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The Chemistry of Aminoglycoside Antibiotics from *Pseudomonas fluorescence*. III.[1] Absolute Configuration of P-2563(P) (Sorbistin A_1) and P-2563(A) (Sorbistin B)

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The absolute configuration of P-2563(P) (**1**) and P-2563(A) (**2**) were elucidated to be 3-O-(4-deoxy-4-propionamido-α-D-glucopyranosyl)-(2*S*,3*S*,4*R*,5*S*)-1,4-diamino-1,4-dideoxyhexitol and 3-O-(4-acetamido-4-deoxy-α-D-glucopyranosyl)-(2*S*,3*S*,4*R*,5*S*)-1,4-diamino-1,4-dideoxyhexitol, respectively, from the absolute configuration of the aminopolyol moiety (**3**),[1] together with the plane structures of **1** and **2** and ^{13}C-NMR studies.

Keywords——aminoglycoside antibiotics; *Pseudomonas fluorescence*; absolute configuration; PMR-spectrometry; ^{13}C-NMR spectrometry

In the previous papers,[1,3] the authors reported that the aminoglycoside antibiotic P-2563 (P) (**1**)[3] consists of a propionyl group, 4-amino-4-deoxy-D-glucose and (2*S*, 3*S*, 4*R*, 5*S*)-1,4-diamino-1,4-dideoxyhexitol (**3**),[1] and that P-2563(A) (**2**)[3] has an acetyl group instead of a propionyl group in **1**. The present paper deals with the whole structures of **1** and **2** as well as with the absolute configurations of them; the structure elucidation has a distinctive feature including full application of proton magnetic resonance (PMR) and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) decoupling experiments.

The positions of acyl and glycosidic linkage were clarified as follows. Propionyl group of **1** was assumed to be combined with amino group of 4-amino-D-glucose as amido linkage from the following facts; (1) the pK_a' values of **1** are 9.6 (1 mol) and 7.2 (1 mol), and those of the depropionyl derivative (**4**)[3] are 8.7 (2 mol) and 6.5 (1 mol); (2) the amide group is observed at 1640 cm^{-1} in the IR spectrum of **1**; (3) the methanolysis of di-N-(*p*-methoxybenzyl) derivative of **1** (**5**)[3] gave amino sugars (**7a, b**)[3] besides di-N-(*p*-methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol (**6**).

The 1′-anomeric hydroxyl of 4-amino-D-glucose was assumed to link to one of the four hydroxyl groups in **3** on the basis of the fact that (1) methanolysis of **1** gave the methylglycoside mixture (**7a, b**) and (2) two amino groups in **3** are primary. In order to determine the linkage between **3** and 4-amino-D-glucose, the spin-decoupling studies of P-2563(P) octaacetate (**8**)[3] was carried out (Fig. 1). In the PMR spectrum of **8** (Table I), propionyl group protons (1.13 ppm, triplet, 3H, J=7 Hz, 2.17 ppm, quartet, 2H, J=7 Hz) observed in the PMR spectrum of **1**[3] were also observed. H-1, H-2 and H-3 in methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-D-glucopyranoside (**9a**)[3] were also observed in **8** (Fig. 1) (H-1′; 4.96 ppm, H-2′; 5.03 ppm, H-3′; 5.54 ppm). Decoupling by irradiation at 5.03 ppm (H-2′) showed a change in the H-3′ proton; the collapse of the triplet (H-3′, 5.54 ppm, J=9 Hz) to a doublet (J=9 Hz), indicated $J_{2'-3'}=J_{3'-4'}=9$ Hz. On the contrary, when H-3′ (5.54 ppm) was irradiated, H-2′

1) Part II: K. Nara, K. Katamoto, S. Suzuki, S. Akiyama, and E. Mizuta, *Chem. Pharm. Bull.* (Tokyo), **26**, 1083 (1978).
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3) Part I: K. Nara, Y. Sumino, K. Katamoto, S. Akiyama, and M. Asai, *Chem. Pharm. Bull.* (Tokyo), **26**, 1075 (1978).

