A-72363 A-1, A-2, and C, Novel Heparanase Inhibitors from *Streptomyces nobilis* SANK 60192

I. Taxonomy of Producing Organism, Fermentation, Isolation, and Structure Elucidation

TOSHIO TAKATSU, MASAAKI TAKAHASHI,[a] YUMI KAWASE,[a] RYUZO ENOKITA,[c] TAKAO OKAZAKI,[c] HIROSHI MATSUKAWA, KANEO OGAWA, YOSHIHARU SAKAIDA, TAKESHI KAGASAKI, TAKESHI KINOSHITA,[b] MOTOWO NAKAJIMA[d] and KAZUHIKO TANZAWA[a]

Biomedical Research Laboratories, [a] Biological Research Laboratories, [b] Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan
[c] Tsukuba Research Laboratories, Sankyo Co., Ltd., 33 Miyukigaoka, Tsukuba-shi, Ibaraki 305, Japan
[d] Institute of molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

(Received for publication May 17, 1995)

Novel heparanase inhibitors, A-72363 A-1, A-2, and C, were isolated from the culture filtrate of *Streptomyces nobilis* SANK 60192 by column chromatography on various resinous adsorbents, followed by preparative anion exchange HPLC. Spectroscopic studies revealed that they are diastereomers of siastatin B, a neuraminidase inhibitor.

It is recognized that heparanase plays a key role both in tumor cell breakdown of basement membranes during metastasis[1,2] and in angiogenesis.[3] Although inhibition of the enzyme by heparin, heparin derivatives, and suramin obstructs pulmonary metastasis of B16 melanoma cells,[4~6] they are substances of either high-molecular weight nature or multi-functional properties. As such, the discovery of specific low-molecular weight heparanase inhibitors will be invaluable towards the studies of tumor angiogenesis and metastasis from both basic and clinical standpoints. In the course of screening for heparanase inhibitors, the complex A-72363 was found in the culture filtrate of *Streptomyces nobilis* SANK 60192.

In this paper, we describe the taxonomy of the producing organism, as well as the fermentation, isolation, and structure elucidation of A-72363 components. Their biological properties will be reported in a subsequent paper.[7]

Materials and Methods

Taxonomic Studies

The producing organism, strain SANK 60192, was isolated from a soil sample collected in Okinawa Prefecture, Japan, 1989. Methods described by WAKSMAN[8] and the International Streptomyces Project (ISP)[9] were used for studies of the morphological and cultural characterization, carbohydrate utilization, and other taxonomic identification. The procedure of HASEGAWA *et al.*[10] was used for chromatographic detection of the isomers of diaminopimelic acid and of major whole-cell sugars.

Chemical Studies

Analytical HPLC was performed with a Shimadzu LC6A system. Preparative HPLC was done by a Senshu Scientific Liquid Chromatograph 6300 system. IR spectra were recorded on a Jasco IRA-302 spectrometer employing KBr pellet samples. UV spectra were recorded using water as solvent with a Hitachi 124 spectrophotometer. Optical rotations were measured in water with a Perkin-Elmer 241 polarimeter. ^{1}H (360 MHz) and ^{13}C (90 MHz) NMR spectra in 1 N ND_4OD were obtained on a Bruker AMX 360 spectrometer. Chemical shifts were calibrated with an internal HDO signal taken as δ 4.75 in ^{1}H NMR, and with a 1,4-dioxane signal taken as δ 67.4 in ^{13}C NMR. FAB-mass spectra were measured on a Jeol JMS-HX100 mass spectrometer.

Results and Discussion

Taxonomy of The Producing Strain

The strain formed primary or secondary verticils of short flexible spore chains (Fig. 1). Observation with a scanning electron microscope revealed the spores to be elliptical with a smooth surface (Fig. 2). No special morphological organs, such as sclerotia, sporangia, or fragmentation of hypha, were observed on the media employed. The culture characteristics on various agar media at 28°C for

Fig. 1. Light micrograph of strain SANK 60192 on potato extract-carrot extract agar at 28°C for 7 days.

