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Antibiotics Aminosidin. II.[1] Some Amino Derivatives of Aminosidin and Their Biological Activity[2]

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Three penta-N-phenylalkylideneaminosidins, two penta-N-phenylalkylaminosidins, and two N-methylaminosidins were prepared, and their antimicrobial activities *in vitro* were determined. Penta-N-phenylalkylideneaminosidins had antimicrobial activity approximately equal to or slightly less than that of aminosidin. However, penta-N-phenylalkylaminosidins and N-methylaminosidins were devoid of antimicrobial activity.

Penta-N-benzylideneaminosidin protected mice from death by *Staphylococcus aureus* and *Escherichia coli* infections, and acute toxicity of the penta-N-benzylideneaminosidin was less than that of aminosidin by a subcutaneous route.

In the preceding paper,[1] we revealed that water-soluble basic antibiotics, aminosidin separated from the fermented broth of *Streptomyces chrestomyceticus*,[4] consisted of two components, I and II, and that aminosidin-I was identical with paromomycin-I, and aminosidin-II with paromomycin-II. Aminosidin-I had 2,6-diamino-2,6-dideoxy-L-idose (neosamine-B) as its partial amino-sugar moiety, and aminosidin-II, 2,6-diamino-2,6-dideoxy-D-glucose (neosamine-C).

In the present work, some penta-N-substituted derivatives of aminosidin were prepared for examination of biological activity, especially to learn the structure-activity relations *in vitro* and *in vivo*.

Miyaki, *et al.*[5] and others[6] published the tetra-N-phenylalkylidene derivatives of kanamycin, and also Fujii, *et al.*[7] reported that penta-N-phenylalkyl derivatives of kanamycin exhibited antimicrobial activity *in vitro*, especially to kanamycin-resistant bacteria.

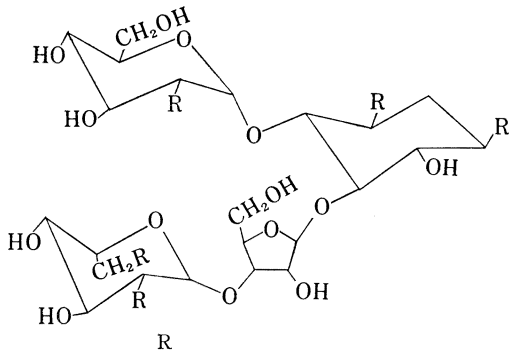
Cooper, *et al.*[8] reported that N-phenylalkylidenegentamicin-C_2 derivatives had a strong antimicrobial activity *in vitro* and the derivatives maintained their blood level. Therefore, aminosidin derivatives considered to be stable under physiological conditions were expected to have biological activities different from kanamycin or gentamicin C_2 derivatives.

Preparation of Aminosidin Derivatives

Biological activity of a minor component, aminosidin-II, in aminosidin complex was similar to that of aminosidin-I. Therefore, aminosidin complex (I) was used as the starting material.

Penta-N-benzylideneaminosidin (II), penta-N-salicylideneaminosidin (III), and penta-N-anisylideneaminosidin (IV) were prepared by the condensation of aminosidin (I) and excess of the corresponding aldehyde. Shiff's bases, released from the reaction mixture as an oily substance, were decanted, and the substances were reacted further with the aldehyde in

1) Part I: H. Taniyama, Y. Sawada, K. Hashimoto, Y. H. Chung, and T. Kitagawa, *Yakugaku Zasshi*, **91**, 1362 (1971).
2) Paper read at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1972.
3) Location: *1-14, Bunkyo-machi, Nagasaki, 852, Japan.*
4) F. Arcamone, C. Bertazzoli, M. Ghione, and T. Scotti, *G. Microbiol.*, **7**, 242, 251 (1959).
5) Miyaki and T. Yasuda, *Annu. Rep. Inst. Food Microbiol.*, **14**, 39 (1961).
6) Brit. Pat. 833851 (1960), (to Bristol Laboratories).
7) A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiotics*, **21**, 340 (1968).
8) D.J. Cooper, J. Weinstein, and J.A. Waitz, *J. Med. Cem.*, **14**, 1118 (1971).



