SORBISTIN, A NEW AMINOGLYCOSIDE ANTIBIOTIC COMPLEX OF BACTERIAL ORIGIN

III. STRUCTURE DETERMINATION

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The structures of sorbistins A_1, A_2, B, C and D have been determined including stereochemistry. Sorbistins A_1, A_2 and B are composed of a 4-acyl-amino-4-deoxy-D-glucose and 1,4-diamino-1,4-dideoxy-D-sorbitol, the latter compound being hitherto undescribed in literature. Sorbistins C and D have the same aglycone of 1,4-diamino-1,4-dideoxy-D-sorbitol, which is linked with D-glucose and 4-amino-4-deoxy-D-glucose, respectively, through a glycosidic bond.

The production, isolation and physicochemical properties of sorbistins have been described in the preceding paper[1]. Sorbistin is a complex of closely related aminoglycoside antibiotics produced by a strain of *Pseudomonas* species. It has been separated into five components, A_1, A_2, B, C and D, the latter two components being bio-inactive. This paper reports the structure determination of sorbistin components, which were found to have a common novel aglycone but differ in the structure of sugar moiety.

General Structural Characteristics

Bio-active sorbistin components A_1(**Ia**), A_2(**Ib**) and B(**Ic**) were obtained as a white amorphous carbonate salt and their physicochemical properties are summarized in Table 1. The molecular formula of $C_{15}H_{31}N_3O_9$ was found for **Ia**, $C_{16}H_{33}N_3O_9$ for **Ib** and $C_{14}H_{29}N_3O_9$ for **Ic** based on the analyses of the carbonates and the crystalline N-di-acetates (**IIa, IIb** and **IIc**). **Ia**, **Ib** and **Ic** gave positive reactions with ninhydrin and anthrone reagents, but were negative to SAKAGUCHI and FEHLING reactions. Their IR spectra were very similar to each other and indicated the presence of hydroxyl and amide groups. As shown in Table 1, the NMR spectrum of Ia hydrochloride indicated the presence of a $-CO-CH_2-CH_3$ group (δ: 1.13 and 2.32 ppm) and one anomeric proton at 5.23 ppm. The spectrum also showed the presence of additional 14 protons (δ: 3.2~4.3 ppm) which are linked to carbons bearing oxygen or ammonium group. The NMR spectra of **Ib** and **Ic** were principally identical with that of **Ia** except that the spectrum of **Ib** showed signals for a $-CO-CH_2-CH_2-CH_3$ (δ: 0.92, 1.62 and 2.30 ppm) group and that of **Ic** for a $-CO-CH_3$ (δ: 2.02 ppm) group instead of the propionyl group in **Ia**.

Two bio-inactive components C (**III**) and D (**IV**) were obtained as amorphous carbonates. Molecular formula for **III** and **IV** was established as $C_{12}H_{26}N_2O_9$ and $C_{12}H_{27}N_3O_8$, respectively, by elemental analysis of the carbonates and of the crystalline N-acetates. The two components gave positive reactions to ninhydrin and anthrone reagents but were negative to FEHLING, TOLLENS and SAKAGUCHI reactions. Their IR spectra indicated the presence of hydroxyl and/or amino groups (3,300~3,400 cm^{-1}) but apparently lacked the band for amide carbonyl group at around 1,640 cm^{-1}. The absence of amide group in components C and D was further substantiated by the NMR spectra as shown in Table 2.

