New Glycosidases Inhibitors, Panosialins D and wD Produced by *Streptomyces* sp. OH-5186

HARUKI YAMADA, KAZURO SHIOMI, QI XU, TAKAYUKI NAGAI, MAKI SHIBATA, IZUMI OYA, YŌKO TAKAHASHI and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

(Received for publication September 1, 1994)

New panosialin analog, panosialins D and wD have been isolated from the culture broth of *Streptomyces* sp. OH-5186. Their structures were elucidated as 5-(13-methylpentadecyl)-1,3-benzenediol bis(sodium sulfate) and 5-(13-methylpentadecyl)-1,3-benzenediol 1-(sodium sulfate), respectively. They showed strong inhibitory activity against α-mannosidase, α-glucosidase, and β-glucosidase. Panosialins wA～wD mixture also showed weak mitogenic activity but suppressed the mitogen induced activity.

It has been reported that carbohydrate chains of glycoproteins on the cell surface are involved in various biological reactions, such as immune responses, metastasis of cancer cells, and viral infections.[1] Therefore processing glycosidase inhibitors have a possibility to regulate these reactions.

In the course of screening for new glycosidase inhibitors from actinomycetes, new panosialin analogs, which we named panosialin D and wD (**4** and **8**, Fig. 1), have been isolated together with known panosialins (**1**～**3** and **5**～**7**, Fig. 1).[2,3] They were produced in the culture broth of *Streptomyces* sp. OH-5186, which was isolated from a desert soil sample.

Panosialins were reported as the inhibitors of viral sialidase, acid phosphatase, and polygalacturonase.[2,3] In the report, they were obtained as a mixture (**1**～**3**) and its monodesulfate (**5**～**7**) that was derived from **1**～**3** chemically. We isolated all of them and renamed them as panosialins A～C (**1**～**3**) and panosialins wA～wC (**5**～**7**, previous name was panosialin-w). Recently, inhibitory activity against HIV protease of purified **1** has been reported.[4]

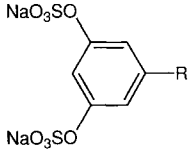
Here, we report the production, isolation, physico-chemical properties, structure elucidation, and biological activities of panosialins. As panosialin-w was chemically derived from the panosialin mixture and there was no description about the preparation and physico-chemical properties of panosialin-w,[2] we also report about panosialins wA～wD in detail. The taxonomy of the producing strain will be reported elsewhere.

Materials and Methods

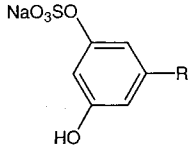
Materials

p-Nitrophenyl-α-D-mannopyranoside (PNP-α-Man), PNP-β-D-mannopyranoside, PNP-α-D-glucopyranoside, PNP-β-D-glucopyranoside, and PNP-β-D-galactopyranoside were purchased from Sigma, and sodium PNP-*N*-acetyl-α-D-neuraminate was from Wako Pure Chemical Industries (Osaka). Jack bean α-mannosidase, *Achatina fulica* β-mannosidase, and jack bean β-galactosidase were purchased from Seikagaku Corporation (Tokyo). Brewers yeast α-glucosidase and almond β-glucosidase were purchased from Sigma. *Arthrobacter*

Fig. 1. Structures of panosialins A～D (**1**～**4**) and wA～wD (**5**～**8**).



	R
Panosialin A (**1**)	$(CH_2)_{12}-CH(CH_3)_2$
Panosialin B (**2**)	$(CH_2)_{14}-CH_3$
Panosialin C (**3**)	$(CH_2)_{13}-CH(CH_3)_2$
Panosialin D (**4**)	$(CH_2)_{12}-CHCH_3$ (branch: CH_2CH_3)



	R
Panosialin wA (**5**)	$(CH_2)_{12}-CH(CH_3)_2$
Panosialin wB (**6**)	$(CH_2)_{14}-CH_3$
Panosialin wC (**7**)	$(CH_2)_{13}-CH(CH_3)_2$
Panosialin wD (**8**)	$(CH_2)_{12}-CHCH_3$ (branch: CH_2CH_3)

ureafaciens sialidase was obtained from Nacalai Tesque (Kyoto). Influenza virus A/PR/8/34 was grown in allantoic sacs of 10-day-old embryonated eggs for 48 hours at 34°C. The allantoic fluid was harvested and clarified at $1,000 \times g$ for 20 minutes, and then the resulting supernatant was centrifuged at $80,000 \times g$ for 45 minutes. The pellet was suspended in phosphate-buffered saline (PBS), pH 7.4, and the suspension was used as influenza virus sialidase. LPS (O127:B8) from *Escherichia coli* was obtained from Sigma and purified by hot phenol method. Concanavalin-A (ConA) and phytohemagglutinin (PHA) were purchased from Sigma.