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I : $-NH_2$

II : $-N=CH-$ 

III : $-N=CH-$ 
OH

IV : $-N=CH-$  $-OCH_3$

V : $-NHCH_3$

VI : $-N(CH_3)_2$

VII : $-NHCH_2-$ 

VIII : $-N(CH_3)-CH_2-$ 

Chart 1

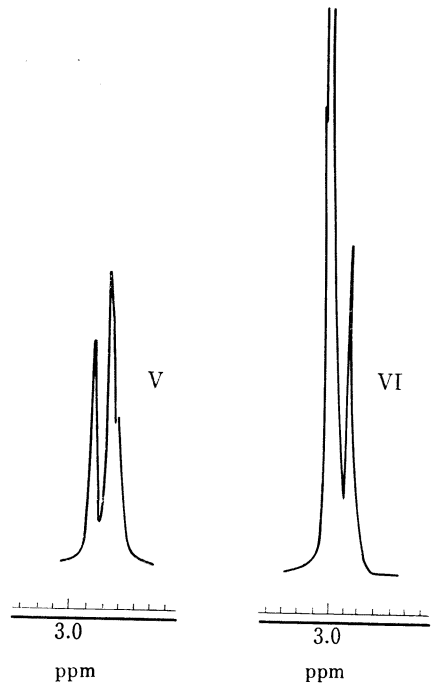

Fig. 1. NMR Spectra of N-Methyl Groups of V and VI in D_2O

methanol-chloroform solution. The reaction mixture was dried up to a viscous residue and then the Shiff's base was obtained as a crystalline powder by reprecipitation.

Deca-N-methylaminosidin (VI) was prepared by reductive methylation[9] of aminosidin and formaldehyde in the presence of platinum dioxide, followed by reprecipitation with water-acetone. Penta-N-benzylaminosidin (VII), synthesized by hydrogenation of penta-N-benzylideneaminosidin (II) over platinum dioxide, was reduced to give the penta-N-benzyl- and penta-N-methylaminosidin (VIII). The VIII was debenzylated to give the penta-N-methylaminosidin (V) in presence of palladium-carbon catalyst. Debenzylation was not easy in spite of the addition of excess catalyst under warming. Therefore, the reaction mixture of the final step was chromatographed over cellulose column and Sephadex G-10 column.

Table I. Chromatographic Comparison of Aminosidin and Its N-Methyl Derivatives

Compound	*Rf* values		
	TLC(silica gel)[a]	TLC(Avicel-SF)[b]	PPC[c]
	n-PrOH–HOAc–pyridine $-H_2O$(15:3:10:10)	*n*-PrOH–HOAc–pyridine $-H_2O$ (9:1:1:10)	*n*-BuOH–HOAc–pyridine $-H_2O$-*t*-BuOH(15:3:10:12:4)
I	0.84	0.21	0.20
II	0.31	0.90	0.27
VI	0.16	0.94	0.29

a) Merck product
b) Funakoshi product
c) Toyo-Roshi No. 51 UH-type

9) P. Claes and H. Vanderhaeghe, *Bull. Soc. Chim. Belges*, **78**, 561 (1969).

Compound V was obtained in about 80% yield from VIII.

These aminosidin derivatives were determined from their ultraviolet (UV) spectra, and infrared (IR) spectra, elemental analysis data, and others. For example, nuclear magnetic resonance (NMR) spectra in heavy water (D_2O) showed the N-methyl protons for the compound VI at δ 3.05—2.80(10×-CH_3), and at δ 2.91—2.63(5×-CH_3) for the compound V based on an anomeric proton respectively. Two N-methylaminosidins were compared by thin-layer chromatography (TLC) and paper chromatography (PPC), and the result is presented in Table I.

Compounds II, III, IV, VII, and VIII were slightly soluble in neutral water, soluble in alcohols, chloroform, and dioxane. Therefore, these compounds were considered to be rather lipophilic in contrast to aminosidin.