Table 1. Physicochemical properties of sorbistins A_1, A_2 and B

	Sorbistin A_1 (**Ia**)		Sorbistin A_2 (**Ib**)		Sorbistin B (**Ic**)	
Analysis	$C_{15}H_{31}N_3O_9 \cdot 1/2\ H_2CO_3$		$C_{16}H_{33}N_3O_9 \cdot 1/2\ H_2CO_3$		$C_{14}H_{29}N_3O_9 \cdot 1/2\ H_2CO_3$	
	(Calc'd)	(Found)	(Calc'd)	(Found)	(Calc'd)	(Found)
C	43.45	43.22	44.79	44.35	42.02	41.92
H	7.53	7.52	7.75	7.83	7.30	7.43
N	9.81	9.49	9.50	9.21	10.14	9.93
$[\alpha]_D$ in H_2O	+78.5°		+79.1°		+85.0°	
pKa	6.8 & 9.4		6.9 & 9.4		6.8 & 9.4	
Titration equivalent	422		434		414	
NMR (60 MHz in D_2O+DCl) δ: (ppm)			0.92 (t) 3H			
	1.13 (t) 3H		1.62 (sex) 2H			
	2.32 (q) 2H		2.30 (t) 2H		2.02 (s) 3H	
	3.2~3.4 (m) 2H		3.2~3.4 (m) 2H		3.2~3.4 (m) 2H	
	3.5~4.3 (m) 12H		3.6~4.4 (m) 12H		3.6~4.3 (m) 12H	
	5.23 (d) 1H		5.25 (d) 1H		5.23 (d) 1H	

Table 2. Physicochemical properties of sorbistins C and D

	Sorbistin C (**III**)		Sorbistin D (**IV**)	
Analysis	$C_{12}H_{26}N_2O_9 \cdot 1/2\ H_2CO_3$		$C_{12}H_{27}N_3O_8 \cdot H_2CO_3$	
	(Calc'd)	(Found)	(Calc'd)	(Found)
C	40.21	39.89	38.70	38.67
H	7.29	7.16	7.25	6.93
N	7.50	7.21	10.42	10.16
pKa	6.8 & 9.4		6.6 (2) & 9.8	
Titration equivalent	379		415	
NMR (60 MHz in D_2O +DCl) δ: (ppm)	3.2~3.4 (m) 2H		3.1~3.4 (m) 2H	
	3.5~4.3 (m) 12H		3.6~4.3 (m) 12H	
	5.22 (d) 1H		5.23 (d) 1H	

The microanalysis and titration data (Table 2) suggested that **III** has two amino groups and **IV** has three. On acetylation with methanolic acetic anhydride, **III** gave a crystalline N-diacetate (**V**), while N-triacetate (**VI**) was obtained from **IV**. The acetate VI thus obtained was found to be identical with N-diacetyl-sorbistin B (**IIc**). These findings coupled with the NMR data suggested that **Ib** might be a mono-N-acetyl derivative of **IV**.

Alkaline Hydrolysis of Ia, Ib and Ic

The hydrolysis of **Ia** in a saturated barium hydroxide solution yielded an acidic material (**VII**) and a basic fragment. The latter was purified by column chromatography and identified as **IV**. The acidic material **VII** was treated with *p*-bromophenacyl bromide in ethanol and the product (**VIII**) was identified as *p*-bromophenacyl propionate[2]. Under similar hydrolytic conditions, **Ib** and **Ic** liberated *n*-butyric acid and acetic acid, respectively, along with the common fragment **IV**. Consequently, **Ia**, **Ib** and **Ic** are all derivatives of **IV** being acylated at one of the amino groups with propionyl, *n*-butyryl and acetyl group, respectively.

Methanolysis of Ia and IV

A methanolic solution of **Ia** was refluxed for 20 hours under continuous bubbling of dry HCl gas. The products were separated by a column of Amberlite CG-50 into an amino sugar fragment as methyl glycoside (**IX**), a basic aglycone part (**X**) and a small amount of IV. The methyl glycoside **IX** was further separated into a major α-methyl glycoside (**IXa**) and a minor β-anomer (**IXb**). **IXa** crystallized from an ethanol solution and analyzed as $C_7H_{15}NO_5$, m.p. 171~173°C[3]. Treatment of **IXa** with acetic anhydride and pyridine gave crystalline N, O-tetraacetate (**XIa**), $C_{15}H_{23}NO_9$, which showed physicochemical properties very similar to those reported for the N,O-tetraacetate of methyl 4-amino-4-deoxy-α-D-glucopyranoside obtained from 4-trehalosamine[4]. The identity was established by NMR and IR spectra and mixed melting point determination with an authentic sample. Similarly the N,O-tetraacetate of **IXb** (**XIb**) was identified as the N,O-tetraacetate of methyl 4-amino-4-deoxy-β-D-glucopyranoside. In a similar manner, the same methyl glycosides, **IXa** and **IXb**, and aglycone **X** were isolated from the methanolyzate of **IV**.

