PANCLICINS, NOVEL PANCREATIC LIPASE INHIBITORS

II. STRUCTURAL ELUCIDATION

KIYOSHI YOSHINARI, MASAHIRO AOKI, TATSUO OHTSUKA, NOBORU NAKAYAMA, YOSHIKO ITEZONO, MASAE MUTOH, JUNKO WATANABE and KAZUTERU YOKOSE

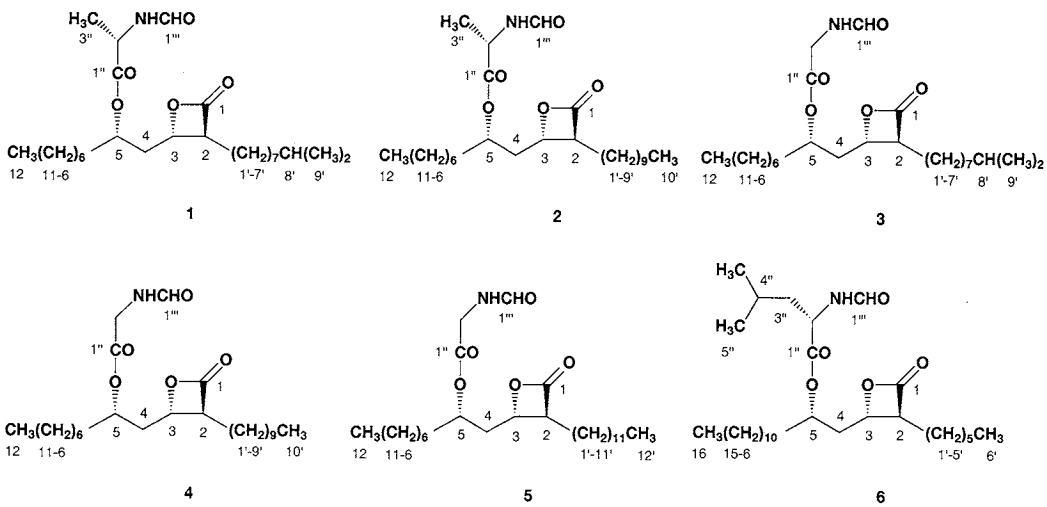
Nippon Roche Research Center, 200 Kajiwara, Kamakura 247, Japan

(Received for publication July 13, 1994)

Panclicins A~E are novel and potent pancreatic lipase inhibitors produced by *Streptomyces* sp. NR 0619. Their structures have been elucidated based on NMR and FAB-MS experiments. The relative configurations have also been determined by NMR experiments. The absolute stereochemistry has been determined by the chiral HPLC analysis of the hydrolysates of panclicins A and B and by modified Mosher's method on a derivative of panclicin A. They are structurally related to β-lactone esterase inhibitors of microbial origin, lipstatin, valilactone, ebelactones and esterastin. Panclicins also contain a β-lactone structure with two alkyl chains, one of which has an *N*-formylalanyloxy or *N*-formylglycyloxy substituent.

In our screening program for novel pancreatic lipase inhibitors, we discovered potent inhibitors, panclicins A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**), in the culture broth of *Streptomyces* sp. NR 0619. Their structures were determined as shown in Fig. 1 based on NMR and MS experiments, hydrolysis and modified Mosher's method on a degradation product of **1** and were related to β-lactone esterase inhibitors of microbial origin, lipstatin[1], valilactone[2], ebelactones[3] and esterastin[4]. In the preceding paper, the taxonomical study and the production, isolation and biological activity of panclicins have been reported[5]. In the present paper, we describe their physico-chemical properties and structural elucidation.

Fig. 1. Structures of panclicins A~E (**1**~**5**) and tetrahydrolipstatin (**6**).



Results

Physico-chemical Properties of Panclicins

The physico-chemical properties of the panclicins are summarized in Table 1. The molecular formulae of **1**, **2**, **3**, **4** and **5** were determined to be $C_{26}H_{47}NO_5$, $C_{26}H_{47}NO_5$, $C_{25}H_{45}NO_5$, $C_{25}H_{45}NO_5$ and $C_{27}H_{49}NO_5$, respectively, from their high resolution FAB-MS data. Their UV spectra showed no characteristic absorption bands. Their IR spectra, which were almost identical to one another, showed absorption bands at 1822, 1738 and 1692 cm^{-1}, suggesting the presence of β-lactone, ester and amide groups, respectively. These physico-chemical properties suggested that the panclicins were structurally quite similar to lipstatin and esterastin, which contained β-lactone, ester, amide and di-substituted olefinic groups. Since the olefinic proton signals, however, were not observed in the ^1H NMR spectra of the panclicins, obviously they were different from these β-lactone esterase inhibitors of microbial origin. The ^1H NMR spectral data for the panclicins shown in Table 2 were quite similar to one another and also similar to those of tetrahydrolipstatin (**6**)[1], a hydrogenated derivative of lipstatin, and valilactone, a microbial β-lactone esterase inhibitor having saturated alkyl chains. Although the ^{13}C NMR spectral data for **1**, **2** and **3** were also similar to those of **6** (Table 3), the panclicins were different from **6** and valilactone having a leucine or valine residue, because hydrolysis of the panclicins did not give leucine or valine but L-alanine or glycine (*vide infra*).