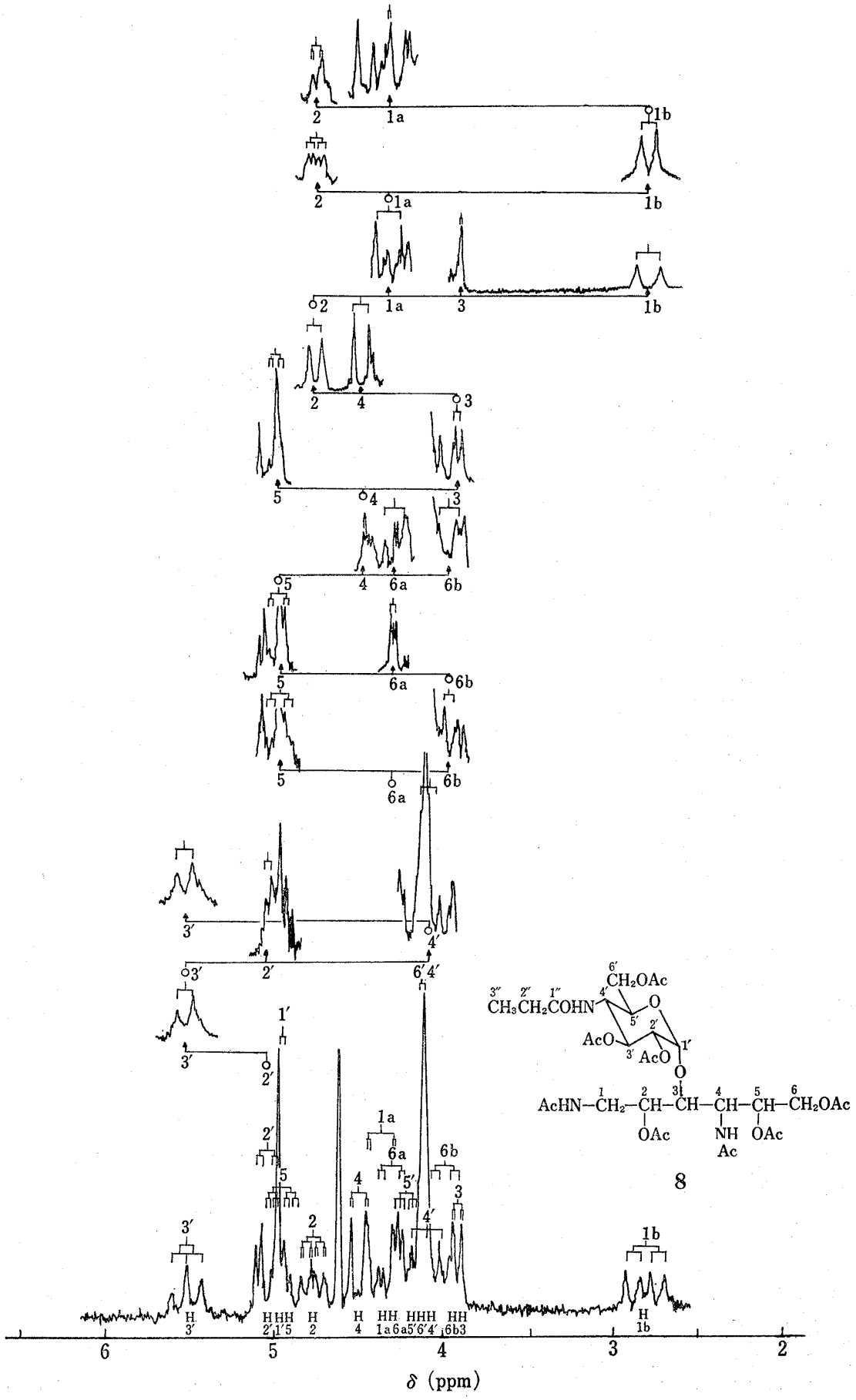


Fig. 1. PMR Spectra of **8** (100 MHz in $CDCl_3+D_2O$)

TABLE I. PMR Spectra of **8** (100 MHz in $CDCl_3+D_2O$)

		H 3′	H 2′	H 1′	H 5	H 2	H 4	H 1a	H 6a	H 5′	H 6′
8	δ (ppm)	5.54	5.03	4.96	4.94	4.74	4.46	4.31	4.29	4.24	4.15
		t	d.d	d	oct	oct	d.d	d.d	d.d	d.t	d
		1H	1H	1H	1H	1H	1H	1H	1H	1H	2H
	J (Hz)	$J_{2'-3'}$ 9.0	$J_{1'-2'}$ 4.0	$J_{1'-2'}$ 4.0	J_{4-5} 9.0	J_{1a-2} 2.0	J_{3-4} 1.5	J_{1a-1b} 14.5	J_{5-6a} 3.0	$J_{4'-5'}$ 9.0	$J_{5'-6'}$ 3.0
		$J_{3'-4'}$ 9.0	$J_{2'-3'}$ 9.0		J_{5-6a} 3.0	J_{1b-2} 8.5	J_{4-5} 9.0	J_{1a-2} 2.0	J_{6a-6b} 12.0	$J_{5'-6'}$ 3.0	
					J_{5-6b} 5.5	J_{2-3} 5.0					

		H 4′	H 6b	H 3	H 1b	H 2″	Ac	H 3″	NH[a] on C-4′	NH[a] on C-4	NH[a] on C-1
8	δ (ppm)	4.14	3.98	3.97	2.78	2.17	1.94 | 2.12	1.13	6.88	6.44	6.36
		t	d.d	d.d	d.d	q	s	t	d	d	d.d
		1H	1H	1H	1H	2H	3H×8	3H	1H	1H	1H
	J (Hz)	$J_{3'-4'}$ 9.0	J_{5-6b} 5.5	J_{2-3} 5.0	J_{1a-1b} 14.5	$J_{2''-3''}$ 7.0		$J_{2''-3''}$ 7.0	$J_{4'-NH}$ 8.0	J_{4-NH} 8.0	J_{1a-NH} 8.0
		$J_{4'-5'}$ 9.0	J_{6a-6b} 12.0	J_{3-4} 1.5	J_{1b-2} 8.5						J_{1b-NH} 4.0

a) In $CDCl_3$.
Abbreviation: s=singlet, d=doublet, t=triplet, d.d=double doublet, d.t=double triplet, oct=octet.

