AN ENZYME INHIBITOR, PANOSIALIN, PRODUCED BY *STREPTOMYCES*

I. BIOLOGICAL ACTIVITY, ISOLATION AND CHARACTERIZATION OF PANOSIALIN

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In the screening of streptomyces culture filtrates for inhibitors of viral sialidase, acid phosphatase and polygalacturonase, a new type of compound was isolated and named panosialin. It had the following ID_{50} values: 9×10^{-6} M, 3.8×10^{-5} M and 3.9×10^{-5} M againt sialidase, acid phosphatase and polygalacturonase respectively. In this paper, characters of the panosialin-producing strains and the isolation of panosialin are described. It was found to bind to protein and to have an action similar to that of detergents.

As reviewed by UMEZAWA[1], this laboratory had recently used the methods of antibiotic research to find specific enzyme inhibitors of microbial origin, inhibitors of proteases[2~11], tyrosine hydoxylase[12~14] and dopamine β-hydroxylase[15~18]. In an attempt to extend this approach to antiviral compounds[19], we have examined culture filtrates of microorganisms for inhibitors of a viral sialidase. In this paper, we report the production, isolation and characterization of panosialin. Structurally, panosialin is a new type of metabolite which inhibits sialidase and various other enzymes under certain conditions.

Materials and Methods

Virus: The viruses were propagated and partially purifiied by a modification of the method of MAYRON[20]. The Narashino strain of Newcastle disease virus (NDV), the Hemagglutinating virus of Japan strain (HVJ) of parainfluenza virus and the PR 8 strain of influenza virus were propagated in 10-day-old embryonated eggs by the inoculation of 0.2 ml of a 1 : 1,000 dilution of infective chorioallantoic fluid per egg. Following incubation at 35°C for 48 hours, the eggs were chilled and the chorioallantoic fluid carefully harvested. After centrifuging at 2,000 r.p.m. for 30 minutes, the supernatant containing the virus was centrifuged at $105,000\times g$ for 3~5 hours. The sediment was resuspended in 0.05 M citrate-phoshate buffer saline, pH 6.0 and recentrifuged at $105,000\times g$ for 2~4 hours. The partially purified virus was resuspended in 0.05 M citrate-phosphate buffer saline of pH 6.0 at 10,000 HA/ml and stored at −60°C. The virus was diluted with the same buffer immediately before use. Sialidase activity was assayed by the procedures described previously[19,21,22] using a bovine sialyllactose (BSL) as the substrate. The liberation of N-acetylneuraminic acid (NANA) from BSL was proportional to the time of the incubation for 0~40 minutes at 37°C, and proportional to the amount of the enzyme added.

Acid phosphatase: Acid phosphatase of wheat germ purchased from Seikagaku Kogyo

Co., Ltd., Tokyo was employed. It was dissolved and diluted with 0.2 M sodium acetate buffer of pH 5.0 immediately before use.

Polygalacturonase: A plant tissue macerating enzyme, 'Macerozyme' obtained from *Rhizopus* sp. purchased from Kinki Yakult Manuf. Co., Ltd., Nishinomiya was employed. It was dissolved and diluted with 0.03 M citrate-phosphate buffer of pH 5.0.

Substrates: Bovine sialyllactose (BSL) was prepared from cow colostrum by a modification of the method of KUHN[23] and 0.1 % BSL solution in 0.05 M citrate-phosphate buffer of pH 6.0 was employed for the experiment. Disodium *p*-nitophenyl phosphate (DPNP) was purchased from Daiichi Pure Chemical Co., Ltd., Tokyo, and 0.1 M DPNP in distilled water was employed. Sodium polygalacturonic acid purchased from Nutritional Biochemicals Co., Ltd., Ohio was employed as a substrate for polygalacturonase study.

Inhibitors: Sodium *n*-alkylsulfates (C_6~C_{18}) were obtained from Banyu Seiyaku Co., Ltd., Tokyo. Dipotassium 5-alkylbenzene 1,3-disulfates were synthesized as described in next paper[24]. Sodium dodecylbenzene sulfonate, sodium deoxycholate, Triton X-100 and Tween 85 were obtained from Tokyo Kasei Co., Ltd., Tokyo.

