
Cephalexin			1.2	4.1	5.8
Cefaclor			0.61	3.6	1.1

Abbreviations: See footnote in Table 4.

Table 8. Effect of configuration of the phenylglycine moiety in the 7-side chain on *in vitro* activity of BMY-28100 and analogs, (Mueller-Hinton agar, 10^6 cfu/ml, 37°C, 18 hours).



Compound	Ar	*	Geometric mean of MIC (μg/ml)		
			GP-S	GP-R	GN
6d (BMY-28100)		D	0.30	0.70	0.92
6e		L	33	>50	>50
6m		D	0.35	1.1	2.1
6m(L)		L	4.2	12.5	29
6o		D	0.20	0.61	7.2
6o(L)		L	3.1	9.5	>50
Cephalexin			1.2	4.1	5.8
Cefaclor			0.61	3.6	1.1

Abbreviations: See footnote in Table 4.

showed that **6k** as well as **6d** were stable under the conditions of MIC determination (for 18 hours at 37°C) in both Mueller-Hinton agar and nutrient agar. At this time, it is not found the reason why the MIC difference of **6k** occurred in both media.

Table 9. Mouse blood levels of BMY-28100 and analogs, (*dd*Y-mice, $n=5$).

Compound	Dose					
	100 mg/kg, po			20 mg/kg, im		
	Cmax (μg/ml)	$T_{1/2}$ (hours)	AUC (μg·hours/ml)	Cmax (μg/ml)	$T_{1/2}$ (hours)	AUC (μg·hours/ml)
6b	30	1.2	28	23	0.37	15
6c	33	1.1	46	16	0.58	13
6d (BMY-28100)	39	1.2	60	27	0.44	18
6f	36	1.9	85	ND	ND	ND
6j	25	1.7	37	21	0.48	13
6l	31	1.6	50	21	0.46	14
6k	175	1.9	353	67	1.0	150
6m	32	0.76	42	15	0.22	7.2
6n	18	1.5	39	16	0.58	8.1
6o	21	0.87	32	9.2	0.48	5.4
Cephalexin	47	1.4	57	26	0.44	16
Cefaclor	32	1.3	42	21	0.45	13

ND: Not determined.

Table 10. Mouse urinary recovery of BMY-28100 and analogs, (*dd*Y-mice, $n=5$).

Compound	% Recovery (Dose: 100 mg/kg, po)				
	0~2 (hours)	2~4 (hours)	4~6 (hours)	6~24 (hours)	Total
6d (BMY-28100)	36	24	5.4	2.7	68
6l	26	23	5.2	4.7	59
6k	119	165	38	35	357
Cefaclor	35	18	8.2	1.5	63

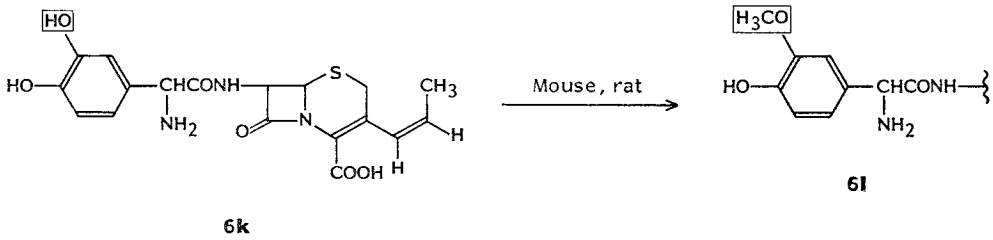
Tables 7 and 8 show the structure-activity relationships on the stereochemistry of the 3- and 7-side chains, respectively. As shown in Table 7, the 3-*cis*-propenyl derivatives (**6d** and **6l**) were more active than the corresponding *trans* isomers (**7d** and **7l**), especially in the Gram-negative activity. Table 8 shows that the L-phenylglycyl derivatives (**6e**, **6m**(L) and **6o**(L)) were much less active than the D-congeners (**6d**, **6m** and **6o**) as was the case in many β-lactam antibiotics[8,10].

Table 9 shows blood level parameters for mice treated orally and intramuscularly with the present series of cephalosporins. In general, they were well absorbed by oral administration. Exceptionally high Cmax (the maximum concentration in the blood achieved) and AUC (area under the drug concentration-time curve) values observed for **6k** suggested that **6k** might be metabolized to a more active substance *in vivo*. Table 10 shows the urinary recovery after oral administration of **6d**, **6l** and **6k**. Again the urinary recovery of **6k** was found unrealistically high.