Glycosidase Assay

Compounds **1**~**4** were dissolved in water. Compounds **5**~**8** were dissolved in methanol. Jack bean α-mannosidase activity was assayed in 0.11 ml of 25 mM sodium citrate buffer, pH 4.5, containing 1.25 munit of the enzyme and 1 mM PNP-α-Man in the presence or absence of various concentrations of samples. The reaction mixture was incubated at 37°C for 15 minutes in 96 wells microtiter plate. Then the reaction was terminated with the addition of 0.19 ml of 0.2 M sodium borate buffer, pH 9.8. The PNP liberated was determined from the absorbance at 405 nm with a Microplate Reader Model 450 (Bio-Rad). Other glycosidase activities were assayed with the appropriate PNP-glycoside substrates at optimum pH in a similar manner as jack bean α-mannosidase.

Anti-complementary Activity

The anti-complementary activity was measured as described previously.[5] The mixture of **1**~**4** in water (50 μl) was mixed with 50 μl each of normal human serum (NHS) and gelatin-veronal-buffered saline, pH 7.4, containing 500 μM $MgCl_2$ and 150 μM $CaCl_2$ (GVB^{2+}). The mixtures were preincubated for 30 minutes at 37°C, and the residual hemolytic complement was determined by a method using IgM-hemolysin-sensitized sheep erythrocytes at 10^8 cells/ml. NHS was incubated with water to provide a control.

Mitogenic Activity

Male ICR mice (12 weeks old) were used for the spleen cell cultures. Single spleen cells were prepared as described previously.[6] Cells were suspended in RPMI-1640 supplemented with antibiotics, 10^{-6} M 2-mercaptoethanol, 12.5 mM HEPES, and 10% heat-inactivated fetal bovine serum, and then cells were cultured at a density of 5×10^6 cells/well with or without test sample and mitogen (LPS, ConA, and PHA) in 0.2 ml culture medium in 96 wells microculture plates (Costar) at 37°C for 2 days under a humidified 5% CO_2 atmosphere. Lymphocyte proliferation was measured by a colorimetric method which is based on the *in situ* reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by viable cells. MTT (1 mg/ml) in RPMI-1640 medium (100 μl) was added and the suspension was incubated for 6 hours at 37°C. The resulting formazan precipitate was dissolved in 100 μl of isopropanol containing 0.04 M HCl followed by addition of equal volume of water, and the absorbance of the solution was determined spectrophotometrically at 570 nm with a Microplate Reader.

Anti-influenza Virus Activity[7]

Confluent monolayers of MDCK cell cultures in 48 wells culture plates were infected with influenza virus A/PR/8/34 at a multiplicity of infection of 0.0035 PFU/cell in 0.25 ml of EAGLE's minimum essential medium containing 0.2% bovine serum albumin, acetyltrypsin (3 μg/ml), and antibiotics (maintenance medium). Then the sample solution (10 μl) was added to the well of the culture plate. The plate was incubated at 37°C for 72 hours under 5% CO_2 atmosphere. Then the monolayers in the culture plate were separated from the medium, washed with PBS, pH 7.4, to remove the dead cells resulting from infection of the influenza virus. The viable cells were determined by MTT method as described above. Anti-influenza virus activity was also estimated by the hemagglutination assay of the medium using chicken erythrocytes.

Antimicrobial Activity

Antimicrobial activity was tested for 16 species of microorganisms. An agar plate containing each microorganism on which 8 mm paper disc containing 50 μg of samples was incubated for 24~48 hours at 27°C or 37°C, on each appropriate condition. Then antimicrobial activity was determined by the diameter of inhibitory zone.