Structure of Aglycone (X)

The aglycone part (**X**) was purified by column chromatography on Amberlite CG-50 and crystallized as the monosulfate, $C_6H_{16}N_2O_4 \cdot H_2SO_4$. Acetylation of **X** in methanol gave the crystalline N-diacetate (**XII**) and in pyridine the N,O-hexaacetate (**XIII**). The physicochemical and spectroscopic characterizations showed a close similarity between **X** and streptamine. However, the microanalyses of **X**, **XII** and **XIII**, as well as the mass spectrum of XIII (M^+, *m/e* 432), indicated that **X** should have two more hydrogen atoms than streptamine, suggesting a diaminodideoxyhexitol structure for **X**.

The location of the two amino groups in **X** was determined to be at C-1 and C-4 of the hexitol by NMR and mass spectra. As shown in Fig. 1, 220 MHz NMR of **XIII** indicated the presence of two non-equivalent methylene and four methine protons in the skeleton of **X**. The resonance pattern was simplified with addition of D_2O, which revealed that one of the amino groups was attached to a terminal methylene group (δ: 3.40 and 3.66 ppm) and the other to a methine group (δ: 4.60 ppm). The

Fig. 1. 220 MHz NMR spectra of **XIII**

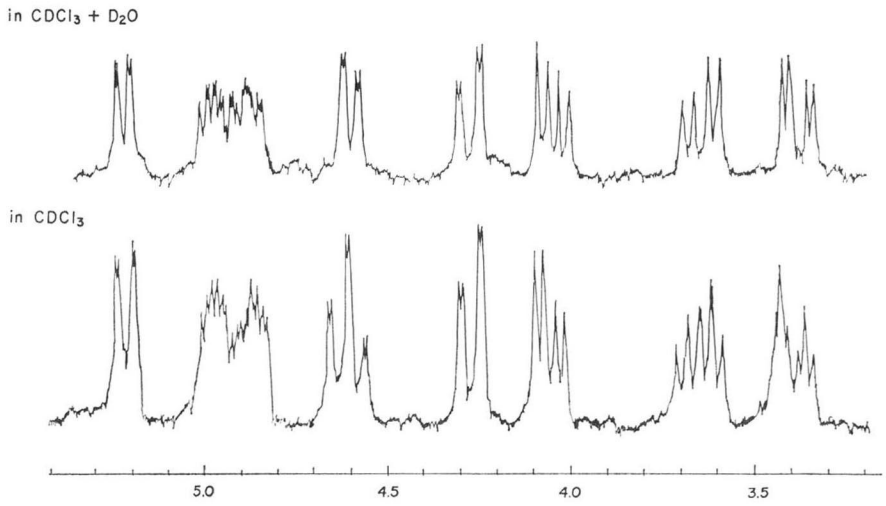
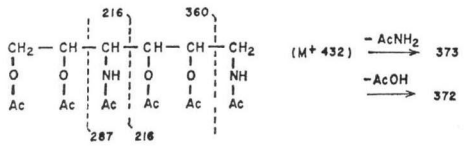
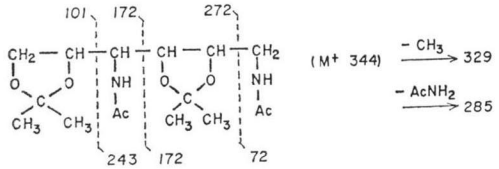


Fig. 2. Structures and major fragment ions of **XIII**, **XIV** and **XV**

N,O-Hexaacetyl derivative of X (**XIII**)



N-Diacetyl-diisopropylidene derivative of X (**XIV**)



N-Diacetyl-O-tetra(trimethylsilyl) derivative of X (**XV**)