(5.03 ppm, double doublet) was collapsed into a doublet (J=4 Hz), indicating $J_{1'-2'}$=4 Hz.

H-4, H-5 and H-6 in **9a** were also observed in **8** (Fig. 1) (H-4′; 4.14 ppm, H-5′; 4.24 ppm, H-6′; 4.15 ppm). H-4′ (4.14 ppm), which had been simplified by addition of D_2O into a triplet (J=9 Hz), was further decoupled into a doublet (J=9 Hz) by irradiation at 5.54 ppm (H-3′), indicating $J_{3'-4'}=J_{4'-5'}$=9 Hz. These coupling constants were also confirmed by the fact that the triplet of H-3′ (J=9 Hz) was collapsed into a doublet (J=9 Hz) upon irradiating at 4.14 ppm (H-4′).

H-5′ and H-6′ were confirmed in the light of the chemical shifts and splitting patterns of H-5 and H-6 protons in **9a** and the integral intensity of the PMR spectrum of **8** (H-5′; 4.24 ppm, 1H, double triplet, $J_{4'-5'}$=9 Hz, $J_{5'-6'}$=3 Hz, H-6′; 4.15 ppm, 2H, doublet, $J_{5'-6'}$=3 Hz).

H-6a, b, H-5 and H-4 in the hexaacetate of **3** (**10**)[1] were also observed in **8** (H-6a; 4.29 ppm, H-6b; 3.98 ppm, H-5; 4.94 ppm, H-4; 4.46 ppm). Decoupling by irradiation at 4.29 ppm (H-6a) caused changes in the H-6b and H-5 protons; the collapses of the octet (H-5, 4.94 ppm) to a double doublet (J=5.5 and 9 Hz) and of the double doublet (H-6b, 3.98 ppm) to a doublet (J=5.5 Hz). When 3.98 ppm (H-6b) was irradiated, H-5 (4.74 ppm) was collapsed into a double doublet (J=3 and 9 Hz). Further, when peak at 4.94 ppm (H-5) was irradiated, H-6a (4.29 ppm) and H-6b (3.98 ppm) were collapsed into a pair of doublets (J=12 Hz), and at the same time, H-4 (4.46 ppm), which had been simplified by addition of D_2O into a double doublet (J=1.5 and 8 Hz), was collapsed into a doublet (J=1.5 Hz). Irradiation of 4.46 ppm (H-4) caused collapse of H-5 (4.94 ppm) into a double doublet (J=3 and 5.5 Hz) and collapse of a double doublet at 3.97 ppm into a doublet (J=5 Hz).

Finally, H-3, H-2, H-1a and H-1b in **10**[1] were present in **8** (H-3; 3.97 ppm, H-2; 4.94 ppm, H-1a; 4.31 ppm, H-1b; 2.78 ppm). When H-3 (3.97 ppm) was irradiated, H-4 (4.46 ppm) was decoupled into a doublet (J_{4-5}=9 Hz), and H-2 (4.74 ppm) into a double doublet (J=2

and 8.5 Hz). On the contrary, when H-2 (4.74 ppm) was irradiated, H-3 (3.97 ppm) was collapsed into a doublet (J_{3-4}=1.5 Hz) and H-1a (4.31 ppm) and H-1b (2.78 ppm), which had been simplified by addition of D_2O into two double doublets (H-1a; J=2.0 and 14.5 Hz, H-1b; J=8.5 and 14.5 Hz), into a pair of doublets (J_{1a-1b}=14.5 Hz). When H-1a (4.31 ppm) was irradiated, H-1b (2.78 ppm) was decoupled into a doublet (J_{1b-2}=8.5 Hz) and H-2 (4.74 ppm) into a double doublet (J_{2-3}=5 and J_{1b-2}=8.5 Hz). On the other hand, when H-1b (2.78 ppm) was irradiated, H-1a (4.31 ppm) was decoupled into a doublet (J_{1a-2}=2 Hz) and H-2 (4.74 ppm) into a double doublet (J_{1a-2}=2 and J_{2-3}=5 Hz). These results are summarized in Table I.