Penta-N-benzylidenekanamycin and penta-N-benzylkanamycin were also prepared by the method described in the literatures.[5,7]

Antimicrobial Activity of Aminosidin Derivatives *in Vitro*

The antimicrobial activity of aminosidin and its derivatives was determined by the agar-streak method (serial double dilutions) on nutrient agar (pH 7.0). Minimum inhibitory concentration (MIC) of aminosidin and its derivatives is shown in Tables II and III. Aminosidin showed antimicrobial activity against gram-positive and -negative bacteria as reported in the preceding paper.[1] Penta-N-phenylalkylideneaminosidins has an activity qualitatively

TABLE II. Antimicrobial Activity of Aminosidin and Kana mycin Derivatives *in vitro*

Test organism	MIC(μg/ml) Compounds										
	I[a]	II[b]	III[b]	IV[b]	V[a]	VI[a]	VII[b]	VIII[b]	IX[a]	X[b]	XI[b]
Staphylococcus aureus ATCC 6538P	0.16	0.16	0.31	0.62	31.2	>996	63	7.8	0.052	0.10	52
Streptococcus faecalis ATCC 10541	3.90	3.90	7.8	3.90	>996	>996	498	126	26	52	420
Bacillus subtilis # 10707	0.08	0.16	0.16	0.16	10.6	>996	63	3.9	0.026	0.052	6.5
Escherichia coli ATCC 26	1.26	1.2	1.2	2.4	252	>996	>996	498	0.41	0.41	830
Klebsiella pneumoniae ATCC 10031	0.32	0.3	0.62	5.0	63	>996	498	498	0.052	0.10	100
Proteus vulgaris ATCC 6897	2.52	2.4	2.4	>10	252	>996	>996	>996	0.21	0.41	830
Pseudomonas aeruginosa BMH # 1	0.98	1.95	1.95	1.95	252	>996	>996	>996	6.5	13	420
Shigella sonnei ATCC 9290	2.52	2.4	2.4	10	252	>996	>996	252	0.41	0.82	830
Salmonella typhosa ATCC 9992	0.63	1.2	1.2	1.2	126	>996	498	498	0.1	0.21	420
Escherichia coli R_3 (R-SM,KM,CP,PRM)	1000	500	125	1000	>996	>996	>996	252	>830	830	830

solvents: *a*) H_2O, *b*) MeOH
R-SM, KM, CP, PRM: resistant to streptomycin, kanamycin, chloramphenicol, and paromomycin
compounds:
I : aminosidin sulfate
II : penta-N-benzylideneaminosidin
III : penta-N-salicylideneaminosidin
IV : penta-N-anisilideneaminosidin
V : penta-N-methylaminosidin acetate
VI : deca-N-methylaminosidin sulfate
VII : penta-N-benzylaminosidin free base
VIII : penta-N-benzyl, and penta-N-methylaminosidin free base
IX : kanamycin sulfate
X : tetra-N-benzylidenekanamycin
XI : tetra-N-benzylkanamycin free base

and quantitatively equal to or slightly less than that of aminosidin. It can be assumed that penta-N-phenylalkylideneaminosidins are rather unstable in solution, liberate the substituents gradually and then exhibit the antimicrobial activity.

TABLE III. Antimicrobial Activity of Aminosidin and Its Derivatives *in Vitro*

Test organism	MIC(μg/ml)		
	I	II	VII
Staphylococcus aureus 209P	2.5	2.5	>50
Sarcina lutea	2.5	2.5	>50
Bacillus subtilis	1.25	1.25	>50
Shigella flexneri	12.5	12.5	>50
Salmonella abortivoequina	6.25	12.5	>50
Escherichia coli B	6.25	1.25	>50
Staphylococcus aureus[a)]	12.5	25	>50

a) requires aminosidin for growth[10)]

On the contrary, penta-N-phenylalkylaminosidins and N-methylaminosidins were less active than aminosidin, but some differences in the extent of the diminution are observed among the derivatives and test bacteria.

In penta-N-methylaminosidin (V), the diminution in activity against all the test bacteria is smaller than that of deca-N-methylaminosidin (VI). This shows that the antimicrobial activity of aminosidin may be related with a hydrogen atom in the amino group of aminosidin. And also the diminution in activity of compounds VII and VIII is smaller than other test bacteria against both *Staphylococcus aureus* and *Bacillus subtilis*.