Chromatographic study revealed that the urine and serum samples of mice administered **6k** by oral route contained a considerable amount of **6l** along with **6k** as shown in Fig. 2. This metabolism was also observed in rats and confirmed by isolation of **6l** from the rat urine sample collected after oral administration of **6k**.

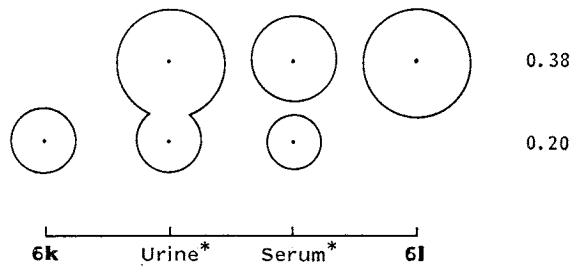
Table 11 shows the *in vivo* activity of cephalosporin derivatives against *S. aureus* Smith and *Escherichia coli* Juhl infections determined after oral and intramuscular administrations. Small differences between oral and intramuscular activities in most compounds of Table 11 indicated their good oral

Fig. 2. Bioconversion of **6k** to **6l**.



6k **6l**

Paper chromatography : Butanol - ethanol - water (4 : 1 : 5)
Bioautography : *Micrococcus luteus* PCI-1001



* Collected after oral administration of compound **6k** to mice

Table 11. *In vivo* activity of BMY-28100 and analogs, (*dd*Y-mice, $n=5$).

Compound	PD_{50} (mg/kg)			
	Staphylococcus aureus Smith		*Escherichia coli* Juhl	
	po	im	po	im
6b	0.30	ND	6.3	ND
6c	0.18	0.15	1.5	0.89
6d (BMY-28100)	0.12	0.10	0.69	0.51
7d	0.20	0.13	7.5	6.0
6f	0.58	ND	5.5	ND
6j	0.17	0.11	3.0	1.7
6k	0.12	0.12	0.56	0.48
6l	0.12	0.10	0.65	0.65
6m	0.12	0.12	4.1	1.9
6n	0.23	0.31	16	9.0
6o	0.18	0.18	9.0	4.7
Cephalexin	0.31	0.24	9.1	6.3
Cefaclor	0.24	0.37	0.63	1.1

ND: Not determined.

absorbability. The 3-vinyl (**6b**) and 3-butenyl (**6f**) derivatives were much less effective against both infections than the 3-propenyl derivative (**6d**). The *trans* propenyl derivative (**7d**) was somewhat less effective than the *cis* isomer (**6d**) against *S. aureus* Smith and more than 10 times less effective against *E. coli* Juhl when administered orally and intramuscularly. The phenyl (**6c**) and 3-chloro-4-hydroxyphenyl (**6j**) analogs of **6d** were as effective as **6d** against the *S. aureus* infection, but 2~4 times less active than **6d** against *E. coli* Juhl. The 3,4-dihydroxyphenyl (**6k**) and 4-hydroxy-3-methoxyphenyl (**6l**) analogs

were as active as **6d** against both infections. Better *in vivo* activity of **6k** than that expected from its MIC value, would be due to the bioconversion of **6k** to **6l** described above.

BMY-28100 (**6d**) was selected as a lead compound in this series for further evaluation[11].

Experimental

Melting points were determined with a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra were recorded on Jasco IRA-1 and UV spectra on Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on a Jeol CL-60HL or on a Varian FT-80A spectrometer.

3-Triphenylphosphoniomethyl Derivatives, **4** (Table 1): General Procedure Illustrated with the Preparation of Diphenylmethyl 7-[D-α-(*N-tert*-butoxycarbonylamino)-α-(4-hydroxyphenyl)acetamido]-3-(triphenylphosphonio)methyl-3-cephem-4-carboxylate Iodide (**4b**)

To a stirred solution of 107.8 g (0.26 mol) of diphenylmethyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate (**1**)[9] and 83.3 g (0.312 mol) of D-α-(*N-tert*-butoxycarbonylamino)-α-(4-hydroxyphenyl)-acetic acid in 1,200 ml of dry THF was added 56 g (0.273 mol) of *N,N'*-dicyclohexylcarbodiimide (DCC) at 5~10°C. The mixture was stirred at room temp for 1.5 hours and concentrated to 300 ml. The concentrate was diluted with 1 liter of EtOAc to separate dicyclohexylurea, which was removed by filtration. The filtrate was washed successively with aq $NaHCO_3$, H_2O and a satd NaCl solution, dried over anhydrous magnesium sulfate and evaporated to dryness to give diphenylmethyl 7-[α-(*N-tert*-butoxycarbonylamino)-α-(4-hydroxyphenyl)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (**3b**) as a foamy solid, which was used without further purification.