A large difference ($\Delta\delta$=153 Hz) of the chemical shifts between C-1 methylene protons (H-1a and H-1b) in **8** may be attributable to an increase in non-equivalence of the methylene protons due to rotational barriers[4] caused by acetylation of **1**. The non-equivalence of the methylene protons was also observed in the ^{13}C-NMR spectrum (Fig. 2). In the off-resonance spectrum of **8**, a double doublet like pattern of C-1 carbon (δ 38.1 ppm) was observed instead of a triplet, indicating a difference of the chemical shifts between H-1a and H-1b.

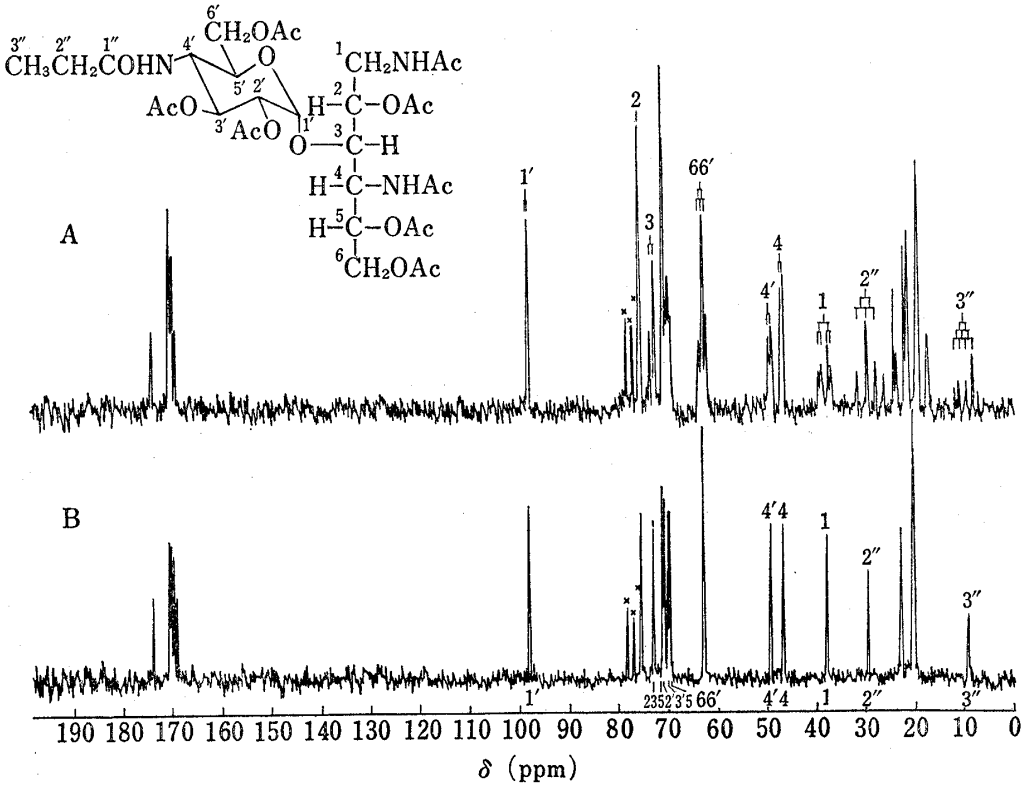


Fig. 2. A. Off-resonance (4.78 ppm irradiation) Spectrum of **8**
B. Proton Decoupled ^{13}C-NMR Spectrum of **8**

TABLE II. Assignment of Methine and Methylene Protons adjacent to Oxygens in **8** and **10**

		H 2	H 3	H 5	H 6a	H 6b
8	δ (ppm)	4.74	3.97	4.94	4.29	3.98
10	δ (ppm)	4.99	5.24	4.91	4.30	4.05

4) L.M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Inc., New York, 1959.

Now, all proton-signals observed in **9a** plus **10** except protons of one acetyl and one methoxy group are similarly observed in **8**, so, the structures of **9a** and **10** may be applied to the elucidation of the structure of **8**.

When the chemical shifts of three *O*-methine protons and one *O*-methylene protons of the aminopolyol moiety in **8** were compared with those of **10**, as shown in Table II, the H-3 proton in **10** showed a downfield shift on acetylation, suggesting that the aminosugar was combined with C_3–OH of the aminopolyol.

The configuration of the glycosidic linkage of **1** was assigned to be α by the coupling constant of the anomeric proton (J=3.5 Hz) and by the molecular rotational evidence of **7a**, **3** and **4** as shown in Table III.