Shiff's base III and the compound VIII have activity against streptomycin, kanamycin, chloramphenicol, and paromomycin-resistant strain of *Escherichia coli*.

TABLE IV. Activity of Aminosidin and Its Derivatives against Experimental Infection by *Staphylococcus aureus* PV-I in Mice

Compounds	Administration route	Dose (mg/kg)	Mortality[a)] (%)	Days survived 50% of mice tested	Dose for survival of 50% of mice tested(mg/kg)
Control	—	—	100	5	—
I	subcutaneous	25	8	>10	
		12.5	25	>10	9
		6.25	83	6	
II		25	0	>10	
		12.5	25	>10	8.5
		6.25	75	7	
VII		25	75	6	
		12.5	75	6	>25
		6.25	83	7	
I	oral	25	66	5	
		12.5	66	6	>25
		6.25	100	7.5	
II		25	58	5	
		12.5	66	7	>25
		6.25	83	7	

a) mortality of mouse on the 10 days after
Compounds II and VII dissolved in a small volume of MeOH was diluted with aseptic water and aminosidin (I) was diluted only with aseptic water.
Mouse: COB_5 strain, 20±1 g, 12 animals/group (6 females and 6 males)
Inoculum: 3.3×10^8 cells/mouse by *i. p.* route

In Table III, the value for *Staphylococcus aureus* which needs aminosidin for its growth shows the minimum concentrations of aminosidin to develop itself at its maximum.[10]

Therapeutic Effects on the Experimental Infections

1) Against *Staphylococcus aureus* PV-I——Therapeutic test was carried out in mice infected with *Staphylococcus aureus* PV-I. Compounds were administered to animals infected at a daily dose of 25, 12.5, 6.25 mg/kg respectively by a single subcutaneous injection for four consecutive days starting 4 hr after the injection. Data in Table IV show that aminosidin (I) and penta-N-benzylideneaminosidin (II) have similar activity by subcutaneous route, but penta-N-benzylaminosidin (VII) is not active at the dosage tested.

Penta-N-benzylideneaminosidin (II) was tested by oral administration in order to examine eventual intestinal absorption. The activity was very low, and penta-N-benzylideneaminosidin (II) seemed not to be absorbed or absorbed only in a very small amount.

2) Against *Staphylococcus aureus* Smith——A test was carried out on mice infected with a strain of *Staphylococcus aureus* Smith. Compounds were administered by single intraperitoneally route starting 2 hr after the infection. Penta-N-benzylideneaminosidin (II) was active as aminosidin, but penta-N-benzylaminosidin (VII) was low active as shown in Fig. 2.

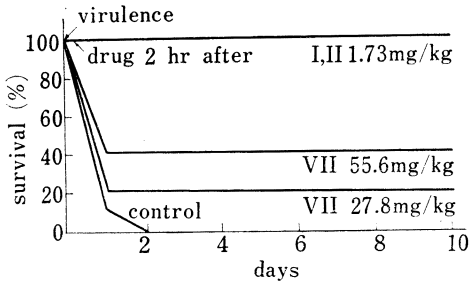

Fig. 2. Activity of Aminosidin and Its Derivatives against Experimental Infection by *Staphylococcus aureus* Smith in Mice(*i.p.*)

mouse: dd strain, male 18±1 g, 5 animals/group
inoculum: 3.3×10^8 cells/mouse

3) Against *Escherichia coli* GN 2411-5——Protective effects on the infection caused by *Escherichia coli* GN-2411-5 in mice were investigated and the results are shown in Table V. Penta-N-benzylideneaminosidin (II) was active, but penta-N-benzylaminosidin (VII) was almost inactive.

TABLE V. Activity of Aminosidin and Its Derivatives for 7 Days after against Experimental Infection by *Escherichia coli* GN 2411-5 in Mice(*i.p.*)

Compound	Dose (mg/kg)						
	6.95	13.9	27.8	55.6	111.2	222.4	Control
I	60	80	100	100	100	—	0
II	20	40	80	80	100	0	
VII	—	—	—	—	0	20	

mouse: dd strain, male, 18± 1 g, 5 animals/group
inoculum: 4.6×10^6 cells/mouse (*i.p.*)

Thus, only penta-N-benzylideneaminosidin (II) among the tested derivatives protected mice from death by microorganism infections. These data confirmed the results obtained *in vitro*.