Results

Production and Isolation of Panosialins

The stock culture of the strain OH-5186 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of starch 2.4%, glucose 0.1%, peptone 0.3%, meat extract 0.3% (Kyokuto Pharmaceutical Industrial Co.), yeast extract 0.5% (Oriental Yeast Co.), and $CaCO_3$ 0.4%, at pH 7.0 before sterilization. Incubation was on a rotary shaker at 27°C for 2 days. Two ml of the seed culture was inoculated into each of thirty 500-ml Erlenmeyer flasks containing 100 ml of a production medium consisting of maltose 5%, fermipan 1.5% (Gist-brocades), Ebios 2.5% (Tanabe Seiyaku Co.), KBr 1.0%, KH_2PO_4 0.05%, and $Mg_2SO_4 \cdot 7H_2O$ 0.05%, at pH 7.0 before sterilization, and the flasks were incubated on a rotary shaker at 37°C for 2 days. The active substances were detected by inhibitory activity against α-mannosidase.

The mycelia obtained from 3.0 liters of the cultured broth were extracted with two 1.3-liter portions of

acetone. The acetone extracts were combined and concentrated under reduced pressure to remove acetone. The concentrate was adjusted to pH 7.0 and absorbed on a Diaion HP 20 column (150 ml). The column was first washed with water and the active substances were eluted using 50% aqueous acetone. The active fractions were combined and concentrated under reduced pressure to remove acetone. The concentrate was diluted three times with water, adjusted to pH 7.0, and extracted with the same volume of ethyl acetate. The ethyl acetate layer was concentrated to dryness to give a brown oil (406 mg). The water layer of the ethyl acetate extraction was adjusted to pH 7.0, extracted with the same volume of 1-butanol, and concentrated to dryness to give a yellow oil (2.07 g).

The oil of the butanol extraction was applied on a silica gel column (60 g, Kieselgel 60, 70~230 mesh, Merck) and eluted with $CHCl_3$-methanol (4:1) and $CHCl_3$-methanol (1:1). The active fractions eluted with $CHCl_3$-methanol (1:1) were combined and concentrated to dryness to give a yellow oil (885 mg). The oil was chromatographed over a Sephadex LH-20 column (1,000 ml) developed with methanol. The active fractions were combined and concentrated to dryness to give 633 mg of white powder mixture of panosialins A~D (**1**~**4**). Then 100 mg of the powder was subjected to preparative HPLC under the following conditions: column, Capcell Pak C_{18} (i.d. 20×250 mm, Shiseido Co.); mobile phase, methanol-50 mM sodium phosphate (pH 7.0) buffer (78:22); flow rate, 8 ml/minute; detection, UV 205 nm. Under these conditions **1**, **2**, **4**, and **3** were eluted in this order. They were rechromatographed with the same column using acetonitrile-50 mM sodium phosphate (pH 7.0) buffer (50:50) and desalted by MCI gel CHP20P (Mitsubishi Kasei) column to yield 9.6 mg of **1**, 13.2 mg of **2**, 7.5 mg of **3**, and 9.9 mg of **4**.

The oil of the ethyl acetate extraction was applied on a silica gel column (10 g, Kieselgel 60, 70~230 mesh) and eluted with $CHCl_3$-methanol (10:1) and $CHCl_3$-methanol (4:1). The active fractions eluted with $CHCl_3$-methanol (4:1) were combined and concentrated to dryness to give a yellow oil (106 mg). The oil was chromatographed over a Sephadex LH-20 column (600 ml) developed with methanol. The active fractions were combined and concentrated to dryness to give 30.4 mg of white powder mixture of panosialins wA~wD (**5**~**8**). The powder was subjected to preparative HPLC under the following conditions: column, Capcell Pak C_{18} (i.d. 20×250 mm); mobile phase, acetonitrile-25 mM sodium phosphate (pH 7.0) buffer (65:35); flow rate, 8 ml/minute; detection, UV 205 nm. Under these conditions **5**, **6**, **8**, and **7** were eluted in this order. Compounds **7** and **8** were rechromatographed with the same column using acetonitrile-25 mM sodium phosphate (pH 7.0) buffer (60:40). The eluates of HPLC were desalted by MCI gel CHP20P column to yield 4.3 mg of **5**, 2.4 mg of **6**, 2.7 mg of **7**, and 1.5 mg of **8**.

Physico-Chemical Properties of Panosialins

The physico-chemical properties of **4**~**8** are shown in Table 1. Strong absorbance at 1230~1240 cm^{-1} in IR spectra indicated their sulfate residue. From the molecular formulae, each compound was thought to be mono- or disodium salt of sulfonic acid.