Assay of viral sialidase (N-acetylneuraminate glycohydrolase, E.C.3.2.1.18) activity: BSL was dissolved in 0.05 M citrate-phosphate buffer (pH 6.0) at 1 mg/ml, and to 0.1 ml of this substrate solution, 0.2 ml of the same buffer, and 0.1 ml of the buffer with or without a test material were added. After 3 minutes at 37°C, 0.1 ml of viral sialidase containing 0.8 enzyme unit was added and the reaction mixture incubated for 30 minutes at 37°C. To stop the reaction 0.25 ml of 0.025 M sodium metaperiodate in 0.125 N sulfuric acid was added. Then, the liberated NANA was determined by the method of AMINOFF[25]. The absorption at 550 mμ was measured and the blank was subtracted from the reading. Activity was expressed as units of enzyme, 1 unit being defined as that amount of enzyme which releases 25 μg NANA from an excess of BSL in 1 hour at 37°C and pH 6.0.

Assay of acid phosphatase (orthophosphoric monoester hydrolases, E.C.3.1.3.2) activity: The reaction system in the method of CHERSI[26] for determination of acid phosphatase was modified. A solution containing 0.02 ml of 0.1 M DPNP solution, 0.05 ml of 0.2 M sodium acetate buffer (pH 5.0) and 0.12 ml of distilled water with or without a test material was incubated for 3 minutes at 37°C, and 0.01 ml of acid phosphatase (1 mg/ml) in the same buffer was added. After 20 minutes incubation at 37°C, the reaction was stopped by adding 1.5 ml of 0.15 N NaOH. The absorption at 420 mμ was measured as described above.

Assay of proteases: The reaction systems for trypsin, papain and pepsin were described previously[3,9].

Assay of polygalacturonase (poly-α-1, 4-galacturonide glycanohydrolase (E.C.3.2.1.15) activity: Polygalacturonase activity was assayed by the cup-plate method of DINGLE[27] which was modified as follows: 10 ml of 1 % sodium polygalacturonic acid solution in 0.03 M citrate-phosphate buffer (pH 5.0) containing 1.5 % agar was poured into Petri dish immediately before use; 0.3 ml of 7.5 % polygalacturonase solution with or without inhibitors was placed in stainless steel cup on the plate and incubated at 27°C; after 18 hours, the plate was developed with 1 N NaOH and the diameter of the transparent zone was plotted against log enzyme concentration.

Inhibition percent: The percent inhibition of an enzymatic reaction was calculated from the experimental data described above, and the probit of the percent inhibition was plotted against the logarithm of concentration of each inhibitor. The concentration of inhibitor required for 50% inhibition of enzyme activity, ID_{50}, was obtained from this plot and shown in Table 1 and Fig. 3.

Panosialin-producing strains: Panosialin was isolated from two strains numbered MC 638-A 2 and MC 860-A1. They were classified as *Streptomyces pseudoverticillus* nov. sp. and *Streptomyces rimosus* forma *panosialinus* nov. forma, respectively, with the characteristics described below.

Strain No. MC638-A2

The strain was isolated from a soil sample collected at Nepal.

1. Morphology: Aerial mycelium (about 1 μ in width) develops from the substrate mycelium and its end is curved or forms a loop bearing spore chains. Pseudowhorls are observed (incomplete verticillus) on some media and in some cases a bundle of aerial mycelia (coremium-like) is formed. More than 10 spores form a chain and the spore is round or oval. Surface of the spore is smooth (Plates 1 and 2).

2. Characteristics on various media:

(1) On sucrose nitrate agar (cultured at 27°C): Growth is good, colorless to pale yellowish brown and the reverse is colorless to pale yellowish brown. Aerial mycelium (AM) is white or slightly gray and occasionally pale blue. No soluble pigment (SP).

(2) On glucose asparagine agar (27°C): Growth is good and pale yellow. AM is abundant and grayish white at the early stage and turns to gray later. Reverse of the growth is pale yellow. No SP.

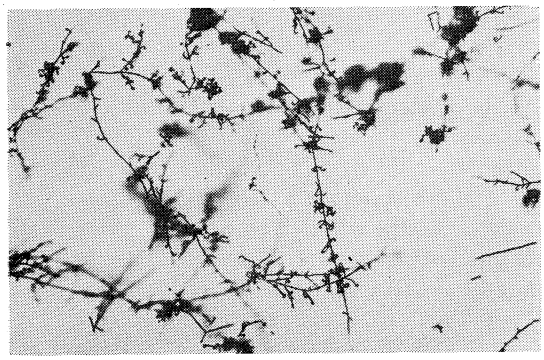
(3) On glycerol asparagine agar (27°C): Growth is weak, colorless to slightly colored as yellowish gray. AM is scant and gray.