Acute Toxicity of Aminosidin Derivatives

Acute toxicity of the compounds II and VII was tested in mice by a subcutaneous injection. Compounds suspended with carboxymethylcellulose (CMC, 1%) was dissolved with aseptic water. 50% Lethal dose (LD_{50}) was obtained by van der Wäden method after 7 days. Acute toxicity of II was less than that of aminosidin (I) as shown in Table VI.

10) L. Corini, R. Rosset, and R.A. Zimmermann, *Science*, **157**, 1314 (1967).

Table VI. Acute Toxicity of Aminosidin and Its Derivatives in Mice(*s.c.*)

Administration route	LD_{50}(mg/kg)		
	I	II	VII
subcutaneous	615.6	>1000	>1000

Compounds II and VII were diluted with aseptic water in the presence of CMC (1%), and aminosidin (I) was diluted with aseptic water, and 0.5 ml of the solution was administrered to each mouse (dd strain, male, 20±1 g, 5 animals/group).

Discussion

Among the N-substituted derivatives of aminosidin, penta-N-benzylideneaminosidin has antimicrobial activity equal to aminosidin *in vivo*, in addition to lower toxicity than aminosidin in mice. Therefore, other penta-N-phenylalkylideneaminosidins prepared in the present series seems to be active *in vivo*. Penta-N-phenylalkylaminosidins and N-methylaminosidins are inactive *in vitro* or *in vivo*. Thus N-substitution affects the biological activity of aminosidin.

Tetra-N-benzylidenekanamycin (X) has the same or half antimicrobial activity comparing with that of kanamycin (IX), while tetra-N-benzylkanamycin (XI) has lower activity. These antimicrobial activities are similar to those of the corresponding aminosidin derivatives as shown in Table II. It would be interesting to study that the bulky N-substituents must have a hindrance effect[7] on cell wall permeability and complex formation with the site of action, that is, ribosomal mRNA complex. The diminution in antimicrobial activity due to N-substituted derivatives can be explained by the hindrance effect.

Samuel *et al.*[11] reported that inactive N-methylneomycin and N-methylparomomycin had an activity by oral route to decrease a serum cholesterol. Therefore, it seems that the activity decreasing serum cholesterol level is different from an antimicrobial activiyt because slight absorption was observed in their water-soluble basic antibiotics.

Experimental

Melting points were determined with an automatic micro-melting point apparatus and are uncorrected. All concentrations were made with a rotary evaporator, and then P_2O_5 was used as a drying agent in a desiccator.

Penta-N-benzylideneaminosidin (II)——Aminosidin (I) sulfate (10 g) was dissolved in H_2O (100 ml), and the solution was adjusted to about pH 12 with 2N NaOH. To the solution benzaldehyde (6 ml) was added under stirring for 30 min. Only the oily substance released in the reaction mixture was decanted and the oily substance was dissolved in a mixture of MeOH–$CHCl_3$ (1: 1) (100 ml). The solution was filtered and to the filtrate benzaldehyde (4 ml) was added under stirring. The filtrate after 12 hr was concentrated to give a viscous substance which was washed with ether (3×30 ml) and H_2O (100 ml). The product was dried in a desiccator to give a white powder. To the solution of the powder in MeOH (30 ml) ether (100 ml) was added to produce a white powder. The reprecipitation by MeOH–ether was performed three times to give a crystalline powder, mp 175—180° (decomp.). Yield, 10.4 g. $[\alpha]_D^{26}$ +12° (c=1, MeOH). *Anal.* Calcd. for $C_{58}H_{65}O_{14}N_5 \cdot 5H_2O$: C, 60.78; H, 6.55; N, 6.10. Found: C, 60.56; H, 6.83; N, 6.32.