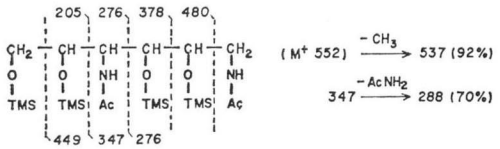
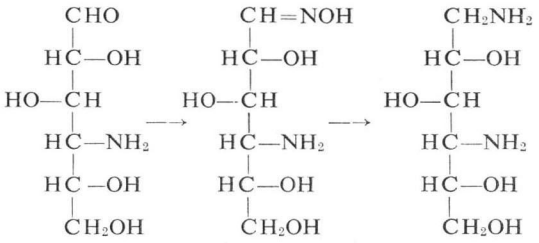


Fig. 3. Synthesis of X



4-Amino-4-deoxy-D-glucose (**XVI**, 4 AG)

X: 1,4-Diamino-1,4-dideoxy-D-sorbitol

splitting of the methine proton (broad doublet after D_2O addition) suggested the latter amino group on C-3 or C-4 rather than C-2 or C-5. Moreover, the treatment of **XII** with dimethoxypropane readily gave a diisopropylidene derivative (**XIV**), indicating that a 1,4-substitution of the two amino groups would be more favorable than 1,3-substitution in order to locate two pairs of vicinal hydroxyl groups in the structure.

Another evidence for the 1,4-substitution was obtained by the mass spectral analyses of three derivatives of **X**, namely, **XIII**, **XIV** and **XV** (N-diacetyl-O-tetratrimethylsilyl derivative of **X**). The structures of these derivatives and their major diagnostic fragment ions are illustrated in Fig. 2. Although the spectra showed low abundance or lack of the M^+ ion in these compounds, the ion peak corresponding to $M^+ - CH_3$ was intense in the mass spectra of **XIV** and XV, and the ions which are less by one acetamide or acetic acid group than the molecular ion were prominent in the spectrum of XIII. The fragment ions produced by a cleavage between C_3–C_4 or C_4–C_5 bond were remarkable in all of these mass spectra. For example, an intense peak at *m/e* 276 in the spectrum of **XV** was assigned to the ion carrying C_1–C_2–C_3 and/or C_4–C_5–C_6 unit, and strong peaks at *m/e* 347 and 205 were readily assignable to C_1–C_2–C_3–C_4 and C_5–C_6 units, respectively. Thus, **X** is 1,4-diamino-1,4-dideoxyhexitol.

Configuration and Synthesis of X

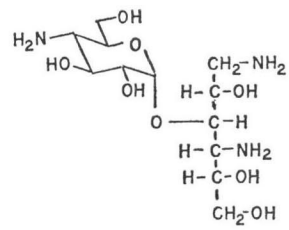
As described above, the structure of 1,4-diamino-1,4-dideoxyhexitol was assigned for aglycone **X**. Since the sugar component of the antibiotic was identified as 4-amino-4-deoxy-D-glucose, one might postulate a D-gluco configuration for aglycone **X** from biosynthetic considerations. Therefore, the preparation of 1,4-diamino-1,4-dideoxy-D-sorbitol was undertaken starting from 4-amino-4-deoxy-D-glucose (**XVI** or 4-AG)[5] which was obtained by mild acid hydrolysis of methyl glycoside **IX**. The reduction of phenylhydrazone or oxime groups is known to be a convenient method for the synthesis of amino compounds in the sugar series[6], but the attempt to synthesize the hydrazone of 4-AG was unsuccessful owing to the alkaline labile nature of the sugar[7]. Therefore, the hydrochloride of 4-AG was reacted with hydroxylamine in a neutral medium to give the oxime which was reduced by catalytic hydrogenolysis to give 1,4-diamino-1,4-dideoxy-D-sorbitol (Fig. 3). The crude product was

purified by a column of Amberlite CG-50 and crystallized as monosulfate, $C_6H_{16}N_2O_4 \cdot H_2SO_4$, m.p. 261.5~263°C, $[\alpha]_D$ −10.3°. Its physicochemical properties along with NMR and IR spectra were consistent with those of **X**, and the identity was confirmed by mixed melting point determination. Accordingly, the aglycone of sorbistin is 1,4-diamino-1,4-dideoxy-D-sorbitol.