(4) On starch inorganic salts agar (27°C): Growth is good and pale yellowish brown. AM is abundant and pale brownish gray. Reverse is pale yellowish brown. No SP.

(5) On tyrosine agar (27°C): Growth is colorless to yellowish gray. AM is none or scant and white or grayish white. Reverse is colorless to yellowish gray. No SP.

(6) On nutrient agar (27°C): Growth is weak. AM is none or scant and grayish white. Reverse is yellowish brown. Yellowish brown SP slightly.

Plate 1. MC 638-A 2 (67×) on yeast malt agar

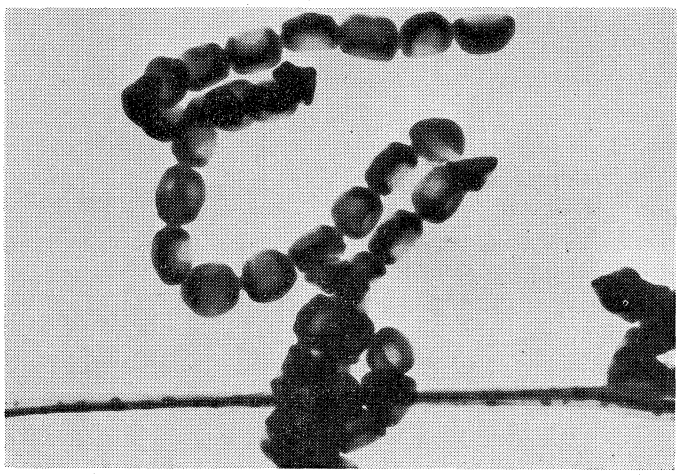


(7) On yeast malt agar (27°C): Growth is good and dark yellow. AM is abundant, and white at the beginning, and gradually turns to gray. Pale yellow SP.

(8) On oat meal agar (27°C): Growth is good and colorless to yellowish brown. AM is gray at the beginning and grayish white later. Reverse is colorless to pale yellow. No SP.

(9) On calcium malate agar (27°C): Growth is colorless to yellowish gray. AM is white at the beginning and turns to grayish white gradually. Reverse is colorless to yellowish gray. No SP.

Plate 2. MC 638-A 2 (2,500×) on yeast malt agar



(10) On LOEFFLER coagulated serum (37°C): No liquefaction.

(11) Peptone nitrate broth (27°C): No reduction to nitrite.

(12) On glucose peptone gelatin medium (20°C and 27 °C): At 27°C, a brownish black pigment is formed and gelatin is liquefied. At 20°C, the brownish black pigment is

formed, but the liquefaction is weak.

(13) On starch agar (27°C): Weakly hydrolyzed zone is shown by iodine-starch reaction.

(14) In milk medium: At 27°C, weak coagulation followed by peptonization is observed. At 37°C, coagulation are weaker than at 27°C.

(15) Growth temperature on yeast malt agar: None at 3°C, ± at 10°C, + at 20°C, ++ at 27°C, ++ at 30°C, ++ at 37°C, ++ at 42°C, ± 48°C (±: weak growth, +: good growth, ++: abundant growth, ++: more abundant growth).

(16) Melanin formation (27°C): No pigment is formed on tyrosine agar, but brown black pigment is formed on peptone yeast agar.

(17) Utilization of carbohydrates on GOTTLIEB and PRIDHAM media: ++ L-arabinose; ++ D-xylose; + D-glucose; ++ D-fructose; + sucrose; ++ inositol; + L-rhamnose; ++ raffinose; ++ D-mannitol; ± or - dulcitol; + inulin; ± or - sorbitol; ± or - without carbohydrate (−, +, ++, ++ are the same as in (15).