Paper chromatography was carried out by using a paper of Toyo Roshi No. 51 UH and a solvent system of BuOH–pyridine–HOAc–H_2O–*t*-BuOH (15: 10: 3: 12: 4), except when mentioned otherwise. *Rf* value of aminosidin (I) is 0.38, while II is 0.57 (detected by ninhydrin reagent). UV λ_{max}^{MeOH} nm (ε): 250 (77000). IR ν_{max}^{KBr} (cm^{-1}): 3400, 2910, 1638, 1594, 1576, 1449, 1380, 1310, 1290, 1210, 945, 753, 690.

Penta-N-salicylideneaminosidin (III)——To a solution of I sulfate (0.1 g) in H_2O (3 ml) adjusted to pH 10—12 by triethylamine, salicylaldehyde (1 ml) was added under stirring. After 2 hr, salicylaldehyde (1 ml) was added. The yellow oily substance released in the reaction mixture after 3 hr was treated in a similar manner as described for the preparation of II to give a yellow powder. Yield, 0.12 g. Recrystallization from MeOH–ether gave a crystalline powder, mp 180—190° (decomp.). $[\alpha]_D^{20}$ −26.6° (c=0.375, MeOH). *Anal.* Calcd. for $C_{58}H_{65}O_{19}N_5 \cdot 5H_2O$: C, 56.82; H, 6.11; N, 5.71. Found: C, 57.14; H, 6.18; N, 5.46. *Rf*:

11) P. Samuel and A. Steiner, *Proc. Soc. Exp. Med.*, **100**, 193 (1969); P. Samuel and W.I. Waithe, *Circulation*, **24**, 578 (1961).

0.72. UV λ_{max}^{MeOH} nm (ε): 237 (28000), 257 (25000). IR ν_{max}^{KBr} (cm^{-1}): 3440, 2940, 1630, 1580, 1520, 1492, 1460, 1400, 1280, 1145, 1025, 755.

Penta-N-anisylideneaminosidin (IV)——To a solution of I sulfate (1 g) in H_2O (5 ml) adjusted to about pH 12 with 2N NaOH, anisaldehyde (3 ml) was added under stirring. After 5 hr, the reaction mixture was evaporated to dryness and the residue was treated in the same manner as described for the preparation of II. Recrystallization from MeOH–ether gave a white powder, mp 165—175° (decomp.). Yield, 1.1 g. $[\alpha]_D^{20}$ +32.6° (c=0.95, MeOH). *Anal.* Calcd. for $C_{63}H_{75}O_{19}N_5 \cdot 5H_2O$: C, 58.37; H, 6.56; N, 5.40. Found: C, 58.92; H, 6.73; N, 5.92. *Rf*: 0.68. UV λ_{max}^{MeOH} nm(ε): 272 (88000), 280 (76000). 288 (60000). IR ν_{max}^{KBr} (cm^{-1}): 3400, 2960, 1640, 1605, 1580, 1510, 1460, 1420, 1310, 1250, 1165, 830.

Deca-N-methylaminosidin (VI)——A solution of I sulfate (1 g) in H_2O (6 ml) was hydrogenated in the presence of 38% HCHO (6 ml) and PtO_2 (0.35 g) to give a negative ninhydrin reaction. The reaction mixture was filtered off and the filtrate was concentrated to dryness. The residue was reprecipitated three times with H_2O–acetone, and then chromatographed over Sephadex LH-20 column (2.5×140 cm). Elution with H_2O into fractions of 6 ml each afforded the pure substance in tubes 32—42. Detection of the fractions was achieved by the Rydon-Smith reagent.[12] Freeze-drying of the fractions 32—42 gave a white, hygroscopic powder, mp 195—210° (decomp.). Yield, 0.98 g. $[\alpha]_D^{25}$ +42° (c=1, H_2O). IR ν_{max}^{KBr} (cm^{-1}): 3400, 1660, 1600, 1470, 1380, 1350, 1030, 765. NMR (D_2O) δ from DSS: 3.05—2.80 (m, 30H, N–Me), 5.41 (d, J=7.0 Hz 2H, two anomeric protons), 6.16 (d, J=4 Hz, 1H, anomeric proton). Reineckate of VI was obtained from aqueous solution and by reprecipitation with a hot H_2O twice, mp 178—185° (decomp.). *Anal.* Calcd. for $C_{53}H_{95}O_{14}N_{35}Cr_5S_{20} \cdot 5H_2O$: C, 27.91; H, 4.61; N, 21.50; Cr, 4.56. Found: C, 27.63; H, 4.55; N, 21.04; Cr, 4.03.