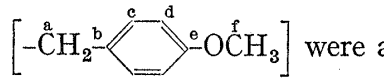
Table III. Configuration of Glycosidic Linkage of **1**

	H-1′		$[M]_D$
	δ (ppm)	$J_{1'-2'}$ (Hz)	
Methyl 4-amino-4-deoxy-α-D-glucopyranoside (**7a**)	5.00	3.0	+29300
Methyl 4-amino-4-deoxy-β-D-glucopyranoside (**7b**)	4.52	7.5	−6600
P-2563(P) (**1**)	5.39	3.5	
1,4-Diamino-1,4-dideoxy-hexitol (**3**)			−300
Synthetic			+29000 −6900
Depropionyl derivative of **1** (**6**)	5.21	3.0	+28100

Table IV. ^{13}C-NMR Spectra of **3** and **12**

	3(δ)[a]	**12**(δ)	**3**—**12**(δ)
C-1	44.1(t)	52.8 or 51.4(t)	−8.7 or −7.3
C-4	55.1(d)	55.3(d)	−0.2
C-6	63.8(t)	63.9(t)	−0.1
C-3	70.8(d)	71.4(d)	−0.6
C-5	73.2(d)	73.3(d)	−0.1
C-2	75.1(d)	72.7(d)	+2.4

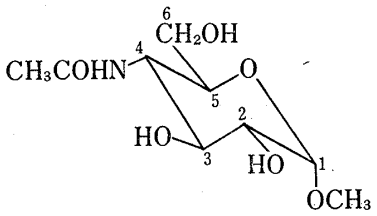
a) δ: ppm from TMS using dioxane (δ=67.4 ppm) as the internal reference.
Abbreviation: d=doublet, t=triplet; multiplicity on off-resonance experiment.

Next, the confirmation of the structure of **1** by the ^{13}C-NMR spectrum was carried out. The assignment and chemical shift of **3** and its mono-N-alkyl derivative, *i.e.* (2*S*, 3*S*, 4*R*, 5*S*)-1-N-(*p*-methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol (**12**), obtained by methanolysis of mono-N-(*p*-methoxybenzyl) derivative of **1**, are shown in Table IV. In the spectrum of **3**, three of six peaks at 44.1 (t), 63.8 (t) and 55.1 (d) ppm were readily assigned to C-1, C-6 and C-4 by the chemical shifts and off-resonance experiment. Each peak at 70.8 (d), 73.2 (d) or 75.1 (d) ppm remained as C 2, 3 or 5. The six peaks of *p*-methoxybenzyl group in **12**  were assigned as follows: C_a; 52.8 or 51.4 (t), C_b; 130.6 (s), C_c; 132.3 (d), C_d; 114.8 (d), C_e; 158.8 (s), C_f; 56.2 (q) ppm. A downfield shift [52.8 or 51.4 (t) ppm] of C-1 from 44.1 (t) ppm in **12** and an upfield shift [72.7 (d) ppm] of an *O*-methine carbon from 75.1 (d) ppm were observed, while other peaks remained relatively constant. It has been

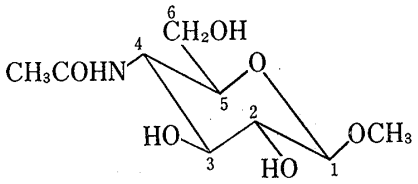
reported by Levy, *et al.*[5] that N-alkyl substituents cause a downfield shift at the carbon of attachment and a small upfield shift (2—4 ppm) at the carbon of α-position (C-2) from C-N-alkyl and that influence of N-alkyl substituents is primarily over a short range. From these facts, remaining three peaks were assigned as shown in Table IV.

Methyl 4-acetamido-4-deoxy-α-D-glucopyranoside (**13a**) was used as a reference compound of the ^{13}C-NMR spectroscopy. The assignments of ^{13}C-NMR spectrum of **13a** (Table V) were carried out by comparing the ^{13}C-NMR spectrum of **13a** with that of its anomer (**13b**) taking

TABLE V. ^{13}C-NMR Spectra of **13a** and **13b**



13a



13b

	13a (δ)[a]	**13b** (δ)	**13b**−**13a**
C-1	100.1(d)	103.9(d)	+3.8
C-2	72.5(d)	74.3(d)	+1.8
C-3	71.5(d)	74.3(d)	+2.8
C-4	52.4(d)	52.5(d)	+0.1
C-5	71.5(d)	75.9(d)	+4.4
C-6	61.7(t)	61.8(t)	+0.1
OCH_3	55.9(q)	58.0(q)	+2.1
$\underline{C}OCH_3$	175.1(s)	175.3(s)	+0.2
$CO\underline{C}H_3$	23.0(q)	22.9(q)	−0.1

a) δ: ppm from TMS using dioxane (δ=67.4 ppm) as the internal reference. Abbreviation: s=singlet, d=doublet, t=triplet, q=quartet: multiplicity on off-resonance experiment.