Structural Elucidation

Planar Structure of Panclicin A (**1**)

Homodecoupling experiments on **1** established proton connectivities in partial structures, A, C1′H_2 (δ_H 1.76)-C2H (δ_H 3.21)-C3H (δ_H 4.30)-C4H_2 (δ_H 2.00 and 2.16)-C5H (δ_H 5.08)-C6H_2 (δ_H 1.62), and B, C3″CH_3 (δ_H 1.46)-C2″H (δ_H 4.65)-NH (δ_H 6.16)-C1‴H (δ_H 8.19). The proton 1‴-H was assigned to the NH–CHO group because it was coupled with the NH and its chemical shift δ_H 8.19 was almost the same as that of the formyl proton in **6**. Since the chemical shifts of protons and carbons in the partial structure A were essentially identical to those of corresponding protons and carbons in **6**, the positions 2 and 3 were determined to be α- and β-positions of the β-lactone, suggested from the IR absorption band at 1822 cm^{-1}, respectively. A carbon signal at δ_C 170.70 was assigned to the ester carbonyl carbon (C-1) of the β-lactone because the chemical shift was almost the same as that of the corresponding carbon in **6** (δ_C

Table 1. Physico-chemical properties of panclicins A~E (**1**~**5**).

	1	**2**	**3**	**4**	**5**
Appearence	Colorless powder	Coloress powder	Colorless oil	Colorless oil	Colorless powder
Molecular formula	$C_{26}H_{47}NO_5$	$C_{26}H_{47}NO_5$	$C_{25}H_{45}NO_5$	$C_{25}H_{45}NO_5$	$C_{27}H_{49}NO_5$
HRFAB-MS	454.3500 $(M+H)^+$ Calcd: 454.3532	454.3513 $(M+H)^+$ Calcd: 454.3532	440.3359 $(M+H)^+$ Calcd: 440.3376	440.3359 $(M+H)^+$ Calcd: 440.3376	468.3663 $(M+H)^+$ Calcd: 468.3689
UV λ_{max}^{MeOH} nm	End	End	End	End	End
IR ν_{max} ($CHCl_3$) cm^{-1}	3450, 1822, 1742, 1692	3450, 1822, 1742, 1692	3450, 1822, 1742, 1692	3450, 1822, 1742, 1692	3450, 1822, 1742, 1692
$[\alpha]_D^{25}$	−26° (*c* 1.27, $CHCl_3$)	−28° (*c* 0.94, $CHCl_3$)	−20° (*c* 0.33, $CHCl_3$)	−23° (*c* 0.30, $CHCl_3$)	−27° (*c* 1.21, $CHCl_3$)
Solubility					
Soluble:	MeOH, $CHCl_3$	MeOH, $CHCl_3$	MeOH, $CHCl_3$	MeOH, $CHCl_3$	MeOH, $CHCl_3$
Insoluble:	H_2O	H_2O	H_2O	H_2O	H_2O

Table 2. ^{1}H NMR spectral data for panclicins A~E (**1**~**5**) and tetrahydrolipstatin (**6**) in $CDCl_3$.