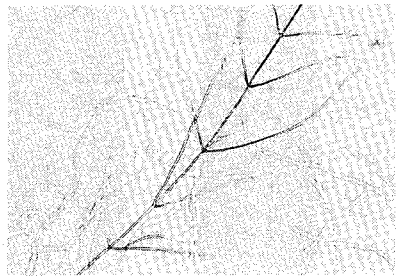


Fig. 2. Scanning electron micrograph of strain SANK 60192 on potato extract-carrot extract agar at 28°C for 7 days.

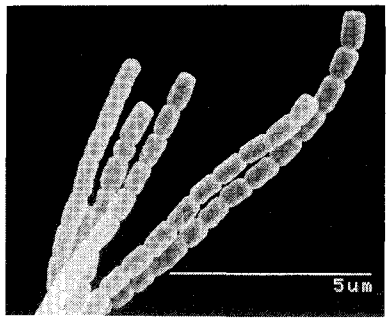


Table 1. Cultural characteristics of strains SANK 60192 and *Streptomyces nobilis* ATCC 19251.

		SANK 60192	ATCC 19251
Yeast extract-malt extract agar (ISP 2)	G	: Abundant, raised, light reddish orange	Good, raised, light brown
	AM	: Moderate, velvety, white	Moderate, velvety, pale orange
	R	: Dull reddish orange	Dark brown
	SP	: None	None
Oatmeal agar (ISP 3)	G	: Abundant, flat, reddish orange	Good, flat, reddish orange
	AM	: Moderate, velvety, pale orange	Moderate, velvety, white
	R	: Yellowish red	Pale red
	SP	: None	None
Inorganic salts-starch agar (ISP 4)	G	: Abundant, flat, reddish orange	Abundant, flat, dull red
	AM	: Good, floccose, pale orange	Good, floccose, pinkish white
	R	: Yellowish red	Dull red
	SP	: None	None
Glycerol-asparagine agar (ISP 5)	G	: Moderate, flat, orange	Moderate, flat, light yellowish orange
	AM	: Poor, white	Poor, white
	R	: Dull orange	Orange
	SP	: None	None
Peptone-yeast extract-iron agar (ISP 6)	G	: Good, raised, pale yellowish brown	Moderate, raised, light brownish gray
	AM	: Poor, white	None
	R	: Yellowish brown	Pale yellowish brown
	SP	: Yellowish brown	Grayish yellow brown
Tyrosine agar (ISP 7)	G	: Good, flat, orange	Moderate, flat, yellowish orange
	AM	: Poor, white	Poor, white
	R	: Orange	Orange to dark brown
	SP	: None	None
Sucrose-nitrate agar	G	: Good, flat, light reddish orange	Good, flat, light brown
	AM	: Moderate, velvety, white	Good, floccose, white
	R	: Light reddish orange	Dull reddish orange
	SP	: None	None
Glucose-asparagine agar	G	: Good, flat, reddish orange	Good, flat, red
	AM	: Good, floccose, pink	Moderate, velvety white
	R	: Light yellowish red	Pale red to red
	SP	: None	None
Nutrient agar (Difco)	G	: Abundant, raised, pale yellowish brown	Good, raised, dull yellowish orange
	AM	: Moderate, velvety, white	Poor, white
	R	: Light brown	Dull yellowish orange
	SP	: None	None
Potato extract-carrot extract agar	G	: Moderate, flat, pale yellow	Moderate, flat, light orange
	AM	: Poor, white	Poor, white
	R	: Pale yellow	Light orange
	SP	: None	None
Water agar	G	: Moderate, flat, pale yellowish orange	Poor, flat, pale brown
	AM	: Poor, white	Poor, white
	R	: Pale yellowish orange	Dull orange
	SP	: None	None

G: Growh, AM: aerial mycelium, R: reverse, SP: soluble pigment.