TABLE VI. ^{13}C-NMR Spectra of **1**, **14**, **13a** and **3**

Carbon	**1** (δ)[a]	**14** (δ)	**13a** (δ)	**3** (δ)
C-1″	179.1(s)	180.4(s)		
C-2″	30.1(t)	27.8(t)		
C-3″	10.4(q)	9.0(q)		
C-1′	101.1(d)		100.1(d)	
C-2′	72.9(d)		72.5(d)	
C-3′	72.4(d) or 7.11		71.5(d)	
C-5′	71.1(d) or 72.4		71.5(d)	
C-6′	61.6(t)		61.7(t)	
C-4′	52.3(d)		52.4(d)	
C-3	80.9(d)			70.8(d)
C-2	74.5(d)			75.1(d)
C-5	73.1(d)			73.2(d)
C-6	63.8(t)			63.8(t)
C-4	54.5(d)			55.1(d)
C-1	43.9(t)			44.1(t)
OCH_3			55.9(q)	
$CO\underline{C}H_3$			23.0(q)	
$\underline{C}OCH_3$			175.1(s)	

a) δ: ppm from TMS using dioxane (δ=67.4 ppm) as the internal reference. Abbreviation: s=singlet, d=doublet, t=triplet, q=quartet; multiplicity on off-resonance experiment.

5) G.C. Levy and G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, 1972.

into account the upfield shifts of anomeric pairs observed in methyl D-glucosides and other methyl glycosides.[6,7]

In the ^{13}C-NMR spectrum of **1**, fifteen carbons were clearly observed and assigned as shown in Table VI. The assignments of the propionyl moiety come from the comparison with the data reported for propionic acid (**14**).[8] The carbon shifts found for the 4-amino-4-deoxy-D-glucopyranoside residue in **1** coincide with those of methyl 4-acetamido-4-deoxy-α-D-glucopyranoside (**13a**).

The carbon shifts observed for the aminopolyol moiety in **1** are compared with those of **3**. Taking into account that a glycosyl unit causes a downfield shift (7—10 ppm)[9] at the carbon of attachment and that the influence of substituents is primarily over a short range, all carbon resonances could most reasonably assigned as shown in Table VI. Thus C-3 in **1** is shifted to lower field by about 10 ppm relative to that in **3** and the C-2 and C-4 carbons are shifted to upfield by about 0.6 ppm; the C-1, C-5 and C-6 carbons, however, remain almost unaffected (Table VII).

Table VII. ^{13}C-NMR Spectra of **3** and Aminopolyol Moiety of **1**

Carbon	**3** (δ)[a]	**1** (δ)	**3**−**1**
C-1	44.1	43.9	+0.2
C-2	75.1	74.5	+0.6
C-3	70.8	80.9	−10.1
C-4	55.1	54.5	+0.6
C-5	73.2	73.1	+0.1
C-6	63.8	63.8	0

a) δ: ppm from TMS using dioxane (δ=67.4 ppm) as the internal reference.

The configuration of the glycosidic linkage between amino-sugar and aminopolyol was confirmed to be α from the fact that chemical shift of C-1′ (101.1 ppm) was more consistent with that (100.1 ppm) of **13a** than that (103.9 ppm) of methyl 4-acetamido-4-deoxy-β-D-glucopyranoside (**13b**).

Considering that the absolute configuration of aglycone is held unaltered by methanolysis with HCl in MeOH, the absolute configuration of **1** and **2**, drawn according to Fischer's rule, are proposed for **1** and **2**, respectively (Chart 1).

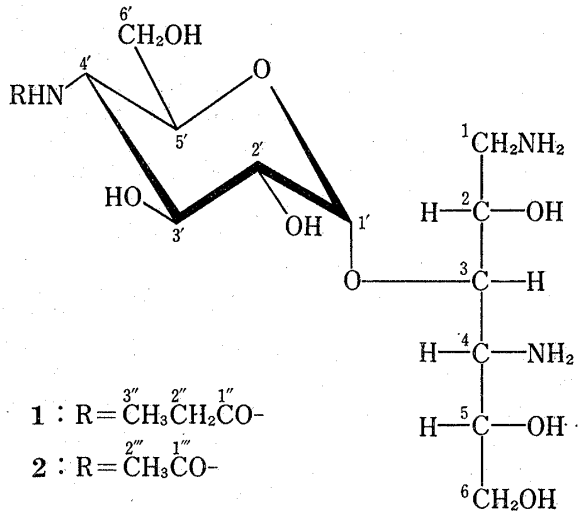


Chart 1

From these results, **1** and **2** were found to be identical with sorbistin A_1 and sorbistin B, respectively, reported independently by Konishi, *et al.*[10]

6) D.E. Dorman and J.D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).
7) A.S. Perlin, B. Casu, and H.J. Koch, *Can. J. Chem.*, **48**, 5296 (1970).
8) R. Hagen and J.D. Roberts, *J. Am. Chem. Soc.*, **91**, 4504 (1969).
9) D.E. Dorman and J.D. Roberts, *J. Am. Chem. Soc.*, **93**, 4463 (1971).
10) M. Konishi, S. Kawata, T. Tsuno, K. Numata, H. Tsukiura, T. Naito, and H. Kawaguchi, *J. Antibiot.*, **29**, 1152 (1976).