As shown in the characters described above, the strain No. MC 638-A 2 belongs to Genus *Streptomyces*, and *S. eurythermus* WAKSMAN and HENRICI and *S. umbrosus* SCHMIDT-KASTNER have many characters in common with this strain. The characters of the strain No. MC 638-A 2 were compared with the description of *S. eurythermus* and with the type culture, ISP 5014 (ETH 6677). The strain MC 638-A 2 was different from *S. eurythermus* in the following properties of the former: no production of soluble pigment in glycerol asparagine medium, tyrosine agar and oat meal agar; the utilization of inositol and L-rhamnose; the formation of pseudowhorls. The strain No. MC 638-A 2 is different from *S. umbrosus* and the type culture ISP 5242 of this species in the following properties of the former: formation of aerial mycelium on calcium malate agar; no soluble pigment in glycerol asparagine agar, tyrosine agar and oat meal agar; utilization of sucrose and raffinose; formation of characteristic pseudowhorls. The strain No. MC 638-A2 produced a ferrimycin-like antibiotic and therefore, it was compared with those forming this type of antibiotics: *S. griseoflavus* WAKSMAN and HENRICI is different from the strain No. MC 638-A 2 in spiny surface of the spores and in its negative utilization of sucrose and raffinose; *S. galilaceus* ETTLINGER *et al.* forms a carmin-red growth; *S. lavendulae* W. & C. has aerial mycelium of lavender color and is different from the strain No. MC 638-A2 in the utilization of carbohydrates. The formation of pseudowhorl is characteristic to the strain No. MC 638-A 2 and therefore, this strain can be classified as a new species which was named *Streptomyces pseudoverticillus* OKAMI *et* HAMADA nov. sp.

Strain No. MC 860-A 1

The strain was isolated from a soil sample collected in Mexico.

1. Morphology: Aerial mycelium (about 1 μ in width) develops from well-branched substrate mycelium. The end of aerial mycelium is straight, waved or spiral shaped, bearing spore chains. More than 10 spores form a chain, and spore is ellipsoid or long ellipsoid. Surface of the spore is smooth (Plates 3 and 4).

2. Characteristics on various media:

(1) On sucrose nitrate agar (27°C): Substrate mycelium is colorless. Aerial mycelium (AM) is white to slightly colored in pale yellowish pink or grayish white. Reverse is colorless to yellowish orange. No soluble pigment (SP).

(2) On glucose asparagine agar (27°C): Growth is elevated and colorless to pale yellow. No or white AM scantly. Reverse is almost colorless to pale yellow. No SP.

(3) On glycerol asparagine agar (27°C): Growth is elevated, colorless to dull yellow. No AM. Reverse is colorless to pale yellow. No SP.

(4) On starch inorganic salts agar (27°C): Growth is thin and yellow. AM is scant and yellowish pink. Reverse is pale yellow. No SP. Strong hydrolysis of starch around the growth.

(5) On tyrosine agar (27°C): Growth is elevated, colorless to dull yellow. AM is none or scant and white. Reverse is colorless to pale dull yellow. No SP.

(6) On nutrient agar (27°C): Growth is elevated, pale yellowish brown. No AM. Reverse is dull yellowish brown. No SP.

(7) On yeast malt agar (27°C): Growth is yellowish brown. AM is white with or without pinkish tinge. Reverse is yellowish brown. No SP.

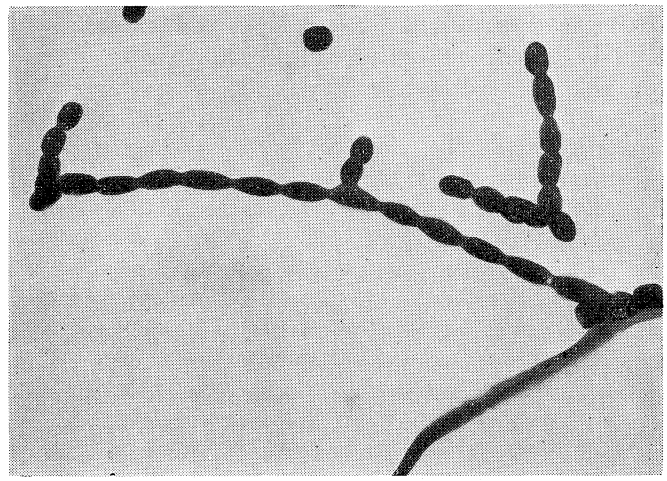
(8) On oat meal agar (27°C): Growth is dark yellow. AM is scant, and white with or without pinkish tinge. Reverse is dull yellow. No SP.

(9) On peptone yeast agar (27°C): Growth is colorless to pale yellowish brown with or without faint blackish tinge. No AM. Reverse is dull yellowish brown with black tinge. The medium turns to slightly blakish brown.