To a solution of **3b** in 1 liter of acetone was added 195 g (1.3 mol) of NaI and the mixutre was stirred at room temp for 30 minutes and evaporated to dryness. The residue was dissolved with 2 liters of EtOAc and the solution was washed with aq $Na_2S_2O_3$, H_2O and a satd NaCl solution and dried over anhydrous magnesium sulfate. The solution was concentrated to 1 liter and the concentrate was cooled to 5°C and mixed with 88.6 g (0.338 mol) of triphenylphosphine under stirring. The mixture was stirred at room temp for 16 hours to separate **4b**, which was collected by filtration, washed with cold EtOAc and ether, and dried *in vacuo* over P_2O_5. Yield 232 g (88%): MP 170~180°C (dec); IR ν_{max}^{KBr} cm^{-1} 1780, 1670, 1490, 1420, 1350, 1240, 1150, 1090; UV λ_{max}^{MeOH} nm (ε) 269 (10,600), 277 (10,000); ^1H NMR (60 MHz, DMSO-d_6) δ 1.42 (9H, s, $C(CH_3)_3$), 3.45 (2H, br s, 2-H), 5.0~5.4 (3H, m, CH_2P and 6-H), 5.7 (1H, m, 7-H), 6.63 (2H, d, J=9 Hz, phenyl-H), 7.1~7.45 (12H, m, phenyl-H), 7.5~7.9 (15H, m, phenyl-H).

Anal Calcd for $C_{52}H_{49}N_3O_7SPI$: C 61.36, H 4.85, N 4.13, S 3.15.
Found: C 61.26, H 4.82, N 4.11, S 3.92.

The Wittig Reaction Products, **5** (Table 2): General Procedure Illustrated with the Preparation of Diphenylmethyl 7-[D-α-(*N-tert*-butoxycarbonylamino)-α-(4-hydroxyphenyl)acetamido]-3-(1-propenyl)-3-cephem-4-carboxylate (**5d**)

To a solution of 101.7 g (0.1 mol) of **4b** in 2 liters of $CHCl_3$ was added a mixture of 1 liter of H_2O and 110 ml (0.11 mol) of 1 N NaOH and the mixture was shaken for 5 minutes. The organic layer was separated, washed with H_2O and subsequently with aq NaCl, and dried over anhydrous magnesium sulfate. The dried organic solution was filtered and concentrated to 500 ml under reduced pressure. The concentrate was cooled and mixed with 200 ml of 90% acetaldehyde under stirring. The mixture was stirred at room temp for 30 minutes and dried over anhydrous magnesium sulfate. The filtrate was chromatographed on a Silica gel column (Wako-gel C-200, 1 kg) by eluting with $CHCl_3$ and $CHCl_3$ - MeOH (99 : 1). The desired fractions were collected and evaporated to dryness. Since the residue still contained a small amount of triphenylphosphine oxide, it was re-chromatographed on a Silica gel column (Kieselgel 60, 300 g) by eluting with toluene - EtOAc (4 : 1). The eluate was monitored with TLC (silica gel, 50% toluene - EtOAc). The desired fractions were combined and evaporated to dryness. The oily residue was triturated with ether - isopropyl ether - *n*-hexane to give 20.5 g (31%) of **5d** melting at 120~130°C (dec): IR ν_{max}^{KBr} cm^{-1} 1780, 1710~1670, 1490, 1360, 1210, 1150; UV λ_{max}^{MeOH} nm (ε) 283 (8,300); ^1H NMR ($CDCl_3$) δ 1.3~1.5 (12H, m, $C(CH_3)_3$ and $C{=}CCH_3$), 3.22 (2H, br s, 2-H), 4.90 (1H, d, J=4.5 Hz, 6-H), 5.15 (1H, s, CHCO), 5.5~5.9 (2H, m, $CH{=}CHCH_3$ and 7-H), 6.07 (1H, d,

J=11 Hz, C*H*=CHCH$_3$), 6.63 (2H, d, J=9 Hz, phenyl-H), 6.91 (1H, s, C*H*-(phenyl)$_2$), 7.09 (2H, d, J=9 Hz, phenyl-H), 7.2~7.5 (10H, m, phenyl-H).