Structure Elucidation of Panosialins D and wD

Chemical shifts in the ^1H and ^{13}C NMR spectra of **4**~**8** are shown in Table 2. HMQC revealed the connectivity of each proton and carbon. The ^1H-^1H

Table 1. Physico-chemical properties of panosialins D (**4**) and wA~wD (**5**~**8**).

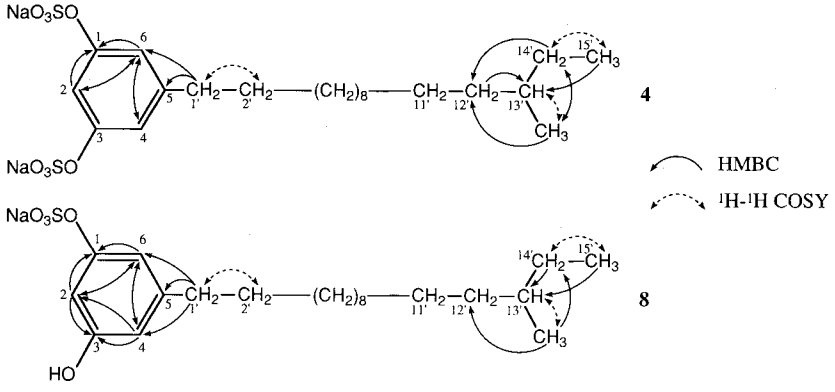
	4	**5**	**6**	**7**	**8**
Appearance	White powder	White powder	White powder	White powder	White powder
MP (°C)	192 (dec.)	176 (dec.)	238 (dec.)	223 (dec.)	207 (dec.)
Molecular formula	$C_{22}H_{36}O_8S_2Na_2$	$C_{21}H_{35}O_5SNa$	$C_{21}H_{35}O_5SNa$	$C_{22}H_{37}O_5SNa$	$C_{22}H_{37}O_5SNa$
FAB-MS (*m/z*)	561 $(M+Na)^+$	445 $(M+Na)^+$	445 $(M+Na)^+$	459 $(M+Na)^+$	459 $(M+Na)^+$
HRFAB-MS					
Found:	515.1747 $(M-Na)^-$	399.2216 $(M-Na)^-$	399.2205 $(M-Na)^-$	413.2371 $(M-Na)^-$	413.2360 $(M-Na)^-$
Calcd:	515.1749 ($C_{22}H_{36}O_8S_2Na$)	399.2205 ($C_{21}H_{35}O_5S$)	399.2205 ($C_{21}H_{35}O_5S$)	413.2362 ($C_{22}H_{37}O_5S$)	413.2362 ($C_{22}H_{37}O_5S$)
UV λ_{max}^{MeOH} nm (ε)	196 (47,700), 212 (sh, 14,800), 275 (1,600)	204 (24,300), 217 (sh, 9,300), 272 (2,500), 278 (sh, 2,300)	202 (12,700), 218 (sh, 4,000), 271 (1,500), 278 (sh, 1,300)	203 (17,500), 217 (sh, 5,500), 272 (1,700), 277 (sh, 1,600)	203 (18,800), 217 (sh, 6,500), 272 (1,700), 278 (sh, 1,600)
IR ν_{max} (KBr) cm^{-1}	3464, 2918, 2850, 1593, 1470, 1281, 1230, 1065, 985	3518, 2918, 2852, 1560, 1465, 1263, 1240, 1072, 993	3496, 2920, 2850, 1566, 1460, 1261, 1230, 1061, 993	3340, 2920, 2850, 1587, 1466, 1261, 1227, 1070, 993	3496, 2924, 2852, 1566, 1441, 1288, 1236, 1061, 997
Solubility					
Soluble:	MeOH, H_2O	MeOH, acetone, H_2O	MeOH, acetone, H_2O	MeOH, acetone, H_2O	MeOH, acetone, H_2O
Insoluble:	$CHCl_3$, acetone	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$

Table 2. ^{1}H and ^{13}C NMR assignments for **4**~**8**.