Experimental

The following instruments were used for obtaining physical data. Melting point, Yanagimoto's microscope hot stage (uncorrected); IR spectra, Hitachi grating infrared spectrophotometer in KBr; PMR spectra, Varian HA-100 spectrometer (TMS as internal standard, δ value); ^{13}C-NMR spectra, Varian XL-100 12 at 25.2 MHz. The samples were examined as 10% solution containing about 2% (v/v) of 1,4-dioxane as an internal reference. The ^{13}C-shifts obtained were converted to the TMS scale.

(2*S*,3*S*,4*R*,5*S*)-Di-N-(*p*-methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol (6)——Di-N-(*p*-methoxybenzyl) derivative of **1** (**5**) (0.2 g) in 6 N HCl in MeOH (20 ml) was refluxed for 12 hr. Evaporation of the reaction mixture left a crude syrup, which was dissolved in water (10 ml), passed through an Amberlite IR-45 (OH) column. The effluent was successively passed through an Amberlite CG-50 (NH_4^+) column (200 ml). The column was eluted with a linear gradient (water to 1.5 N NH_4OH, each 700 ml). The eluates were monitored by TLC [silica gel (Merck), BuOH–AcOH–water=3:1:1; detected by Rydon–Smith reagent). The fractions having an *Rf* 0.41 were combined and evaporated to a syrup. The syrup was neutralized with aqueous HCl and crystallized with aqueous MeOH to colorless prisms (**6**, 35 mg), mp 134—135°. Mass Spectrum (MS) *m/e*: 420 (M^+). *Anal.* Calcd. for $C_{22}H_{32}N_2O_6 \cdot 2HCl \cdot 3/2H_2O$: C, 50.77; H, 7.11; N, 5.38; Cl, 13.65. Found: C, 50.72; H, 6.99; N, 5.49; Cl, 13.81.

Methyl 4-Amino-4-deoxy-α-D-glucopyranoside (7a) and Methyl 4-Amino-4-deoxy-β-D-glucopyranoside (7b)——A mixture (500 mg) of **7a** and **7b** in water (10 ml) obtained by the methanolysis of **1** was passed through an Amberlite CG-50 (50% NH_4^+ form) column (200 ml). The column was eluted with a linear gradient (water to 0.5 N NH_4OH, each 700 ml). The eluates were monitored by TLC [silica gel (Merck), pyridine–acetone–AcOH–water=15:3:1:2; detected by ninhydrin reagent]. The fractions having an *Rf* 0.48 were combined, evaporated to a crude powder (350 mg). The crude powder was dissolved in water (1 ml). PrOH was added to the solution to turbidity. After standing overnight in a refrigerator, the colorless crystals (**7a**) which separated were collected and recrystallized from the same solvent, mp 167—168°, $[\alpha]_D^{25}$ +152° (c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3375, 1600, 1425, 1360, 1200, 1150, 1120, 1090, 1050, 1015, 975, 900, 860. PMR (D_2O) δ: 2.84 (1H, m), 3.57 (3H, s), 3.30—4.20 (5H, m), 4.98 (1H, d, J=3.5 Hz). *Anal.* Calcd. for $C_7H_{15}NO_5$: C, 43.52; H, 7.77; N, 7.25. Found: C, 43.44; H, 7.81; N, 7.08.

The fractions having an *Rf* 0.56 were combined, evaporated to a crude powder (80 mg). The crude powder was dissolved in water (0.5 ml). PrOH was added to the solution to turbidity. After standing overnight in a refrigerator, the colorless crystals (**7b**) which separated were collected and recrystallized from the same solvent, mp 95—97°, $[\alpha]_D^{25}$ −37° (c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3350, 1600, 1450, 1390, 1210, 1160, 1110, 1080, 1050, 890. PMR (D_2O) δ: 2.80 (1H, t, like), 3.40—4.30 (5H, m), 4.52 (1H, d, J=8.0 Hz). *Anal.* Calcd. for $C_7H_{15}NO_5$: C, 43.52; H, 7.77; N, 7.25. Found: C, 43.50; H, 7.82; N, 7.10.