Position	**1**	**2**	**3**	**4**	**5**	**6**
	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)	δ_H (*J*/Hz)
2	3.21 (dt, *J*=5, 8)	3.21 (dt, *J*=5, 8)	3.21 (dt, *J*=5, 8)	3.21 (dt, *J*=5, 8)	3.21 (dt, *J*=5, 8)	3.22 (dt, *J*=5, 8)
3	4.30 (dt, *J*=9, 5)	4.31 (dt, *J*=9, 5)	4.33 (dt, *J*=9, 5)	4.33 (dt, *J*=9, 5)	4.33 (dt, *J*=9, 5)	4.29 (dt, *J*=9, 5)
4a	2.00 (dt, *J*=15, 5)	2.01 (dt, *J*=15, 5)	2.02 (dt, *J*=15, 5)	2.02 (dt, *J*=15, 5)	2.02 (dt, *J*=15, 5)	2.00 (dt, *J*=15, 5)
4b	2.16 (dt, *J*=15, 9)	2.16 (dt, *J*=15, 9)	2.13 (dt, *J*=15, 9)	2.14 (dt, *J*=15, 9)	2.14 (dt, *J*=15, 9)	2.17 (dt, *J*=15, 9)
5	5.08 (ddt, *J*=9, 5, 7)	5.08 (ddt, *J*=9, 5, 7)	5.13 (ddt, *J*=9, 5, 7)	5.13 (ddt, *J*=9, 5, 7)	5.13 (ddt, *J*=9, 5, 7)	5.03 (ddt, *J*=9, 5, 7)
6	1.62 (m)	1.62 (m)	1.62 (m)	1.60 (m)	1.60 (m)	1.63 (m)
7	1.28~1.35 (m)	1.25~1.35 (m)	1.28~1.35 (m)	1.23~1.35 (m)	1.23~1.35 (m)	1.25~1.35 (m)
8	(7-H~11-H)	(7-H~11-H)	(7-H~11-H)	(7-H~11-H)	(7-H~11-H)	(7-H~15-H)
9						
10						
11						
12	0.88 (t, *J*=6.5)	0.88 (t, *J*=6.5)	0.88 (t, *J*=6.5)	0.88 (t, *J*=6.5)	0.88 (t, *J*=6.5)	
13	—	—	—	—	—	
14	—	—	—	—	—	
15	—	—	—	—	—	
16	—	—	—	—	—	0.88[a] (t, *J*=6.6)
1′	1.76 (m)	1.76 (m)	1.76 (m)	1.76 (m)	1.76 (m)	1.76 (m)
2′	1.28~1.35 (m)	1.28~1.35 (m)	1.28~1.35 (m)	1.28~1.35 (m)	1.28~1.35 (m)	1.28~1.35 (m)
3′	(2′-H~6′-H)	(2′-H~9′-H)	(2′-H~6′-H)	(2′-H~9′-H)	(2′-H~11′-H)	(2′-H~5′-H)
4′						
5′						
6′						0.88[a] (t, *J*=6.5)
7′	1.15 (br q, *J*=7)		1.15 (br q, *J*=7)			—
8′	1.51 (m)		1.51 (m)			—
9′	0.86 (d, *J*=6.5)		0.86 (d, *J*=6.5)			—
10′	—	0.88 (t, *J*=6.5)	—	0.88 (t, *J*=6.5)		—
11′	—	—	—	—		—
12′	—	—	—	—	0.88 (t, *J*=6.5)	—
2″	4.65 (dq, *J*=7, 7)	4.65 (dq, *J*=7, 7)	4.03 (dd, *J*=18, 5.5)	4.04 (dd, *J*=18, 5.5)	4.03 (dd, *J*=18, 5.5)	4.69 (dt, *J*=9, 5)
			4.13 (dd, *J*=18, 5.5)	4.13 (dd, *J*=18, 5.5)	4.13 (dd, *J*=18, 5.5)	
3″	1.46 (d, *J*=7)	1.46 (d, *J*=7)	—	—	—	1.25~1.35 (m)
4″	—	—	—	—	—	(3′-H and 4′-H)
5″	—	—	—	—	—	0.97 (d, *J*=6.5)
						0.97 (d, *J*=6.5)
1‴	8.19 (br s)	8.19 (br s)	8.26 (br s)	8.25 (br s)	8.26 (br s)	8.22 (br s)
NH	6.16 (br d, *J*=7)	6.19 (br d, *J*=7)	6.09 (br s)	6.08 (br s)	6.14 (br s)	5.90 (br d, *J*=9)

[a] Interchangeable.

Table 3. ^{13}C NMR spectral data for panclicins A (**1**), B (**2**), C (**3**) and tetrahydrolipstatin (**6**).