No.	4*		5**		6**		7**		8**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Benzenediol sulfate moiety										
1	151.5 s		159.2 s		159.1 s		159.2 s		158.8 s	
2	112.0 d	7.05 dd (8.0, 8.0)	107.3 d	6.60 dd (2.0, 2.1)	107.3 d	6.60 dd (2.2, 2.2)	107.3 d	6.60 dd (2.1, 2.1)	107.0 d	6.59 dd (2.2, 2.2)
3	151.5 s		155.0 s		155.0 s		155.0 s		154.7 s	
4	119.1 d	6.93 d (8.0)	113.9 d	6.62 dd (1.5, 2.0)	113.9 d	6.62 dd (1.5, 2.2)	113.2 d	6.42 dd (1.5, 2.1)	113.6 d	6.62 dd (1.5, 2.2)
5	146.2 s		146.3 s		146.3 s		146.3 s		146.0 s	
6	119.1 d	6.93 d (8.0)	113.2 d	6.43 dd (1.5, 2.1)	113.2 d	6.42 dd (1.5, 2.2)	113.9 d	6.62 dd (1.5, 2.1)	112.9 d	6.41 dd (1.5, 2.2)
Alkyl moiety										
1′	35.3 t	2.53 t (7.7)	37.2 t	2.50 t (7.7)	37.2 t	2.50 t (7.7)	37.2 t	2.50 t (7.7)	36.9 t	2.50 t (7.8)
2′	30.9 t	1.50 m	32.7 t	1.58 m	32.7 t	1.58 m	32.7 t	1.58 m	32.4 t	1.58 m
3′										
4′										
5′										
6′	29.3	1.13~1.19 m	30.7	1.28 m	30.7	1.28 m	30.7	1.28 m	30.6	1.28 m
	~30.0 t	(16H)	~31.3 t	(16H)	~31.1 t	(20H)	~31.3 t	(18H)	~31.1 t	(16H)
7′										
8′										
9′										
10′										
11′	27.0 t	1.13 m	28.8 t	1.28 m					28.2 t	1.27 m
12′	36.5 t	1.01 m, 1.21 m	40.6 t	1.17 m			28.8 t	1.28 m	37.8 t	1.06 m, 1.28 m
13′	34.2 d	1.19 m	29.5 d	1.50 m	33.4 t	1.28 m	40.5 t	1.17 m	35.7 d	1.28 m
13′-Me	19.0 q	0.72 d (7.0)	23.3 q	0.88 d (6.5)					19.6 q	0.84 (6.7)
14′	29.4 t	0.99 m	23.3 q	0.88 d (6.5)	24.2 t	1.28 m	29.5 d	1.51 m	30.4 t	1.12 m
14″-Me							23.3 q	0.88 d (6.5)		
15′	11.1 q	0.73 t (7.5)			14.7 q	0.88 t (7.0)	23.3 q	0.88 d (6.5)	11.7 q	0.86 t (7.2)

* Solvent: D_2O. ** Solvent: CD_3OD. The coupling constants (Hz) are in parentheses.

Fig. 2. Structure elucidations of panosialins D (**4**) and wD (**8**).



COSY and HMBC of **4** suggested symmetric 5-ethyl-1,3-benzenediol moiety as shown in Fig. 2. The ^{13}C chemical shifts of C-11′~C-15′ of **4** coincided with those of 3-methylpentyl moiety of 3-methyloctane.[8] The data of ^{1}H-^{1}H COSY and HMBC also supported the 3-methylpentyl moiety. Comparing the molecular formulae and the chemical shifts of **4** and **1**,[4] it was suggested that two sulfates bonded to benzenediol and the remaining eight methylenes (δ_C 29.3~30.0, δ_H 1.13~1.19) connected ethylbenzenediol and methylpentyl moieties. Therefore the structure of **4** was elucidated as 5-(13-methylpentadecyl)-1,3-benzenediol bis(sodium sulfate) (Fig. 1).

The ^{1}H and ^{13}C NMR spectra of **8** was similar to those of **4** in 13-methylpentadecyl moiety. But ^{13}C NMR showed that benzenediol moiety was asymmetric, which suggested one sulfate of **4** was removed in **8**. Finally the structure of **8** was elucidated as 5-(13-methylpentadecyl)-1,3-benzenediol 1-(sodium sulfate) by ^{1}H-^{1}H COSY and HMBC (Fig. 2).

Biological Properties of Panosialins

Inhibitory Activity of Glycosidases

Inhibitory activity of each compound for various glycosidases are shown in Table 3. Panosialins mainly inhibited α-glucosidase, β-glucosidase, and α-mannosidase. Compounds **1**~**3** and **6**~**8** showed the most potent inhibitory activity against α-glucosidase among the enzymes tested, whereas **4** and **5** each showed the most potent inhibitory activity against β-glucosidase.

Table 3. Inhibitory activity of panosialins against various glycosidases.