14 days are shown in Table 1. Strain SANK 60192 grew relatively well on both organic and synthetic media. The substrate mycelium was well developed and became pale yellow and turned orange to reddish orange in color. Aerial mycelium was well formed on ISP medium 4 and glucose-asparagine agar, but it often showed moderate growth on other media, and varied in mass color from white to pale orange. A pale yellowish brown soluble pigment was produced on ISP medium 6. The physiological properties of strain SANK 60192 are shown in Table 2. Nitrate reduction and xanthine decomposition were negative. Utilization of carbon sources is shown in Table 3. The whole-cell analysis of the strain showed the presence of LL-diaminopimelic acid and the absence of characteristic sugars, and it was classified as chemotype I/NC.

Based on the taxonomic properties described above, the strain SANK 60192 is considered to be a member of the genus *Streptomyces*. By comparison of the characteristics of strain SANK 60192 with the descriptions of various streptomycete species, especially the members of such a whorl-form group, *Streptomyces nobilis* was selected as the most closely related species, from its branching manner and aerial mass color. A direct comparison of strain SANK 60192 and the type strain ATCC 19251 of *S. nobilis* showed that they were very closely related. Although a few culture characteristics of the strain SANK 60192 differed from the type strain, in terms of nitrate reduction, melanoid pigment production in ISP medium 7, and L-arabinose utilization, as shown in Tables 1, 2, and 3, these differences were not significant enough to conclude that these two strains were different species. Therefore, the strain SANK 60192 was identified as *Streptomyces nobilis* SANK 60192.

The strain SANK 60192 has been deposited in the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Ibaraki Prefecture, Japan, with the accession number of FERM BP-4000.

Table 2. Physiological properties of strains SANK 60192 and *Streptomyces nobilis* ATCC 19251.

	SANK 60192	ATCC 19251
Hydrolysis of starch	Positive	Positive
Liquefaction of gelatin	Positive	Positive
Reduction of nitrate	Negative	Positive
Coagulation of milk	Positive	Positive
Peptonization of milk	Positive	Positive
Temperature range for growth (Medium 1)	12～35℃	12～39℃
Optimum temperature for growth (Medium 1)	23～30℃	23～32℃
Sodium chloride resistance (Medium 1)	10%	7%
Decomposition of:		
Casein	Positive	Positive
Tyrosine	Positive	Positive
Xanthine	Negative	Negative
Production of melanoid pigment on:		
(Medium 2)	Negative	Negative
(Medium 3)	Positive	Positive
(Medium 4)	Negative	Positive

Medium 1: Yeast extract-malt extract agar (ISP 2).
Medium 2: Tryptone-yeast extract broth (ISP 1).
Medium 3: Peptone-yeast extract-iron agar (ISP 6).
Medium 4: Tyrosine agar (ISP 7).

Fermentation

One loopful of the growth of strain SANK 60192 was introduced into 500-ml Erlenmeyer flasks containing 100 ml of a medium composed of glucose 6.0%, soybean meal 1.2%, Nisshoku gluten meal 1.2%, Mikunipeptone 0.5%, Oriental yeast extract 0.5%, $MgSO_4 \cdot 7H_2O$ 0.2%, KH_2PO_4 0.05%, $CaCO_3$ 0.3%, and Nissan Disfoam CB 442 0.02%. The pH of the medium was adjusted to 7.3 before sterilization. The flasks were cultivated on a rotary shaker at 28°C for 72 hours. After inoculation of 1.5 liters of the seed culture into a 30-liter jar-fermentor containing 15 liters of the medium described above, fermentation was carried out for 168 hours at 28°C under an aeration rate of 15 liters/minute at an inner pressure of 0.5 kg/cm^2. The progresses of the fermentation and isolation were monitored by heparanase inhibitory activity, measured by the method described in the preceding paper.[11]

Isolation

The purification procedure of the complex, A-72363 is outlined in the flow diagram as shown in Fig. 3.