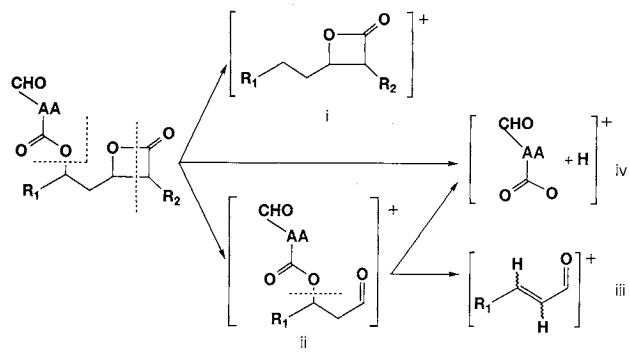
Position	1[a]	2[a]	3[a]	Position	6[b]
	δ_C	δ_C	δ_C		δ_C
1	170.70	170.17	169.18	1	170.73
2	57.10	57.09	57.09	2	57.07
3	74.98	75.00	74.97	3	74.74
4	39.00	38.92	38.98	4	38.73
5	73.04	73.04	73.04	5	72.63
6	34.20	34.20	34.09	6	34.07
12	14.06	14.06[c]	14.01	16	14.00[d]
1′	27.64	27.63	27.61	1′	27.67
7′	38.93	29.51	38.92		
8′	27.96	31.89	27.94		
9′	22.65×2	22.68	22.57×2		
10′		14.10[c]		6′	14.10[d]
1″	172.12	172.13	170.67	1″	171.93
2″	47.10	47.10	40.12	2″	49.76
3″	18.40	18.38		3″	41.44
				4″	24.93
				5″	21.78, 22.87
1‴	160.35	160.38	160.82	1‴	160.83
7～11 and 2′～6′	25.11, 26.79, 27.32×2, 29.07, 29.24, 29.31, 29.75, 31.71×2	22.60, 25.11, 26.79, 29.07, 29.24, 29.30, 29.55, 29.60, 29.65, 31.72	25.13, 26.77, 27.29×2, 29.03, 29.26, 29.29, 29.73, 31.68×2	7～15 and 2′～5′	22.53, 22.69, 25.12, 26.73, 28.99, 29.35×2, 29.46, 29.57, 29.63×2, 31.51, 31.93

[a] Assignments were established by DEPT and HMQC experiments.
[b] See ref 1.
[c,d] Interchangeable.

170.73). ^{13}C-^{1}H long range couplings between C-1 and 2-H and between C-1 and 1′-H obtained by HMBC experiments confirmed the assignment. The partial structure B was assigned to an *N*-formylalanine residue because L-alanine was obtained by acid hydrolysis of **1** (*vide infra*). The carbon signal at δ_C 172.12 was assigned to the carbonyl carbon (C-1″) in the alanine residue because of ^{13}C-^{1}H long range couplings between this carbon and 2″-H and between this carbon and 3″-H. The partial structure B was linked to C-5 in the partial structure A through an ester bond because of a ^{13}C-^{1}H long range coupling between C-1″ and 5-H.

Intense signals at δ_H 1.28～1.35 (20H) suggested the presence of long alkyl chains. A triplet methyl signal (δ_H 0.88, 3H, t, J=6.5 Hz) suggested one of them was an *n*-alkyl group and two degenerated doublet methyl signals (δ_H 0.86, 6H, d, J=6.5 Hz) suggested the terminal of one of them had an isopropyl structure. The isopropyl group was located at the terminal of the alkyl chain attached to C-2 by HOHAHA experiments on **1**, since a relayed correlation was observed from 2-H to the doublet methyl protons (9-H′), whereas only a very weak correlation was observed from 2-H to 5-H and no correlation was observed from 2-H to the triplet methyl protons 12-H. On the other hand, the *n*-alkyl chain was attached to C-5 because of a relayed correlation from 5-H to the triplet methyl signal (12-H) through 6-H (δ_H 1.62) and the intense

Fig. 2. FAB-MS fragmentation of panclicins A~E (**1**~**5**) and tetrahydrolipstatin (**6**).



	$[M+H]^+$	i	ii	iii	iv	AA	R_1	R_2
1	454	337	272	155	118	Ala	C_7H_{15}	$C_{10}H_{21}$
2	454	337	272	155	118	Ala	C_7H_{15}	$C_{10}H_{21}$
3	440	337	258	155	104	Gly	C_7H_{15}	$C_{10}H_{21}$
4	440	337	258	155	104	Gly	C_7H_{15}	$C_{10}H_{21}$
5	468	365	258	155	104	Gly	C_7H_{15}	$C_{12}H_{25}$
6	496	337	370	211	160	Leu	$C_{11}H_{23}$	C_6H_{13}

signals at δ_H 1.28~1.35.

Although the length of each alkyl chain was not clarified from the NMR experiments, this information was revealed by FAB-MS experiments (Fig. 2). The comparison of the FAB-MS spectrum of **1** with that of **6** yielded quite useful information on the alkyl chains as well as on the amino acid structure. Panclicin A gave characteristic fragment ions at *m/z* 337, 272, 155 and 118, which were assigned to structures i, ii, iii and iv, respectively, by comparing the values with those of the corresponding fragment ions of **6** at *m/z* 337, 370, 211 and 160. The fragmentation pathways were established as shown in Fig. 2 by collisionally activated dissociation experiments on **1** and **6**. The collision of the $(M+H)^+$ ion gave the product ions i, ii and iv, and the collision of the fragment ion ii gave the product ions iii and iv. The fragment ion at *m/z* 118 assigned to the fragment ion iv confirmed the presence of the *N*-formylalanine residue in **1**. The fragment ion at *m/z* 272.1829 (Calcd 272.1862 for $C_{14}H_{26}NO_4$) assigned to the fragment ion ii established that R_1 was C_7H_{15}. Taking the molecular formula of **1**, $C_{26}H_{47}NO_5$, into consideration, this fragment ion also indicated that R_2 was $C_{10}H_{21}$. The fragment ions at *m/z* 337 and 155 assigned to the fragment ions i and iii confirmed the length of each alkyl chain R_1 and R_2. The planar structure of **1** was, thus, established as shown in Fig. 1.