Glycosidase	IC_{50} (M)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
α-Mannosidase (jack bean)	9.4×10^{-6}	7.8×10^{-6}	3.3×10^{-6}	2.9×10^{-6}	6.8×10^{-6}	9.5×10^{-6}	8.5×10^{-6}	5.9×10^{-6}
β-Mannosidase (*Achatina fulica*)	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>2.2\times10^{-5}$	$>2.2\times10^{-5}$	$>2.1\times10^{-5}$	$>2.1\times10^{-5}$
α-Glucosidase (yeast)	1.4×10^{-6}	8.4×10^{-7}	6.0×10^{-7}	4.5×10^{-6}	1.9×10^{-6}	1.5×10^{-6}	3.4×10^{-8}	2.8×10^{-8}
β-Glucosidase (almond)	5.3×10^{-6}	6.9×10^{-6}	3.3×10^{-6}	2.7×10^{-6}	2.4×10^{-7}	1.5×10^{-6}	2.3×10^{-6}	1.1×10^{-6}
β-Galactosidase (jack bean)	2.1×10^{-6}	2.1×10^{-6}	1.7×10^{-5}	1.5×10^{-5}	$>2.2\times10^{-5}$	$>2.2\times10^{-5}$	$>2.1\times10^{-5}$	$>2.1\times10^{-5}$
Sialidase (influenza virus)	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>2.2\times10^{-5}$	$>2.2\times10^{-5}$	$>2.1\times10^{-5}$	$>2.1\times10^{-5}$
Sialidase (*Arthrobacter*)	1.2×10^{-5}	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	$>1.8\times10^{-5}$	2.1×10^{-5}	$>2.2\times10^{-5}$	2.0×10^{-5}	1.6×10^{-5}

Table 4. Mitogenic activity of panosialins.

Mitogen (μg/ml)		**1**		**2**		**3**		**4**		Mixture of **5**~**8**	
		0.45 (μg/ml)	1.14	0.45 (μg/ml)	1.14	0.45 (μg/ml)	1.14	0.45 (μg/ml)	1.14	0.45 (μg/ml)	1.14
ConA	2.3	0.99[1)]	0.38	0.89	0.53	0.64	0.63	0.69	0.39	0.62	1.03
	0.2	0.79	0.62	0.97	0.79	1.01	0.97	1.05	0.53	0.56	1.63
	0.02	1.02	0.62	1.13	0.84	1.1	1.96	1.13	0.68	0.51	1.69
LPS	0.45	0.9[1)]	0.51	0.93	0.78	0.89	0.84	0.95	0.65	1.14	1.52
	0.05	0.83	0.67	0.9	0.77	0.86	0.88	0.88	0.72	0.4	1.42
	0.005	0.91	0.57	0.95	0.67	0.85	0.72	0.93	0.5	0.32	1.24
PHA	22.7	0.82[1)]	0.63	0.79	0.67	0.81	0.85	0.77	0.52	1.17	1.28
	2.3	0.59	0.67	0.61	0.48	0.6	0.73	0.5	0.53	0.24	0.62
	0.23	0.89	0.75	0.97	0.75	0.9	0.92	0.84	0.74	0.54	0.78
No mitogen		0.99[2)]	0.63	1.1	0.69	0.96	0.8	1.04	0.59	1.22	1.73

[1)] Values represent as ratio to panosialin free control in the presence of each mitogen.
[2)] Values represent as ratio to panosialin free control in the absence of each mitogen.

Mitogenic Activity and Anti-complementary Activity

Mitogenic activity of **1**~**4** and a mixture of **5**~**8** are shown in Table 4. Compound **1**~**4** did not show any mitogenic activity to mouse spleen cells at a final concentration of 0.45 μg/ml, and showed cytotoxicity at 1.14 μg/ml. However **1**~**4** decreased mitogen stimulus of 2.3 μg/ml of PHA at 0.45 μg/ml. The mixture of **5**~**8** showed mitogenic activity at 0.45 μg/ml and 1.14 μg/ml, and the 0.45 μg/ml concentration of the mixture inhibited the mitogenic activity of all tested concentrations of ConA, 0.05 μg/ml and 0.005 μg/ml of LPS, and 2.3 μg/ml and 0.23 μg/ml of PHA. These results suggest that the mixture of **5**~**8** suppresses immunoresponse of T cell and B cell at low concentration.