Anal Calcd for $C_{38}H_{37}N_2O_7S\cdot\frac{1}{2}H_2O$: C 65.04, H 5.76, N 6.32, S 4.82.
Found: C 65.10, H 5.73, N 6.13, S 5.23.

3-Alkenyl-7-arylglycylaminocephalosporins, **6** and **7** (Table 3)

General procedure accompanied with separation of the *Z* isomer (**6**) and the *E* isomer (**7**) regarding the 3-alkenyl side chain is illustrated by the preparation of **6d** and **7d**, and that accompanied with separation of the D isomer and L isomer regarding the 7-arylglycyl moiety by the preparation of **6d** and **6e** as shown below.

7-[D-α-Amino-α-(4-hydroxyphenyl)acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acid (**6d**, BMY-28100) and Its *E* Isomer (**7d**)

A mixture of 20 g (0.03 mol) of **5d** and 60 ml of TFA was stirred at room temp for 30 minutes and then diluted with 500 ml of ether and 500 ml of isopropyl ether. The precipitate separated was collected by filtration and washed with ether. To a solution of the precipitate in 50 ml of MeOH was added 90 ml of 1 M solution of sodium 2-ethylhexanoate (SEH) in EtOAc to afford a precipitate, which was collected by filtration, washed with EtOAc and ether, and dried *in vacuo* over P_2O_5 to give 11.9 g of crude **6d** containing some amount of **7d**. The crude **6d** was dissolved in 50 ml of 0.01 M phosphate buffer (pH 7.2) - methanol solution (85 : 15) and the solution was adjusted to pH 6 with 6 N HCl and chromatographed on a preparative HPLC (prepPAK-500/C$_{18}$, System 500, Waters). The column was eluted with phosphate buffer solution (0.01 M, pH 7.2) containing 15% MeOH and the eluate was monitored by analytical HPLC. The major component of the first 4 liters fraction was **6d** and that of the second 1 liter fraction was **7d**. The first fraction was concentrated to 2 liters. The concentrate was adjusted to pH 3 with dilute HCl and the solution was charged on a column containing Diaion HP-20 (1 liter). The column was washed with 6 liters of H_2O until the pH of the wash became 7 and then eluted with 4 liters of 30% aq MeOH. The eluate was monitored by HPLC and the desired fraction (*ca.* 2.5 liters) was concentrated to 50 ml below 40°C under reduced pressure, during which time crystalline precipitate separated out. The concentrate was kept to stand at 0°C for 2 hours and the precipitated crystals were collected by filtration, washed with 80% aq acetone and then with acetone and dried *in vacuo* over P_2O_5 to give 4.09 g of **6d** melting at 218~220°C (dec). The mother liquor was concentrated to 10 ml, treated with 20 ml of acetone and allowed to stand overnight in a refrigerator to afford crystalline precipitate, which was collected by filtration and dried *in vacuo* over P_2O_5 to give 670 mg of the second crop of **6d** as prisms. The total yield of **6d** was 4.76 g (40%): IR ν_{max}^{KBr} cm^{-1} 1750, 1680, 1560, 1520, 1460, 1390, 1350, 1270, 1235; UV λ_{max} (pH 7 phosphate buffer) nm (ε) 228 (12,300), 279 (9,800); ^{1}H NMR (D_2O+NaHCO$_3$) δ 1.71 (3H, d, J=6 Hz, CCH$_3$), 3.27 (1H, d, J=18 Hz, 2-H), 3.59 (1H, d, J=18 Hz, 2-H), 5.18 (1H, d, J=4.5 Hz, 6-H), 5.22 (1H, s, CHCO), 5.73 (1H, d, J=4.5 Hz, 7-H), 5.5~6.0 (1H, m, vinyl-H$_b$), 6.02 (1H, d, J=11 Hz, vinyl-H$_a$), 6.98 (2H, d, J=9 Hz, phenyl-H), 7.41 (2H, d, J=9 Hz, phenyl-H).