Stereochemistry of Panclicin A (1)

The stereochemistry of the β-lactone moiety, C-2 and C-3, was determined to be *trans* by difference NOE experiments on **1**, in which NOEs were observed between 2-H and 4-H and between 1′-H (δ_H 1.75) and 3-H. The relation of the positions 3 and 5 was clarified to be *syn* (3*S**,5*S**) by comparing the coupling patterns of 4-Ha and 4-Hb with the corresponding protons' coupling patterns of **6** (2*S*,3*S*,5*S*) and its 5*R* isomer **7**† (2*S*,3*S*,5*R*), which were synthesized stereospecifically[6] (Fig. 3). Obviously the coupling patterns

† The ^1H NMR spectra of **6** and **7** were sent by Dr. W. VETTER and Prof. A. FISCHLI of F. Hoffmann-La Roche Research Center.

Fig. 3. 400 MHz ^{1}H NMR coupling patterns of 4-Ha and 4-Hb of panclicin A (**1**) and tetrahydrolipstatin (**6**) (2*S*, 3*S*, 5*S*) and its 5*R* isomer **7** (2*S*, 3*S*, 5*R*).

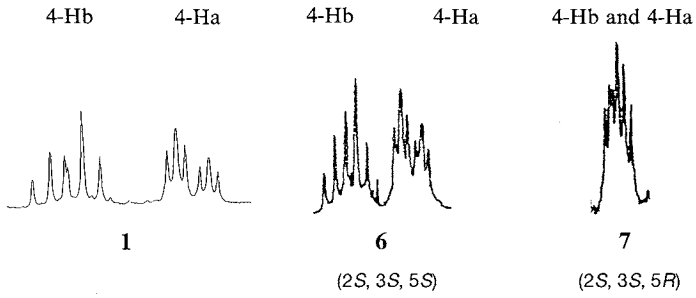
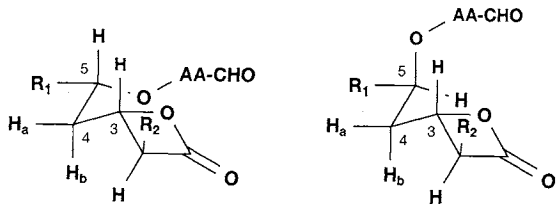


Fig. 4. Stable conformations provided by COSMIC force energy calculation using MEMESIS[7,8]. A: panclicin A (**1**) and tetrahydrolipstatin (**6**), B: 5*R* isomer of **1** and **7** (5*R* isomer of **6**).



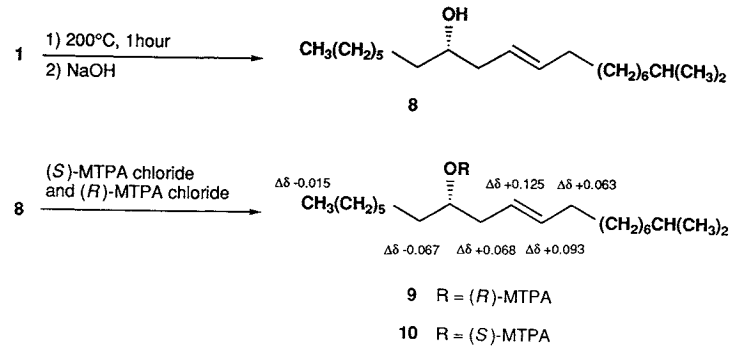
A: **1** and **6** (2*S*, 3*S*, 5*S*) B: 5*R* isomer of **1** and **7** (2*S*, 3*S*, 5*R*)

AA-CHO: *N*-formylalanine (**1** and its 5*R* isomer) or *N*-formylleucine (**6** and **7**), R_1: $(CH_2)_6CH_3$ (**1** and its 5*R* isomer) or $(CH_2)_{10}CH_3$ (**6** and **7**), R_2: $(CH_2)_7CH(CH_3)_2$ (**1** and its 5*R* isomer) or $(CH_2)_5CH_3$ (**6** and **7**).

of 4-Ha and 4-Hb of **1** were not similar to those of **7** but those of **6**. The large coupling constants, $J_{3\text{-H}-4\text{-Hb}}=J_{4\text{-Hb}-5\text{-H}}=9$ Hz, indicated antiperiplanar relations between 3-H and 4-Hb and between 4-Hb and 5-H, which were consistent with the stable conformation provided by COSMIC force energy calculation on **1** (2*S**,3*S**,5*S**) using NEMESIS[7,8] (Fig. 4). On the other hand, the stable conformation for the 5*R* isomer (2*S**,3*S**,5*R**) of **1** obtained by the calculation had a synclinal relation between 4-Hb and H-5.