Penta-N-benzylaminosidin (VII)——A solution of II (5.2 g) in MeOH (100 ml) was hydrogenated in the presence of PtO_2 (0.75 g). After 3 hr, the reaction mixture was filtered by the aid of carbon. The filtrate was concentrated to give a white powder, which was further washed with ether to give VII in yield of 5.1 g, mp 145—150° (decomp.). $[\alpha]_D^{26}$ +11° (c=1, MeOH). *Anal.* Calcd. for $C_{58}H_{75}O_{11}N_5 \cdot 5H_2O$: C, 60.25; H, 7.35; N, 6.06. Found: C, 60.31; H, 7.21; N, 6.43.

TLC (silica-gel G, Merck): *Rf* 0.87 to 0.27 for II, solvent system: BuOH–pyridine–HOAc–H_2O–*t*-BuOH (15: 10: 3: 12: 4). UV λ_{max}^{MeOH} nm (ε): 252 (1440), 259 (1140), 265 (1070), 269 (530). IR ν_{max}^{KBr} (cm^{-1}): 3440, 2960, 1672, 1600, 1555, 1495, 1450, 1383, 1020, 850, 735, 698.

Penta-N-benzyl and Penta-N-methylaminosidin (VIII)——A solution of VII (0.7 g) in HOAc (20 ml) was hydrogenated in the presence of 37% HCHO (1.5 ml) and PtO_2 (0.2 g) for 15 hr. The filtrate from the reaction mixture was concentrated to dryness to give a white powder corresponding to VIII in yield of 0.7 g, mp 150—155° (decomp.). $[\alpha]_D^{26}$ +60.4° (c=1.125, MeOH). *Anal.* Calcd. for $C_{63}H_{85}O_{14}N_5 \cdot 5H_2O$: C, 61.71; H, 7.76; N, 5.71. Found: C, 61.43; H, 7.57; N, 5.63. TLC: *Rf* 0.46 to 0.70 for VII, solvent system: MeOH–HOAc–acetone (9: 1: 2). UV λ_{max}^{EtOH} nm(ε): 253 (1830), 259 (1480), 265 (1040), 269 (690). IR ν_{max}^{KBr} (cm^{-1}): 3400, 2980, 1655, 1560, 1450, 1405, 1020, 740, 695.

Penta-N-methylaminosidin (V)——A solution of VIII (0.5 g) in HOAc (20 ml) was hydrogenated in the presence Pd-C (10%, 1.5 g) at 50°. After 1 hr, the reaction temperature was returned to room temperature, and then the reaction was continued for 24 hr. The filtrate from the reaction mixture was concentrated to dryness. The residue was chromatographed over cellulose (Whatman) column (3×27 cm) using a solvent system for PPC. Fractionation into 2 ml in each tube afforded the pure substance in tubes 62—72. The pooled fractions were freeze-dried to give a white hygroscopic powder, mp 155—160° (decomp.). $[\alpha]_D^{26}$ +37° (c=1.15, H_2O). IR ν_{max}^{KBr} (cm^{-1}): 3440, 1580, 1400, 1330, 1015, 920. NMR (D_2O): δ 2.91—2.63 (15H, N-Me), δ 5.44 (d, J=7.0 Hz, 2H,) δ 5.97 (d, J=4 Hz, 1H, anomeric).

p-Hydroxybenzene-*p'*-azobenzenesulfonate was prepared in H_2O followed by recrystallization from hot H_2O to yellow crystals, mp indefinite. *Anal.* Calcd. for $C_{88}H_{105}O_{34}N_{15}S_5 \cdot 5H_2O$: C, 48.77; H, 5.31; N, 9.70; S, 7.39. Found: C, 48.96; H, 5.48; N, 9.53; S, 7.08.

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12) H.N. Rydon and P.W. Smith, *Nature*, **169**, 922 (1953).