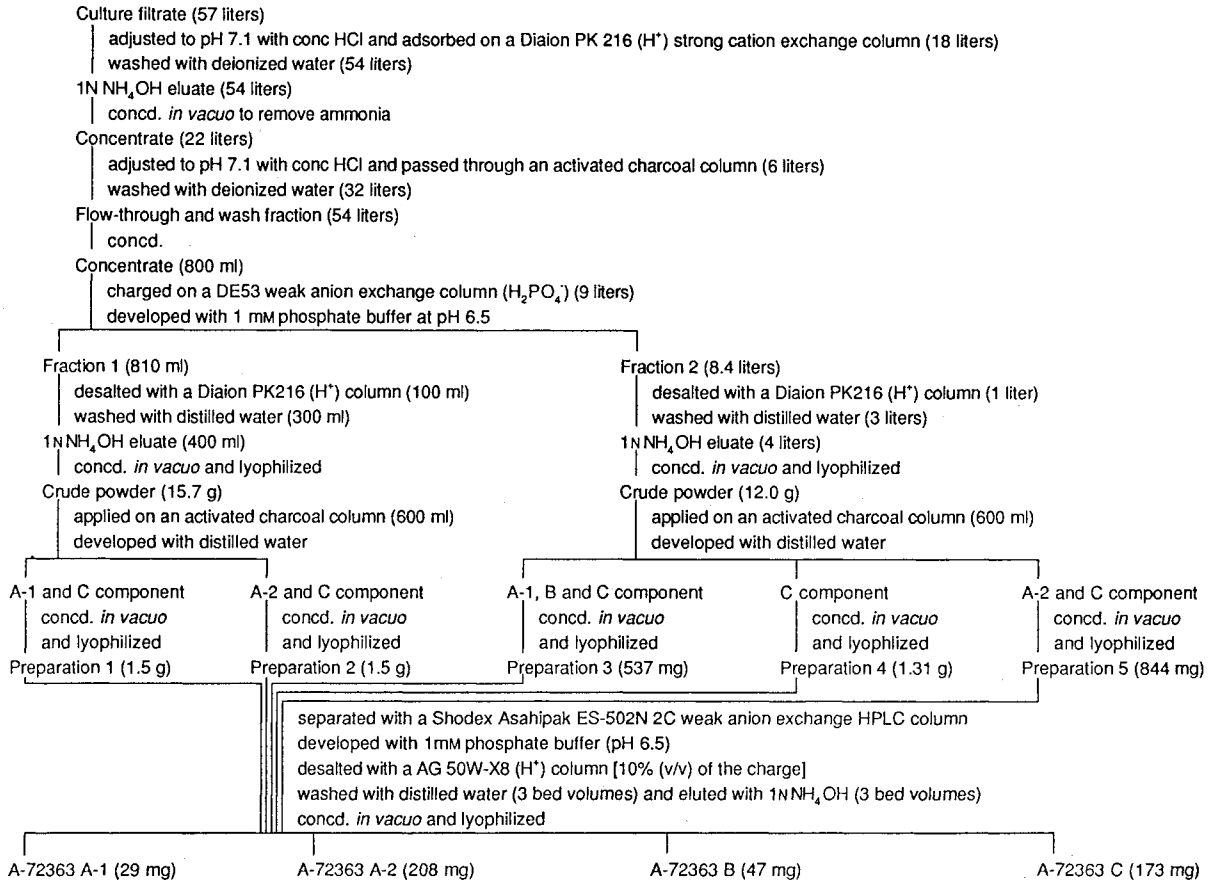
The filtrate (57 liters) from the cultured broth was adjusted to pH 7.1 with conc HCl and applied on a Diaion PK 216 (H^+) column (Mitsubishi Chemical Ind.

Table 3. Carbohydrate utilization of strains SANK 60192 and *Streptomyces nobilis* ATCC 19251.

	SANK 60192	ATCC 19251
D-Glucose	+	+
L-Arabinose	−	+
D-Xylose	±	±
Inositol	+ +	+ +
D-Mannitol	+ +	+ +
D-Fructose	±	+
L-Rhamnose	−	−
Sucrose	−	−
Raffinose	+	+
Control	−	−

+ +: Strongly positive utilization.
+: Positive utilization.
±: Utilization doubtful.
−: Negative utilization.

Fig. 3. Isolation procedure for the A-72363 complex.