The absolute configuration of the alanine residue was determined to be L by the chiral HPLC analysis[9] of the acid hydrolysate of **1**. D-Alanine and L-alanine were separated over a reversed phase C_{18} silica gel column (Capcell pak C_{18}, 4.6×250 mm; Shiseido Co. Ltd.) developed with a solution of 2 mM *N*,*N*-dipropylalanine and 1 mM cupric acetate at a flow rate of 1 ml/minute. D-Alanine and L-alanine had retention times of 5.1 and 7.2 minutes, respectively. Hydrolysis of **1** gave the latter peak. The absolute configuration of the position 5 was determined by modified Mosher's method on a degradation product **8**, which was prepared from **1** by decarboxylation followed by alkaline hydrolysis (Fig. 5). The alcohol **8** was separately treated with (*S*)- and (*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) chlorides to yield (*R*)- and (*S*)-MTPA esters, **9** and **10**, respectively. Detailed analysis of the ^{1}H NMR spectral data of the MTPA esters revealed significant and systematic differences between the proton chemical shifts of the MTPA esters (Fig. 5). When the molecular models of **9** and **10** with 5*S* configuration were constructed, the $\Delta\delta$ ($\delta_{10}\sim\delta_9$) values for the protons oriented toward the left side of the MTPA plane were negative, while those oriented toward the right side of the plane were positive. These data indicated the

Fig. 5. Conversion of panclicin A (**1**) to MTPA esters **9** and **10** and their chemicalshift differences $\Delta\delta$ ($\delta_{10}-\delta_9$) ppm.



S configuration at the position 5 according to modified Mosher's method. Therefore, the absolute configurations of **1** were determined to be 2*S*, 3*S*, 5*S* and 2'*S*, which are the same as those of **6**.

Structures of Panclicins B (**2**), C (**3**), D (**4**) and E (**5**)

Since the ^{1}H NMR spectral data for **2**, **3**, **4** and **5** were almost identical to those of **1**, except for the proton signals assignable to the amino acid residues and the terminal methyl groups of the alkyl chains, their structures were determined by comparing their MS and ^{1}H NMR spectral data with those of **1**. The amino acid residue of **2** was suggested to be alanine from its proton signals at δ_H 4.65 (1H, dq, J=7, 7 Hz) and 1.46 (3H, t, J=7 Hz), and it was established to be L-alanine by the HPLC analysis of the hydrolysate of **2**. The amino acid contained in **3**, **4** and **5** was suggested to be glycine from doublet of doublets' signals at δ_H *ca.* 4.0 (1H, dd, J=5.5, 18 Hz) and *ca.* 4.1 (1H, dd, J=5.5, 18 Hz). The fragment ion at *m/z* 104 in the FAB-MS spectra of **3**, **4** and **5** also indicated the presence of *N*-formylglycine. The presence of the glycine residues in **3** and **5** was confirmed by the HPLC analysis of their hydrolysates under the same conditions as those for alanine.

Because the ^{1}H NMR spectrum of **2** showed two triplet methyl signals at δ 0.88 (6H, t, J=6.6 Hz) but did not show any doublet methyl signal other than that of the alanine residue, both alkyl chains were determined not to be branched. Both of the alkyl chains in **4** and **5** were also determined not to be branched by the analysis of their ^{1}H NMR spectra. On the other hand, one of the alkyl chains in **3** was determined to have an isopropyl terminal, since a triplet methyl signal (δ_H 0.88, 3H, t, J=6.5 Hz) and two degenerated doublet methyl signals (δ_H 0.86, 6H, d, J=6.5 Hz) were observed in the ^{1}H NMR spectrum. The isopropyl group was located at the terminal of the alkyl chain attached to C-2 by HOHAHA experiments on **3**.

The lengths of each alkyl chain R_1 and R_2 in **2**, **3**, **4** and **5** were also determined as shown in Fig. 2 by the analysis of their FAB-MS data. The relative configurations at positions 2, 3 and 5 of **2**, **3**, **4** and **5** were suggested to be the same as those of **1**, because the ^{1}H coupling patterns of 2-H, 3-H, 4-H and 5-H were identical with those of **1**. The optical rotations of these compounds, which were similar to **1**, suggested that these compounds have the same absolute configurations as those of **1**. Their structures were, thus, established as shown in Fig. 1.