(10) In peptone nitrate medium (27°C and 37°C): Growth is colorless to pale yellow. No AM. No SP or slightly yellow SP. Nitrate is reduced to nitrite (detected by red color with GRIES reagent).

Plate 3. MC 860-A 1 (67×) on yeast malt agar

Plate 4. MC 860-A 1 (2, 100×) on yeast malt agar



(11) On gelatin medium (20°C and 27°C): Growth is colorless to pale yellow. No AM. No liquefaction at 37°C, but positive liquefaction of crateri-form at 20°C. No SP.

(12) In milk medium (defatted, 27°C and 37°C): Growth is colorless to pale yellow. No SP. No coagulation and no peptonization at 27°C, but at 37°C positive coagulation followed by the gradual peptonization.

(13) Growth temperature on yeast malt agar: None at 3°C, − at 10°C, + at 20°C, + at 27°C, ++ at 30°C, ++ at 37°C, + at 42°C, − at 46°C, − at 51°C, (−: no growth, +: good growth, ++: abundant growth).

(14) Utilization of carbohydrates in GOTTLIEB and PRIDHAM media: + L-arabinose; + D-xylose; ± D-glucose; ± D-fructose; ± sucrose; ± inositol; ± L-rhamnose; ± raffinose; ± D-mannitol; ± no carbohydrate (±: doubtful weak growth, +: good growth).

The strain No. MC 860-A1 as described above belongs to Genus *Streptomyces* and among known species it is most closely related to *S. rimosus*. Similarity was confirmed by comparison with the type culture, *S. rimosus* ATCC 10, 970 (IMRU 3558). Consequently it was classified *Streptomyces rimosus* forma *panosialinus* OKAMI et HAMADA, nov. forma, pertaining to the production of panosialin.

Results and Discussion

Production and Isolation of Panosialin

Production and isolation of panosialin were studied in most detail with the strain

No. MC 638-A2. Panosialin was produced by the shaken culture or by tank fermentation in media containing various kinds of carbon sources and nitrogen sources. For example, the strain No. MC638-A2 was shaken-cultured in a medium containing 1 % glucose, 1 % starch, 1.5 % soybean meal, 0.1 % K_2HPO_4, 0.1 % $MgSO_4 \cdot 7H_2O$, 0.3 % NaCl, 0.0007 % $CuSO_4 \cdot 5H_2O$, 0.0001 % $FeSO_4 \cdot 7H_2O$, 0.0008 % $MnCl_2 \cdot 4H_2O$, 0.0002 % $ZnSO_4 \cdot 7H_2O$. The production of panosialin was determined against viral sialidase. Maximum production was reached after 4~7 days in the shaken culture. After 48 hours in shaken culture, 700 ml of the broth was inoculated into 130 liters of the same medium in a 200-liter stainless steel fermentor and production reached a maximum after 68 hours under aeration and strirring at 27°C. The culture filtrate generally showed 50 % inhibition against viral sialidase activity (Narashino) when 0.003~0.0035 ml was added to the reaction mixture. The cultured broth was filtered and the filtrate (105 liters) was extracted with 80 liters of *n*-butanol. After washing with 2% aqueous potassium chloride, the butanol extract was concentrated under reduced pressure to 2 liters, and 47.5 g of precipitate was collected. Three μg of the crude powder thus obtained showed 50 % inhibition of the sialidase. The mycelium (wet weight 3 kg) was extracted twice with 10 liters of methanol and the methanol solution was concentrated to 2 liters, which was extracted with 2 liters of *n*-butanol. Concentration of the butanol extract to 190 ml yielded 15 g of the precipitate of such activity that 4.2 μg showed 50 % inhibition. The crude powders obtained from the filtrate and the mycelium were mixed and dissolved in 520 ml of hot water. After overnight in cold room, 30.3 g of lightly yellowish crystals were obtained. Thirty g of the crystals was recrystallized from 3,000 ml distilled water, yielding 23.2 g of white crystals of panosialin. Panosialin is an acidic compound with the peculiar characteristics that its sodium salt is soluble in cold water but the potassium salt is insoluble. Therefore, the potassium salt of panosialin was easily crystallized from water after addition of potassium chloride to water. The addition of potassium chloride raised the crystallization yield. Moreover, addition of 1.0% potassium chloride to the culture filtrate increased the yield of the extraction into *n*-butanol.