Ltd., Japan). After washing the column with deionized water, the active substance was eluted with 1 N NH_4OH. To remove ammonia, the eluate was concentrated *in vacuo* and adjusted to pH 7.1 with conc HCl; then it was rapidly chilled to 4°C. Since the active compound was very unstable at room temperature, especially under acidic circumstances, all of the purification thereafter was performed at 4°C, except for HPLC. The concentrate was passed through a column of activated charcoal (Wako Pure Chemical Ind., Ltd., Japan, chromatographic grade), and the column was washed with deionized water. The flow-through and wash fractions were combined and concentrated, and then the concentrate was equilibrated with a 1 mM phosphate buffer system at pH 6.5. The preparation was chromatographed on a DE 53 column (Whatman Paper Ltd., England) with 1 mM phosphate buffer at pH 6.5 to resolve two fractions based on the separation from impurities. Both fractions were desalted with a Diaion PK 216 (H^+) column in the same manner as the first step. Succeeding evaporation *in vacuo* and lyophilization afforded 15.7 g and 12.0 g of crude powder, respectively. These preparations were applied on columns of activated charcoal, and the columns were developed with deionized water. Since HPLC detection of the components was practicable after the charcoal column, the performance of the chromatographic processes thereafter was monitored by an analytical HPLC system with a weak anion exchange column, Shodex Asahipak ES-502N 7C (Showa Denko K. K., 7.6 × 100 mm), equilibrated with 1 mM phosphate buffer at pH 6.5 and eluted at a flow rate of 1 ml/minute. The components, designated as A-72363 A-1, A-2, B, and C, in the order of their elution on the HPLC system, were detected by UV absorption at 210 nm. Five fractions obtained from the activated charcoal columns were finally charged repetitiously on a preparative HPLC column (Shodex Asahipak ES-502N 2C, 21.5 × 100 mm, Showa Denko K. K.), equilibrated with 1 mM phosphate buffer at pH 6.5 and developed at a flow rate of 5 ml/minute. The combined four pure preparations were desalted with AG 50W-X8 (H^+) Resin (Bio-Rad Laboratories, 200 ~ 400 mesh). Evaporation *in vacuo* and lyophilization of these solutions yielded 29 mg of A-72363 A-1, 208 mg of A-2, 47 mg of B, and 173 mg of the C component as is in pure form.

Physicochemical Properties and Structure Elucidations

A-72363 A-1, A-2, B, and C are all water-soluble, amphoteric white powders, and they exhibit end-absorption in the UV spectra. The molecular formulae of all the components were revealed to be the same: $C_8H_{14}N_2O_5$ (MW 218), based on their quasi-molecular ions in positive and negative high-resolution FAB-mass spectra. The signals in the ^1H NMR spectra and ^{13}C NMR spectra of these compounds accounted for the existence of 9 protons and 8 carbons, respectively (see Table 4). Assignments of the signals were carried out by DEPT and HETCOR. In the ^{13}C NMR spectra, two resonances around δ_C 175 and 180 were assigned to an amide and a carboxyl group, which is consistent with IR absorption bands at 1590 and 1673 cm^{-1} and with interaction of the compounds with DE53. These data suggested that A-72363 A-1, A-2, B, and C were stereoisomers of each other.

Comparison of ^1H and ^{13}C NMR spectra with those of siastatin B revealed that component B was identical with siastatin B, a potent neuraminidase inhibitor.[12] Thus, further studies for the structure elucidation were performed on the A-72363 A-1, A-2, and C components.

The structures of these compounds, shown in Fig. 4, were mainly elucidated using ^1H-^1H COSY, correlation spectroscopy *via* long-range coupling (COLOC),[13] long-range selective proton decoupling (LSPD), and NOESY experiments. In each compound, connectivity from C-2 to C-6 was straightforwardly revealed by a ^1H-^1H COSY experiment. C-H long-range couplings from H-2a and H-2b to C-6 proved the presence of a six-membered ring. The presence of an acetyl group at C-6 and of a carboxyl group at C-3 were also confirmed by their C-H long-range couplings, as shown in Fig. 5.

Table 4. ^1H and ^{13}C NMR assignments for A-72363 A-1, A-2, B (siastatin B), and C.