Anal Calcd for $C_{18}H_{19}N_3O_5S\cdot\frac{1}{2}H_2O$: C 54.26, H 5.06, N 10.55, S 8.05.
Found: C 54.17, H 5.11, N 10.36, S 8.21.

The second fraction from the preparative HPLC was concentrated to 500 ml. The concentrate was adjusted to pH 3 with dilute HCl and chromatographed on an Diaion HP-20 column (100 ml) by eluting with 1 liter each of H_2O and 30% MeOH. The latter eluate (about 300 ml) was concentrated to 10 ml and lyophilized to give 290 mg of the crude *trans* isomer **7d** (55% pure). This material was dissolved in 100 ml of 50% MeOH and treated with activated carbon. The filtrate was concentrated to a volume of 20 ml and allowed to stand overnight at 5°C. Compound **7d** crystallized as colorless prisms which were collected by filtration and dried *in vacuo*. Yield 290 mg (2%): MP 230°C (dec); IR ν_{max}^{KBr} cm^{-1} 1760, 1680, 1590, 1550, 1520, 1450, 1390, 1350, 1240; UV λ_{max} (pH 7 phosphate buffer) nm (ε) 228 (13,000), 292 (16,900); ^{1}H NMR (60 MHz, D_2O+Na_2CO_3) δ 1.89 (3H, d, J=6 Hz, C=CCH$_3$), 3.60 (2H, s, 2-H), 5.13 (1H, d, J=4.5 Hz, 6-H), 5.20 (1H, s, CHCO), 5.68 (1H, d, J=4.5 Hz, 7-H), 5.99 (1H, dq, J=16 and 6 Hz, vinyl-H$_b$), 6.54 (1H, d, J=16 Hz, vinyl-H$_a$), 6.98 (2H, d, J=9 Hz, phenyl-H), 7.41 (2H, d, J=9 Hz, phenyl-H).

7-[D- and L-α-Amino-α-(4-hydroxyphenyl)acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acids (**6d** and **6e**)

A mixture of 5 g (7.62 mmol) of **5e** and 20 ml of TFA was stirred at room temp for 30 minutes and the mixture was diluted with 100 ml of ether and 100 ml of isopropyl ether. The precipitate separated was collected by filtration. To the solution of the precipitate in 20 ml of MeOH was added 23 ml (23 mmol) of 1 M solution of SEH in EtOAc and the mixture was diluted with 300 ml of EtOAc to afford the precipitate, which was collected by filtration, washed with ether and dried *in vacuo* over P_2O_5 to give 2.88 g of crude Na salt of a mixture of **6d**, **6e**.

The above experiment was repeated and the combined crude Na salt (5 g) was dissolved in 50 ml of MeOH, and the solution was acidified with 10 ml of 1 N HCl and chromatographed on a reverse-phase column packed with 400 ml of the packing of prepPAK-C_{18} cartridge (Waters). The column was washed with H_2O and eluted with 10% MeOH. The eluate was collected under monitoring with HPLC (25% MeOH - pH 7 phosphate buffer). At first the eluate contained **6e** predominantly, then the content of **6d** increased to give **6d**-rich fractions and finally **6e** became a major component again. The **6e**-rich fractions (the first and third parts of the above eluate) were combined and concentrated to 300 ml. The concentrate was re-chromatographed on a column with the same packing by eluting with H_2O and 10% MeOH. The heart-cut fractions containing **6e** of 10% MeOH eluate were collected and concentrated to 10 ml and cooled. The resulting crystalline solid was collected by filtration, washed with cold water and acetone and dried *in vacuo* over P_2O_5 to give 250 mg of **6e**. The mother liquor and the side fractions containing **6e** were combined and the mixture was again chromatographed similarly to afford 113 mg of a second crop of **6e**. The total yield of **6e** was 363 mg (9%): MP 200°C (dec); IR ν_{max}^{KBr} cm^{-1} 1760, 1690, 1590, 1520, 1400, 1360, 1270; UV λ_{max} (pH 7 phosphate buffer) nm (ε) 229 (13,000), 279 (9,900); ^1H NMR (60 MHz, $D_2O+Na_2CO_3$) δ 1.71 (3H, d, J=6 Hz, =CHCH_3), 3.36 (1H, d, J=18 Hz, 2-H), 3.67 (1H, d, J=18 Hz, 2-H), 4.68 (1H, s, CHCO), 5.21 (1H, d, J=4.5 Hz, 6-H), 5.59 (1H, d, J=4.5 Hz, 7-H), 5.5~6.0 (1H, m, vinyl-H_b), 6.02 (1H, d, J=11 Hz, vinyl-H_a), 6.96 (2H, d, J=9 Hz, phenyl-H), 7.39 (2H, d, J=9 Hz, phenyl-H).