(2*S*,3*S*,4*R*,5*S*)-1-N-(*p*-Methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol (12)——To a solution of **1** (4 g) (10 mmol) in water (50 ml) was added NaOH (0.6 g) and *p*-anisaldehyde (1.3 g) (10 mmol). The solution was stirred at room temperature for 30 min. The reaction mixture was evaporated *in vacuo* to a crude powder. The product was dissolved in EtOH (100 ml) and the solution was diluted with ethyl ether (500 ml) to obtain a white precipitate. The precipitate in tetrahydrofuran–MeOH (3:2) (200 ml) was reduced with $NaBH_4$ (2 g). The product (2.0 g) was dissolved in water (50 ml), passed through an Amberlite CG-50 (NH_4^+) column (300 ml). The column was eluted with a linear gradient (water to 1.5 N NH_4OH, each 700 ml). The eluates were monitored by TLC [silica gel (Merck), BuOH–AcOH–H_2O=3:1:1; detected by Molish–Udransky reagent). The fractions having an *Rf* 0.22 were combined and evaporated *in vacuo* to leave a crude powder of mono-N-(*p*-methoxybenzyl) derivative of **1** (1.2 g). The powder (0.5 g) in MeOH saturated with HCl (50 ml) was refluxed for 18 hr. Evaporation of the mixture left a crude powder. The crude powder was dissolved with water (20 ml) and the solution was passed through an Amberlite CG-50 (NH_4^+) column (200 ml). The column was eluted with a linear gradient (water to 1.5 N NH_4OH, each 700 ml). The eluates were monitored by TLC [silica gel (Merck), BuOH–AcOH–H_2O=3:1:1; detected by UV light]. The fractions having an *Rf* 0.31 were combined and evaporated *in vacuo* to dryness. The residue was dissolved in water (5 ml); the solution was passed through a Dowex 1×2 (OH) column. The effluent was evaporated to a syrup. After standing overnight in a refrigerator, the colorless crystals (**12**, 50 mg) were obtained, mp 102—104°. *Anal.* Calcd. for $C_{14}H_{24}N_2O_5 \cdot 1/2H_2O$: C, 54.37; H, 8.09; N, 9.06. Found: C, 54.69; H, 8.05; N, 9.10.

(2*S*,3*S*,4*R*,5*S*)-1-N-(*p*-Methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol Hexaacetate (15)——**12** (30 mg) in pyridine (2 ml) was acetylated with Ac_2O (1 ml) to afford a white powder (35 mg). PMR ($CDCl_3$) δ: 1.92—2.18 (3H×6, s, $COC\underline{H}_3$), 3.42, 3.66 (H-1a, H-1b, J_{1a-2}=4 Hz, J_{1b-2}=7 Hz, J_{1a-1b}=13 Hz), 3.76 (3H, s, $-OC\underline{H}_3$), 4.00, 4.28 (H-6a, H-6b, J_{5-6a}=2 Hz, J_{5-6b}=5 Hz, J_{6a-6b}=14 Hz), 4.32—4.58 (2H, N–$C\underline{H}_2$–C_6H_4–OCH_3), 4.56 (H-4, J_{4-5}=9 Hz, J_{3-4}=4 Hz, J_{4-NH}=10 Hz), 4.88 (H-5, J_{4-5}=9 Hz, J_{5-6a}=2 Hz, J_{5-6b}=5 Hz), 5.13 (H-2, J_{2-3}=8 Hz, J_{1a-2}=4 Hz, J_{1b-2}=7 Hz), 5.26 (H-3, J_{2-3}=8 Hz, J_{3-4}=4 Hz). *Anal.* Calcd. for $C_{26}H_{36}N_2O_{11}$: C, 56.5; H, 6.52; N, 5.07. Found: C, 56.3; H, 6.62; N, 5.01.

Methyl 4-Acetamido-4-deoxy-α-D-glucopyranoside (13a)——Methyl 4-acetamido-2,3,6-tri-O-acetyl-4-deoxy-α-D-glucopyranoside (100 mg) in 1 N NH_3/MeOH (10 ml) was allowed to stand at 3—5° for 16 hr. The reaction mixture was evaporated *in vacuo* to dryness. The product was dissolved with water (10 ml), passed

through a Dowex 1×2 (OH) column. The effluent was evaporated *in vacuo* to dryness; the residue was crystallized from MeOH–EtOAc to colorless crystals (**13a**, 35 mg), mp 183—185°, $[\alpha]_D^{24}$ +158° (c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3300, 1620, 1570, 1200, 1080, 1045, 920, 690. PMR (D_2O) δ: 2.08 (3H, s, $COC\underline{H}_3$), 3.45 (3H, s, $-OC\underline{H}_3$), 3.00—3.85 (6H, m), 4.87 (1H, d, J=3.6 Hz). *Anal.* Calcd. for $C_9H_{17}NO_6$: C, 46.0; H, 7.23; N, 5.96. Found: C, 45.95; H, 7.33; N, 5.88.

Methyl 4-Acetamido-4-deoxy-β-D-glucopyranoside (13b)——By essentially the same procedure as above, methyl 4-acetamido-2,3,6-tri-O-acetyl-4-deoxy-β-D-glucopyranoside (100 mg) was de-O-acetylated to afford colorless needles (**13b**, 30 mg), mp 300—302°, $[\alpha]_D^{24}$ +2° (c=1.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3350, 1640, 1555, 1090, 1060, 945. PMR (D_2O) δ: 2.10 (3H, s, $-COC\underline{H}_3$), 3.64 (3H, s, $-OC\underline{H}_3$), 3.10–3.80 (6H, m), 4.42 (1H, d, J= 7.2 Hz).

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