	A-1		A-2	
No.	δ_C	δ_H	δ_C	δ_H
2	41.9 t	2.93 dd (6.4, 13.5)[a]	45.4 t	2.57 t (11.6)[a]
		2.98 dd (4.7, 13.5)[b]		3.11 dd (3.4, 11.6)[b]
3	49.1 d	2.62 ddd (4.7, 6.4, 7.3)	48.1 d	2.49 ddd (3.4, 11.1, 11.6)
4	70.0 d	4.22 dd (3.1, 7.3)	72.1 d	3.87 dd (3.0, 11.1)
5	69.8 d	3.73 dd (3.1, 6.1)	69.8 d	3.86 br. t (~2)
6	63.9 d	4.74 d (6.1)	64.3 d	4.51 d (1.6)
3-Carboxyl				
	180.8 s		181.3 s	
6-*N* Acetyl				
	22.9 q	2.03 s	22.8 q	2.08 s
	175.0 s		174.2 s	

	B		C	
No.	δ_C	δ_H	δ_C	δ_H
2	41.5 t	3.00 t (12.5)[a]	38.0 t	3.06 dd (9.9, 13.0)[a]
		2.90 dd (4.7, 12.5)[b]		2.76 dd (4.4, 13.0)[b]
3	49.1 d	2.49 ddd (2.3, 4.7, 12.5)	48.3 d	2.55 dt (4.4, 9.9)
4	72.3 d	4.38 br. t (~2.5)	70.6 d	4.27 br. t (~4)
5	70.8 d	3.49 dd (3.0, 9.7)	68.9 d	3.79 t (4.1)
6	62.4 d	4.60 d (9.7)	62.9 d	4.80 d (4.1)
3-Carboxyl				
	180.1 s		180.6 s	
6-*N* Acetyl				
	22.9 q	2.03 s	23.2 q	2.05 s
	175.6 s		175.2 s	

a: Axial proton, b: equatorial proton.

Fig. 4. Structures of A-72363 A-1, A-2, B (siastatin B), C, and nagstatin.

a: Relative configuration, b: absolute configuration.

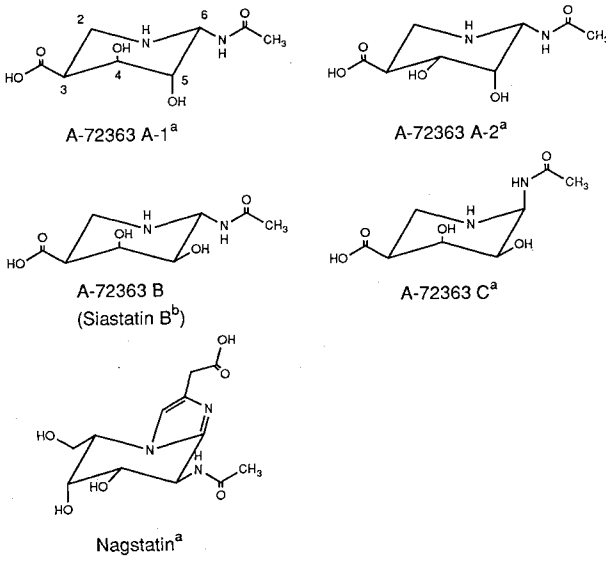
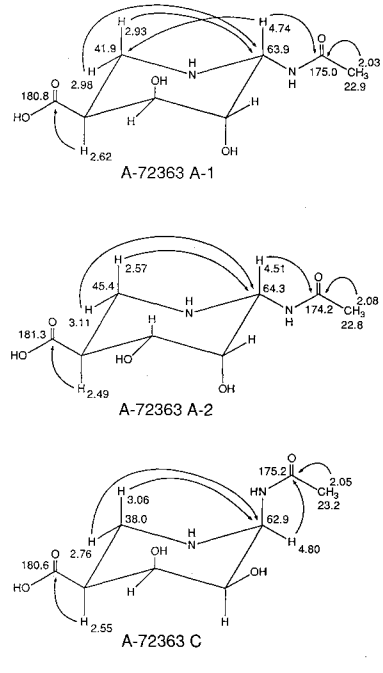