Properties of Potassium Panosialin

The most purified panosialin which was obtained by recrystallization from 1 % aqueous potassium chloride showed the following properties: white crystals melting in the range of 250.5~261.5°C with decomposition, has no optical activity, λ_{max} at 265 mμ ($E^{1\%}_{1cm}$ 5, Fig. 1).

The infrared absorption spectrum showed a band at 1200~1300 cm^{-1} corresponding to the sulfate (Fig. 2).

The results of the elemental analysis were as follows:

Calcd. for $C_{21}H_{34}O_8S_2K_2$: C 45.30, H 6.16, O 22.99, S 11.51;
Calcd. for $C_{22}H_{36}O_8S_2K_2$: C 46.29, H 6.36, O 22.42, S 11.23;
Found: C 45.54, H 6.15, O 22.95, S 11.04.

One mmole (560 mg) of potassium panosialin was hydrolyzed in mixture of 40 ml of distilled water and 0.8 ml of 1 N HCl at 50°C for 30 hours and the hydrolyzate was extracted with ether. From the aqueous layer, by addition of barium chloride, 446 mg (about 2 mmoles) of barium sulfate was obtained. The evaporation of the ether

Fig. 1. Ultraviolet spectrum of potassium salt of panosialin in 1 % aqueous sodium chloride.

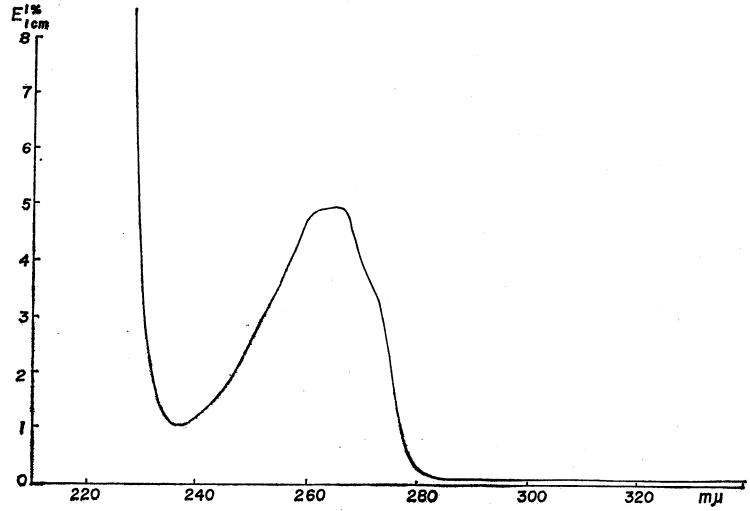
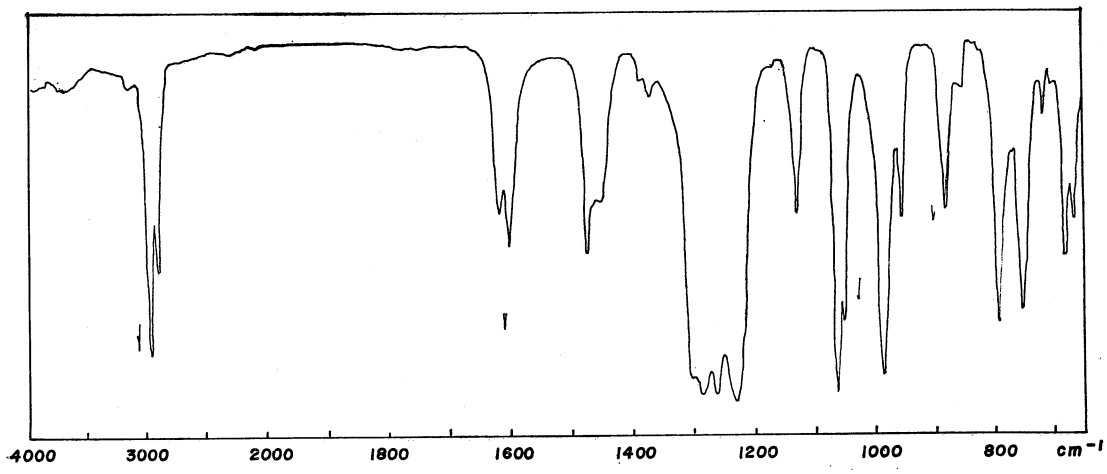
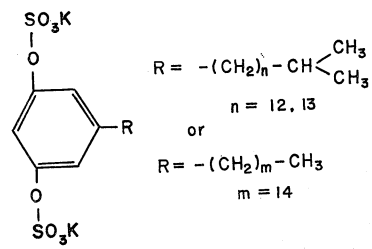