Structure of Sorbistin D (IV)

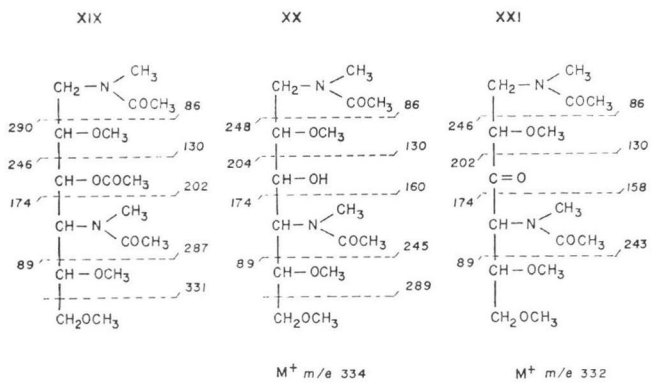
It has been shown that **IV** consists of 4-AG (**XVI**) and 1,4-diamino-1,4-dideoxy-D-sorbitol (**X**). Since IV is devoid of reducing activity and the three amino groups of **IV** are not substituted, the 4-AG moiety should be glycosidically linked to one of the four hydroxyl groups of **X**. The anomeric proton of **IV** gives rise to a doublet at δ 5.23 ppm with a spacing (J: 3.0 Hz) similar to that of the anomeric proton of **IXa**, which allowed an assignment of α-glucopyranoside configuration for the 4-AG moiety. The assignment was also supported by the modified HUDSON's rule[8]: the molecular rotation observed for **IV** was +30,200, which is in agreement with the calculated value of +31,100 for an α-glycoside in contrast to the calculated value of −7,300 for a β-glycoside.

In order to determine the site of binding of 4-AG to the aglycone, N-triacetyl-sorbistin D (**VI**) was treated with excess of methyl iodide in the presence of sodium hydride in anhydrous DMF solution[9] and the permethyl derivative (**XVII**) thus obtained was hydrolyzed with 6 N HCl. Subsequent separation of the products by Amberlite CG-50 chromatography afforded the N,O-pentamethyl derivative of **X** (**XVIII**). The NMR spectrum of **XVIII** indicated the presence of two N-methyl (one six-proton singlet at δ 2.27 ppm) and three O-methyl protons (three three-proton singlets at δ 3.22, 3.27 and 3.32 ppm). Acetylation of **XVIII** in pyridine yielded N,O-triacetate (**XIX**) while the reaction in methanol gave N-diacetate (**XX**). The CrO_3 oxidation of **XX** in pyridine afforded an oxo-compound (**XXI**) in a good yield[10]. The mass spectra of **XIX**, **XX** and **XXI** were analyzed and, as illustrated in Fig. 4, prominent ions mainly arose from fissions between the C_2–C_3 and C_3–C_4 linkages. In the spectrum of **XXI**, fragment ions at *m/e* 130 and 202 derived from the splitting of C_2–C_3 bond appeared as the base peak and the second biggest peak, respectively, suggesting the ease of α-cleavage due to



Sorbistin D (IV)

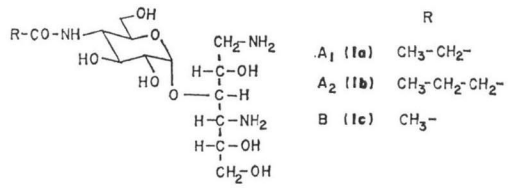
Fig. 4. Structures of **XIX**, **XX** and **XXI** and their fragment ions



the presence of C-3 keto group in the molecule. It was concluded therefore that the hydroxyl group at the C-3 position must be unsubstituted in **XVIII**, and accordingly the 4-AG portion is glycosidically linked to the C-3 position of the aglycone **X**. Thus, the structure of sorbistin D is as shown above:

Structure of Sorbistins A_1, A_2 and B (Ia, Ib and Ic)