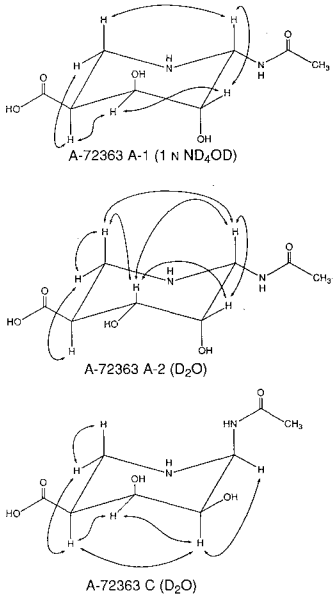
Fig. 5. Informative C-H long-range couplings.



The ^1H and ^{13}C chemical shifts of position 2 ($\delta_H \sim 2.9$ and $\delta_C \sim 42$) and 6 ($\delta_H \sim 4.8$ and $\delta_C \sim 63$) suggested that these carbons should be connected *via* a nitrogen atom, and thus the position 6 was characterized as an anomeric center with two adjacent nitrogen atoms. Therefore, the six-membered ring was characterized as piperidine, and the acetyl group attached to C-6 was ascribed as an *N*-acetyl. The relative configurations of these compounds were determined by the observations of NOEs (Fig. 6) and the values of the ^1H-^1H coupling constants (Table 4).

Accordingly, as shown in Fig. 4, the structures of A-72363 A-1, A-2, and C were elucidated to be 5-episiastatin B, 4,5-diepisiastatin B, and 6-episiastatin B, respectively. Considering the coupling constants of H-2a to H-3 (6.4 Hz) and H-2b to H-3 (4.7 Hz) for A-1 component, it may be possible to say that the conformation of piperidine in this case is not a typical chair form. UMEZAWA *et al.* had reported siastatin A as another neuraminidase inhibitor,[12] but A-72363 A-1, A-2, and C are clearly distinct from it due to their lack of neuraminidase inhibitory activity as described in the subsequent paper.[7] Although 3-episiastatin B and 3,4-diepisiastatin B had been synthesized by NISHIMURA *et al.* as influenza virus neuraminidase inhibitors,[14] A-72363 A-1, A-2, and C possess different optical rotations: $[\alpha]_D^{23}$ +29.0° (*c* 1.0, H_2O), $[\alpha]_D^{23}$ −31.0° (*c* 1.0, H_2O), and $[\alpha]_D^{27}$ −59° (*c* 0.66, H_2O), respectively, and their configurations established by exact NMR studies were distinct from those of 3-episiastatin B and 3, 4-diepisiastatin B. Therefore, A-72363 A-1, A-2, and C were demonstrated to be novel compounds.

Fig. 6. Observed NOEs.



( ): Solvent used.

During another purification trial, nagstatin (Fig. 4), which is known to be a potent *N*-acetyl-*β*-D-glucosaminidase inhibitor,[15,16] was isolated from the culture filtrate and identified (data not shown). It is interesting to note that the A-72363 producing strain, which was designated as a different species from the siastatin B or nagstatin producers, produces a variety of glycosidase inhibitors in the same culture.

Acknowledgment

The authors are grateful to Dr. NAGANAWA of the Institute of Microbial Chemistry, Tokyo, Japan, for providing high-resolution ^1H and ^{13}C NMR spectral data of siastatin B.