Mixture of **1**~**4** did not show anti-complementary activity at a final concentration of 100 μg/ml (data not shown).

Anti-influenza Virus Activity

Compound **1** showed a little anti-influenza virus activity at 3.8 μg/ml as a final concentration. Compounds **2**~**8** showed no anti-influenza virus activity up to 3.8 μg/ml (data not shown).

Antimicrobial Activity

Compounds **1**~**8** showed weak antimicrobial activities at high concentration (50 μg/disk, paper disk method) against *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans*, *Mucor racemosus*, *Piricularia oryzae*, and *Xanthomonas oryzae*. However the same concentration of **1**~**8** did not affect against *Bacteroides fragilis*, *Escherichia coli*, *Mycobacterium smegmatis*, and *Pseudomonas aeruginosa*.

Discussion

The mixture of panosialins A~C has been reported as the inhibitor of viral sialidase.[2] In the present study, isolated panosialins showed higher inhibitory activity against α-mannosidase, α-glucosidase, and β-glucosidase than influenza virus sialidase. Monosulfated panosialins seemed to enhance the inhibitory activity against β-glucosidase in comparison with disulfated panosialins. Panosialins wA~wD mixture also showed mitogenic activity and suppressed the activities induced by ConA, PHA, and LPS. Because panosialins wA~wD mixture suppressed the mitogenic activity higher than panosialins A~D, disulfation might prevent the suppression.

It is reported that the proliferation of lymphocytes is regulated by the carbohydrate chains of cell-surface glycoproteins.[9] These results suggest that panosialins modulate the carbohydrate chains of lymphocyte surface glycoproteins.

Acknowledgments

We thank Ms. AKIKO HATANO and Ms. NORIKO SATO of School of Pharmaceutical Sciences, Kitasato University for measurements of NMR spectra, and also thank Ms. AKIKO NAKAGAWA and Ms. CHIKAKO SAKABE of School of Pharmaceutical Sciences, Kitasato University for measurements of mass spectra.

References

1) PAULSON, J. C.: Glycoproteins: what are the sugar chains for? Trends Biochem. Sci. 14: 272~276, 1989
2) AOYAGI, T.; M. YAGISAWA, M. KUMAGAI, M. HAMADA, Y. OKAMI, T. TAKEUCHI & H. UMEZAWA: An enzyme inhibitor, panosialin, produced by *Streptomyces*. I. Biological activity, isolation and characterization of panosialin. J. Antibiotics 24: 860~869, 1971
3) KUMAGAI, M.; Y. SUHARA, T. AOYAGI & H. UMEZAWA: An enzyme inhibitor, panosialin, produced by *Streptomyces*. II. Chemistry of panosialin, 5-alkylbenzene-1,3-disulfates. J. Antibiotics 24: 870~875, 1971
4) DOLAK, L. A.; E. P. SEEST, J. I. CIALDELLA, G. P. LI & M. J. BOHANON (The Upjohn Company): Compounds used for the inhibition of HIV-protease. PCT WO 93/04055, March 4, 1993
5) YAMADA, H.; H. KIYOHARA, J.-C. CYONG & Y. OTSUKA: Studies on polysaccharides from *Angelica acutiloba*. Part 1. Fractionation and biological properties of polysaccharides. Planta Med. 50: 163~167, 1984
6) YAMADA, H.; H. KIYOHARA, J.-C. CYONG, N. TAKEMOTO, Y. KOMATSU, H. KAWAMURA, M. ABURADA & E. HOSOYA: Fractionation and characterization of mitogenic and anti-complementary active fractions from kampo (Japanese herbal) medicine "Juzen-Taiho-To". Planta Med. 56: 386~391, 1990
7) NAGAI, T.; Y. MIYAICHI, T. TOMIMORI, Y. SUZUKI & H. YAMADA: In vivo anti-influenza virus activity of plant flavonoids possessing inhibitory activity for influenza virus sialidase. Antiviral Res. 19: 207~217, 1992
8) LINDEMAN, L. P. & J. Q. ADAMS: Carbon-13 nuclear magnetic resonance spectrometry. Chemical shifts for the paraffins through C9. Anal. Chem. 43: 1245~1252, 1971
9) BOWLIN, T. L. & P. S. SUNKARA: Swainsonine, an inhibitor of glycoprotein processing, enhances mitogen induced interleukin 2 production and receptor expression in human lymphocytes. Biochem. Biophys. Res. Commun. 151: 859~864, 1988