Discussion

We determined the structures of novel and potent pancreatic lipase inhibitors, **1**~**5**, whose biological

activities were reported in the preceding paper[5]. An *N*-formylalanine residue is contained in **1** and **2**, whereas an *N*-formylglycine residue is contained in **3**~**5** instead. The *N*-formylglycine residue was thought to be associated with more effective inhibitory activity than the *N*-formylalanine residue, because **3** ($IC_{50}=0.62\,\mu M$), **4** ($IC_{50}=0.66\,\mu M$) and **5** ($IC_{50}=0.89\,\mu M$) were three or four times more potent inhibitors than **1** ($IC_{50}=2.9\,\mu M$) and **2** ($IC_{50}=2.6\,\mu M$) . In contrast, the isopropyl group of the alkyl chain in **1** and **3** did not affect the activity.

The structures of the panclicins are related to those of the β-lactone esterase inhibitors of microbial origin, lipstatin, valilactone, ebelactones and esterastin. Among these known inhibitors, only valilactone, produced by *Streptomyces albolongus*, has saturated alkyl chains but no unsaturated alkyl chains. The length of each alkyl chain in valilactone, however, is shorter than those of the panclicins, and it has an *N*-formylvaline residue in place of an *N*-formylalanine or *N*-formylglycine residue. Since lipstatin, a potent pancreatic lipase inhibitor produced by *Streptomyces toxytricini*, was unstable due to its olefinic functionality, its hydrogenated derivative **6** was synthesized to overcome the drawback. Tetrahydrolipstatin (**6**) was not more unstable, and it also showed potent inhibitory activity against pancreatic lipase. We isolated stable and potent pancreatic lipase inhibitors having no olefinic functionality from a microbial broth without chemical modification. The inhibitory activities of **3**, **4** and **5** were two times more potent than that of **6** ($IC_{50}=1.2\,\mu M$), which reduces dietary triglyceride adsorption[1] and is being clinically investigated as the agents for obesity. Therefore, the panclicins, especially **3**, **4** and **5** are also expected to be useful as antiobesic agents.

Experimental

General Procedures

UV and IR spectra were recorded on a Kontron Uvicon 860 UV spectrometer and on a Hitachi 270-30 infrared spectrometer, respectively. Fast atom bombardment mass spectra (FAB-MS) were obtained with JEOL-DX-300 and JEOL-HX-110 spectrometers using *m*-nitrobenzyl alcohol as a matrix. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded on a JEOL-GSX-400 NMR spectrometer with TMS as an internal standard.

Preparation of **8**

Panclicin A (7 mg) was heated at 200°C for 1 hour under Ar. The reaction product was dissolved in 1 N NaOH (0.25 ml) and stirred at room temperature for 19 hours. The solution was diluted with water (4 ml) and extracted with dichloromethane (4 ml). The extract was washed with H_2O and dried over anhydrous Na_2SO_4. The extract was evaporated under reduced pressure, and the residue was separated by preparative TLC (Rf 0.38) (Merck, Kieselgel $60F_{254}$) developed with hexane-EtOAc (5:1) to give **8** (1.7 mg). HREI-MS *m/z* calcd for $C_{21}H_{40}$: 292.3130, found: 292.3128 $(M-H_2O)^+$; ^1H NMR (400 MHz, CD_3COCD_3) δ 0.86 (6H, d, $J=5$ Hz, $CH(CH_3)_2$), 0.88 (3H, t, $J=5$ Hz, CH_3), 1.05~1.62 (23H, br), 1.99~2.27 (6H, m, $CH_2CH(OH)$ and $CH_2CH{=}CHCH_2$), 3.27 (1H, d, $J=4$ Hz, CHOH), 3.53 (1H, m, CHOH), 5.47 (2H, m, CH=CH).

Preparation of **9**

To a solution of **8** (0.75 mg) in pyridine (200 μl) was added (*S*)-MTPA chloride (2 μl), and the mixture was allowed to stand at room temperature for 19 hours under Ar. The solution was diluted with water and extracted with diethyl ether. The ether layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, and the residue was separated by preparative TLC (Rf 0.65) developed with hexane-EtOAc (5:1) to give **9** (0.2 mg). FAB-MS *m/z* 549 $(M+Na)^+$, 531 $(M+Na-H_2O)^+$; ^1H NMR (400 MHz, $CDCl_3$); δ 0.860 (6H, d, $J=7$ Hz, $CH(CH_3)_2$), 0.880 (3H, t, $J=7$ Hz, CH_3), 1.100~1.600 (23H, br), 1.620 (2H, m, CH_2CH(OMTPA)), 1.910 (2H, br q, $J=7$ Hz, $=CHCH_2CH_2$), 2.275 (2H, br t, $J=7$ Hz, CH(OMTPA)$CH_2CH=$), 3.560 (3H, br s, OMe), 5.080 (1H, quintet, $J=7$ Hz, CH(OMTPA)), 5.210 (1H, br dt, $J=15$ Hz, 7 Hz, CH(OMTPA)$CH_2CH=$), 5.420 (1H, br dt, $J=15$ Hz, 7 Hz, $=CHCH_2CH_2$), 7.400 (3H, m, Ph) and 7.550 (2H, m, Ph).