References

1) KRAMER, R. H.; K. G. VOGEL & G. L. NICOLSON: Solubilization and degradation of subendothelial matrix glycoproteins and proteoglycans by metastatic tumor cells. J. Biol. Chem. 257: 2678~2686, 1982
2) NAKAJIMA, M.; T. IRIMURA, D. DI FERRANTE, N. DI FERRANTE & G. L. NICOLSON: Heparan sulfate degradation: Relation to tumor invasive and metastatic properties of mouse B16 melanoma sublines. Science 220: 611~613, 1983
3) VLODAVSKY, I.; G. KORNER, R. ISHAI-MICHAELI, P. BASHKIN, R. BAR-SHAVIT & Z. FUKS: Extracellular matrix-resident growth factors and enzymes: Possible involvement in tumor metastasis and angiogenesis. Cancer Metastasis Rev. 9: 203~226, 1990
4) IRIMURA, T.; M. NAKAJIMA & G. L. NICOLSON: Chemically modified heparins as inhibitors of heparan sulfate specific endo-*β*-glucuronidase (heparanase) of metastatic tumor cells. Biochemistry 25: 5322~5328, 1986
5) SAIKI, I.; J. MURATA, M. NAKAJIMA, S. TOKURA & I. AZUMA: Inhibition by sulfated chitin derivatives of invasion through extracellular matrix and enzymatic degradation by metastatic melanoma cells. Cancer Res. 50: 3631~3637, 1990
6) NAKAJIMA, M.; A. DECHAVIGNY, C. E. JOHNSON, J. HAMADA, C. A. STEIN & G. L. NICOLSON: Suramin: A potent inhibitor of melanoma heparanase and invasion. J. Biol. Chem. 266: 9661~ 9666, 1991
7) KAWASE, Y.; M. TAKAHASHI, T. TAKATSU, M. ARAI, M. NAKAJIMA & K. TANZAWA: A-72363 A-1, A-2, and C, novel heparanase inhibitors from *Streptomyces nobilis* SANK 60192. II. Biological activities. J. Antibiotics 49: 61~64, 1996
8) WAKSMAN, S. A. (*Ed.*): The Actinomycetes Vol. 2. Classification, Identification and Description of Genera and Species. Williams & Wilkins Co., Baltimore, 1961
9) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of Streptomyces species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
10) HASEGAWA, T.; M. TAKIZAWA & S. TANIDA: A rapid analysis for chemical grouping of aerobic actinomycetes.

J. Gen. Appl. Microbiol. 29: 319~322,1983

11) SHIOZAWA, H.; M. TAKAHASHI, T. TAKATSU, T. KINOSHITA, K. TANZAWA, T. HOSOYA, K. FURUYA, S. TAKAHASHI, K. FURIHATA & H. SETO: Trachyspic acid, a new metabolite produced by *Talaromyces trachyspermus*, that inhibits tumor cell heparanase: Taxonomy of the producing strain, fermentation, isolation, structural elucidation, and biological activity. J. Antibiotics 48: 357~362, 1995

12) UMEZAWA, H.; T. AOYAGI, T. KOMIYAMA, H. MORISHIMA, M. HAMADA & T. TAKEUCHI: Purification and characterization of a sialidase inhibitor, siastatin, produced by Streptomyces. J. Antibiotics 27: 963~969, 1974

13) KESSLER, H.; C. GRIESINGER, J. ZARBOCK & H. R. LOOSLI: Assignment of carbonyl carbons and sequence analysis in peptides by heteronuclear shift correlation *via* small coupling constants with broadband decoupling in t_1 (COLOC). J. Magn. Res. 57: 331~336, 1984

14) NISHIMURA, Y.; Y. UMEZAWA, S. KONDO, T. TAKEUCHI, K. MORI, I. KIJIMA-SUDA, K. TOMITA, K. SUGAWARA & K. NAKAMURA: Synthesis of 3-episiastatin B analogues having anti-influenza virus activity. J. Antibiotics 46: 1883~1889, 1993

15) AOYAGI, T.; H. SUDA, K. UOTANI, F. KOJIMA, T. AOYAMA, K. HORIGUCHI, M. HAMADA & T. TAKEUCHI: Nagstatin, a new inhibitor of *N*-acetyl-β-D-glucosaminidase, produced by *Streptomyces amakusaensis* MG846-fF3. Taxonomy, production, isolation, physico-chemical properties and biological activities. J. Antibiotics 45: 1404~1408, 1992

16) AOYAMA, T.; H. NAGANAWA, H. SUDA, K. UOTANI, T. AOYAGI & T. TAKEUCHI: The structure of nagstatin, a new inhibitor of *N*-acetyl-β-D-glucosaminidase. J. Antibiotics 45: 1557~1558, 1992