Fig. 2. Infrared spectrum of potassium salt of panosialin in KBr disk.



extract followed by the crystallization from *n*-hexane yielded 280 mg of colorless crystals. A methanol solution showed a maximum at 281 and 276 mμ ($E^{1\%}_{1cm}$ 46.36 and 47.73) and the result of the elemental analysis (found C 78.98, H 11.16, O 10.42) suggested that 2 moles of sulfric acid was removed by the hydrolysis. Gas chromatography and mass spectroscopy indicated that the crystals were a mixture of the homologs. Thus, panosialin obtained above was a mixture. The main components had the formula of $C_{21}H_{34}O_8S_2K_2$ and $C_{22}H_{36}O_8S_2K_2$ (*ca.* 2 : 1). Thus, in this paper, 560 was assigned to the mean molecular weight of panosialin which was used for the biological experiments.

As reported in another paper[24], panosialin is a mixture of the compounds having the structure indicated at the right.



Panosialin gives positive anisaldehyde-sulfuric

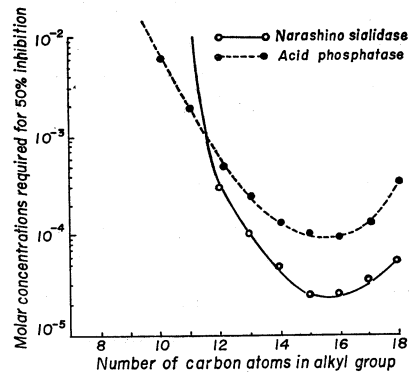
acid, iodine and pH indicators and negative ninhydrin, RYDON-SMITH, SAKAGUCHI and EHRLICH reactions. On high-voltage paper electrophoresis (3,000 V, 15 minutes) using formic acid-acetic acid-water (25 : 75 : 900), panosialin moved 1.5 cm toward the anode. On thin-layer chromatography using silica gel G (E. Merck), panosialin gave a single spot at Rf 0.36 with ethyl acetate - methanol (4 : 1) and 0.26 with *n*-butanol - butyl acetate - acetic acid - water (4 : 2 : 1 : 1).

Potassium panosialin is soluble in hot water, acetic acid, dimetylsulfoxide, dimethylformamide, 0.1 N HCl, and scarcely soluble in cold water, methanol, ethanol, butanol, acetone, ethyl acetate, chloroform, ethyl ether, petroleum ether, benzene and hexane. It is extracted from its aqueous solution with butanol and the yield of the extraction is raised by addition of potassium chloride. Sodium panosialin is soluble in water, and therefore patassium panosialin is soluble in sodium chloride solution. Potassium panosialin is also soluble in water containing NH_4^+, Fe^{+++}, Cu^{++} or Mn^{++} but not in that containing Ba^{++}, Mg^{++} or Zn^{++}.

The Activity of Panosialin, Panosialin-w, *n*-Alkylbenzene-disulfates, *n*-Alkylsulfates and Other Detergents to Inhibit Viral Sialidase, Acid Phosphatase and Polygalacturonase

The molar concentrations of *n*-alkyl (C_8~C_{18}) sulfates for 50% inhibition of the viral sialidase and acid phosphatase reaction are shown in Fig. 3. The inhibitory activity of panosialin, its analogs and other detergents for the enzyme reactions are shown in Table 1.

Fig. 3. Concentrations of sodium-*n*-alkylsulfates for 50 % inhibition of narashino sialidase and acid phosphatase.



Panosialin, panosialin-w which was obtained by removal of one of the sulfuric acid groups from panosialine and *n*-alkyl (C_{13}, C_{15}, C_{19}) benzene-1, 3-disulfates which were prepared by synthesis[24] were tested for activity and compared with the activities of detergents such as sodium dodecyl sulfate, sodium dodecylbenzene sulfonate,

Table 1. The inhibitory activity of panosialin, panosialin-w, 5-alkylbenzene-1, 3-disulfates and detergents to inhibit viral sialidase, acid phosphatase and polygalacturonase