Preparation of **10**

To a solution of **8** (0.75 mg) in pyridine (200 μl) was added (*R*)-MTPA chloride (2 μl), and the mixture was treated in the same manner as for the preparation of **9** to give **10** (0.5 mg). FAB-MS *m/z* 549 $(M+Na)^+$, 531 $(M+Na-H_2O)^+$; ^1H NMR (400 MHz, $CDCl_3$); δ 0.860 (6H, d, $J=7$ Hz, $CH(CH_3)_2$), 0.865 (3H, t, $J=7$ Hz, CH_3), 1.100~1.550 (23H, br), 1.553 (2H, m, CH_2CH(OMTPA)), 1.973 (2H, br q, $J=7$ Hz, $=CHCH_2CH_2$), 2.343 (2H, br t, $J=7$ Hz, CH(OMTPA)$CH_2CH=$), 3.560 (3H, br s, OMe), 5.095 (1H, quintet, $J=7$ Hz, *CH*(OMTPA)), 5.335 (1H, br dt, $J=15$ Hz, 7 Hz, CH(OMTPA)$CH_2CH=$), 5.513 (1H, br dt, $J=15$ Hz, 7 Hz, $=CHCH_2CH_2$), 7.400 (3H, m, Ph) and 7.550 (2H, m, Ph).

Acknowledgments

We thank Dr. W. VETTER and Prof. A. FISCHLI of F. Hoffmann-La Roche Research Center for kindly sending us the NMR spectra of **6** and its isomer **7**. We also thank Prof. H. SETO and Dr. H. MORISAKI of the Institute of Molecular and Cellular Biosciences, The University of Tokyo, for useful suggestions on the collisionally activated dissociation experiments.

References

1) WEIBEL, E. K.; P. HADVARY, E. HOCHULI, E. KUPFER & H. LENGSFELD: Lipstatin, an inhibitor of pancreatic lipase, produced by *Streptomyces toxytricini*. I. Producing organism, fermentation, isolation and biological activity. J. Antibiotics 40: 1081~1085, 1987
2) KITAHARA, M.; M. ASANO, H. NAGANAWA, K. MAEDA, M. HAMADA, T. AOYAGI, H. UMEZAWA, Y. IITAKA & H. NAKAMURA: Valilactone, an inhibitor of esterase, produced by *Actinomycetes*. J. Antibiotics 35: 1647~1650, 1987
3) UMEZAWA, H.; T. AOYAGI, K. UOTANI, M. HAMADA, T. TAKEUCHI & S. TAKAHASHI: Ebelactone, an inhibitor of esterase, produced by *Actinomycetes*. J. Antibiotics 33: 1594~1596, 1980
4) UMEZAWA, H.; T. AOYAGI, T. HAZATO, K. UOTANI, F. KOJIMA, M. HAMADA & T. TAKEUCHI: Esterastin, an inhibitor of esterase, produced by *Actinomycetes*. J. Antibiotics 31: 639~641, 1978
5) MUTOH, M.; N. NAKADA, S. MATSUKUMA, S. OHSHIMA, J. WATANABE, K. YOSHINARI & M. ARISAWA: Panclicins, novel pancreatic lipase inhibitors. I. Taxonomy, fermentation, isolation and biological activity. J. Antibiotics 47: 1369~1375, 1994
6) BARBIER, P. & F. SCHNEIDER: Syntheses of tetrahydrolipstatin and absolute configuration of tetrahydrolipstatin and lipstatin. Helv. Chim. Acta. 70: 196~202, 1987
7) MORLEY, S. D.; R. J. ABRAHAM, I. S. HAWORTH, D. E. JACKSON, M. R. SAUNDERS & J. G. VINTER: COSMIC (90): An improved molecular mechanics treatment of hydrocarbon and conjugated systems. J. Compt. Aided Mol. Design 5: 475~504, 1991
8) TADAMICHI, S.; K. KOBAYASHI, M. UBUKATA & H. OSADA: Absolute configuration of Epiderstatin, a new glutarimide antibiotic produced by *Streptomyces pulveraceus*. J. Antibiotics 45: 1963~1965, 1992
9) WEINSTEIN, S.; M. H. ENGEL & P. E. HARE: The enantiomeric analysis of a mixture of all common protein amino acids by high-performance liquid chromatography using a new chiral mobile phase. Anal. Biochem. 121: 370~377, 1982