Similarly, **6d**-rich fractions (the second part of the eluate) were re-chromatographed by eluting with 10% MeOH. The desired fractions containing **6d** were collected and concentrated to 10 ml and cooled in a refrigerator. The resulting crystalline solid was collected by filtration to give 1 g (25%) of **6d**, which was identical with the product obtained from **5d**.

Isolation of **6l** from the Urine of Rats Fed **6k**

Six male Wistar rats (400~600 g) were placed in steel metabolic cages after the oral administration of **6k** at the dose of 100 mg/kg and urine was collected over a period of 24 hours. The rats were fed their regular diet and given water during the experiment.

The urine (*ca.* 90 ml) was adjusted to pH 3 with 1 N HCl and filtered to remove a precipitate. The filtrate was chromatographed on a column packed with 300 ml of Diaion HP-20 by eluting with 2 liters of H_2O and 2 liters of 30% MeOH under monitoring with HPLC. The fractions containing the bioactive components of the 30% MeOH eluate were collected, concentrated to 10 ml and lyophilized to give 390 mg of brown solid. A solution of the solid in 20 ml of H_2O was chromatographed on a column packed with 200 ml of the packing of a prepPAK-C_{18} cartridge (Waters) by eluting with H_2O, 5% MeOH, and 10% MeOH, successively. The first half of the 5% MeOH eluate was concentrated to 5 ml and lyophilized to give 44 mg of **6k** (70% pure) containing impurities derived from urine. The second half of the 5% MeOH eluate was concentrated to 5 ml and lyophilized to give 36 mg of product, which was a mixture of **6k**, **6l** and impurities derived from urine. The eluate with 10% MeOH (*ca.* 600 ml) was concentrated to 5 ml and lyophilized to give 38 mg of **6l** (70% pure by HPLC), which was re-chromatographed on a column of the same packing as above (40 ml) by eluting with H_2O, 5% MeOH and 10% MeOH. The desired fractions eluted with 10% MeOH were combined and concentrated to 5 ml and lyophilized to give 16 mg of powder which was identical with **6l** by comparison of IR, UV, ^1H NMR and HPLC.

Determinaton of MICs

MICs were determined on solid medium by the standard 2-fold agar dilution method in Mueller-

Hinton agar (Difco) or in nutrient agar (Eiken) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml.

Blood Level and Urinary Recovery in Mice

Five male *dd*Y-mice, weighing 18 to 22 g, were given an antibiotic solution by oral or intramuscular administration. Blood samples were collected from the orbital sinuses at 0.5, 1, 2, 3, 4, 5, 6 and 7 hours after oral administration or at 10, 20, 30, 40, 50, 60, 90 and 120 minutes after intramuscular administration and assayed by the paper disc-agar diffusion method using *Micrococcus luteus* PCI-1001 as an assay organism. The half life ($T_{1/2}$, hours) and area under the drug concentration-time curve (AUC, μg·hours/ml) were calculated by the method of LEITNER *et al.*[12]. Urine specimens were collected in four fractions (0 to 2, 2 to 4, 4 to 6 and 6 to 24 hours) after administration and assayed by the procedure same as that in the blood level experiment.

Protective Effect

Organisms were cultured overnight at 37°C in brain heart infusion broth and suspended in 5% hog mucin (American Laboratory, Omaha, Neb.). Male *dd*Y-mice were infected intraperitoneally with about 100 times of the median lethal dose of the pathogen. Five mice at each dose level were individually given an antibiotic solution orally or intramuscularly just before the bacterial challenge. The 50% protective dose (PD_{50}, mg/kg) was calculated by the method of LITCHFIELD and WILCOXON[13], from survival rate recorded on 7 days after the bacterial infection.

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