Detergents	ID_{50} (M)				
	PR-8	Narashino	HVJ	Acid phosphatase	Poly-galacturonase
Panosialin	5.0×10^{-5}	1.0×10^{-5}	4.3×10^{-5}	3.8×10^{-5}	3.9×10^{-5}
Panosialin-w[1]	6.3×10^{-4}	1.3×10^{-5}	6.3×10^{-5}	4.9×10^{-5}	5.7×10^{-5}
C_{13} [2]	1.3×10^{-4}	2.8×10^{-5}	5.7×10^{-5}	7.6×10^{-5}	4.9×10^{-5}
C_{15} [2]	5.6×10^{-5}	1.4×10^{-5}	5.0×10^{-5}	3.9×10^{-5}	2.2×10^{-5}
C_{19} [2]	4.6×10^{-5}	1.0×10^{-5}	4.7×10^{-5}	4.2×10^{-5}	1.5×10^{-5}
SDS [3]	9.0×10^{-4}	3.0×10^{-4}	2.7×10^{-4}	5.0×10^{-4}	4.3×10^{-4}
SDBS [4]	2.3×10^{-4}	9.0×10^{-5}	9.6×10^{-5}	1.3×10^{-4}	8.9×10^{-5}
DOC [5]	$>10^{-2}$	1.8×10^{-3}	1.4×10^{-3}	$>10^{-2}$	$>10^{-3}$
Tween-85 [6]	0	0	0	0	0
Triton X-100 [6]	0	0	0	0	0

1) The compound which was obtained by removal of one sulfuric acid group from panosialin. 2) Number of carbon atoms in 5-alkylbenzene-1,3-disulfates. 3) Sodium dodecyl sulfate. 4) Sodium dodecylbenzene sulfonate. 5) Sodium deoxycholate. 6) Inhibition percent at 500 μg/ml.

sodium deoxycholate, Tween-85 and Triton X-100. As shown in Fig. 3, the activities of *n*-alkylsulfates were dependent on the number of carbon atoms and increased with increase of carbon number up to 15~16. Pentadecyl- and hexadecylsulfate showed the strongest inhibition. As shown by 50% inhibition concentrations in Table 1, among dipotassium 5-alkylbenzene-1, 3-disulfates, pentadecylbenzene-1, 3-disulfate and panosialin were the most potent inhibitors.

Panosialin was not found in the screening studies of inhibitors of proteases, when casein and hemoglobin, and the culture filtrate were mixed before addition of the enzyme. In this case, panosialin must bind with the substrate (protein) and showed no inhibition. When panosialin was incubated with enzymes such as trypsin, papain and pepsin before addition of the substrate, panosialin showed a strong inhibition.

Biological Activities of Panosialin

After we isolated panosialin from *Streptomyces pseudoverticillus* and *S. rimosus*, we obtained the same compound from five other strains of streptomyces which belonged to other species. Therefore, panosialin is thought to be distributed widely among *Streptomyces*. However, it is a new finding to isolate this type of compound from microorganisms.

Panosialin at 100 μg/ml showed no inhibition against growth of Gram-negative bacteria in a nutrient medium. It showed a weak inhibition against Gram-positive bacteria with a minimal inhibitory concentration as follows: *Staphylococcus aureus* FDA 209 P, 50 μg/ml; *Sarcina lutea* PCI 1001, 50 μg/ml; *Micrococcus flavus*, 12.5 μg/ml; *Corynebacterium brevis* 1810, 100 μg/ml; *Bacillus subtilis* B 558, 12.5 μg/ml.

Panosialin had relatively low toxicity. Oral administration of 2,350 mg/kg or the intraperitoneal injection of 140 mg/kg did not cause the death of mice.

Against the NWS strain of influenza virus, panosialin showed inhibition of virus multiplication when it was mixed with the virus and intracerebrally administered. However, the effect of the intraperitoneal injection of panosialin on the infection of PR 8 strain in mouse lung was not significant. *In vitro*, myxoviruses were readily disrupted by panosialin and the electron microscopy showed the extensive disruption of the particles. The effect of panosialin for disruption of the virus particles was found to be more potent than sodium deoxycholate (DOC) and sodium dodecyl sulfate (SDS).

At the beginning of the study, panosialin was thought to be a macromolecular compound because it did not appear to dialyze. However, ultracentrifugation did not show a single molecular weight but indicated a distribution from 70,000 to 1,000,000. This is thought to be due to the aggregation of panosialin molecules. Aggregates thus formed should be polianionic macromolecules. Therefore, the panosialin is thought to exhibit the biological activities of macromolecular polyanionic compounds.

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