It has been shown that **Ia**, **Ib** and **Ic** are, respectively, mono-N-propionyl, mono-N-*n*-butyryl and mono-N-acetyl derivatives of **IV**. The position of the acyl group in these antibiotics was determined by the mass spectral analysis of N-diacetyl-O-hexa(trimethylsilyl) derivatives of **Ia** and **Ic** (**XXIIa** and **XXIIc**). The molecular ions were not obtained with **XXIIa** and **XXIIc** as in the case reported with other trimethylsilylated sugars[11], and the $M^+ - CH_3$ ions (*m/e* 896 in **XXIIa**, *m/e* 884 in **XXIIc**) were the highest peak observed with both compounds. As illustrated in Fig. 5, the peaks due to the aglycone moiety (*m/e* 463, 205, 72) were essentially the same in the spectra of **XXIIa** and **XXIIc** but differences seen in the fragment ions of 4-AG moiety. A relatively intense peak at *m/e* 420 in the spectrum of **XXIIc** was assigned to N-acetyl-O-tri(trimethylsilyl)-4-AG moiety, while the corresponding ion in the spectrum of **XXIIa** was observed at 14 mass units higher (*m/e* 434) indicating the propionyl group is attached to the 4-AG moiety. Therefore, the site of acylation in sorbistins was determined to be the 4′-amino group in the 4-AG moiety and the structures shown below are assigned to sorbistins A_1, A_2 and B (**Ia**, **Ib**, **Ic**).



Structure of Sorbistin C (III)

On acid methanolysis, **III** was rather easily cleaved to a methyl glycoside of neutral sugar and the basic aglycone **X**, the former being identified as methyl D-glucoside. As shown in Fig. 6, the mass spectrum of N-diacetyl-O-hepta(trimethylsilyl) derivative of **III** (**XXIII**) indicated a significant ion peak at *m/e* 451 which was assigned to the tetra(trimethylsilyl)-glucose moiety, along with the ions due to the aglycone part (*m/e* 463, 205 and 72). Thus, the structure of sorbistin C (**III**) was determined to be 3-O-[α-D-glucopyranosyl]-1,4-diamino-1,4-dideoxy-D-sorbitol.

Fig. 5. Mass spectra of **XXIIa** and **XXIIc**

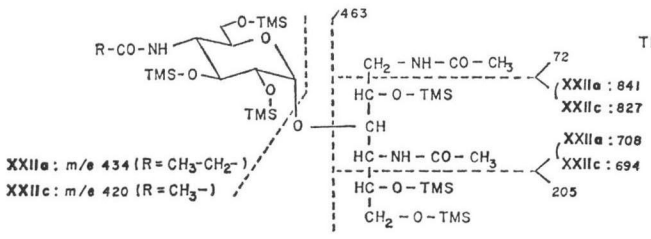
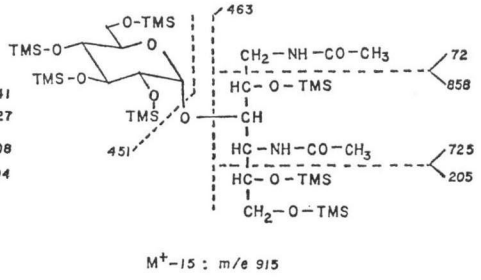


Fig. 6. Structure and fragment ions of **XXIII**



Discussion

A large number of aminoglycoside antibiotics have been discovered and their structures determined. With only a few exceptions this class of antibiotics contain an aminocyclitol moiety as aglycone. Sorbistins are the first example of aminoglycoside antibiotics that have a straight-chain basic aglycone, 1,4-diamino-1,4-dideoxy-D-sorbitol, which is *per se* a novel aminohexitol compound.

As reported in a companion paper[1], sorbistins A_1, A_2 and B inhibit various gram-positive and gram-negative bacteria while sorbistins C and D are inactive. Since the bio-active sorbistins are the N-acylated derivatives of sorbistin D, the acylation of the amino group of 4-amino-4-deoxy-D-glucose moiety should be essential for manifesting the antimicrobial activity of sorbistins. This presents a sharp contrast to the N-acylation effect in the aminocyclitol aminoglycoside antibiotics where a substantial loss of activity generally results from the acylation of amino sugar moieties[12].

Experimental

Thin-layer chromatography (TLC) procedure

TLC was carried out on silica gel plates (60 F_{254}, E. Merck, Darmstadt) using the following solvent systems.

System No.	Solvent system	
S-114	MeOAc - *n*-PrOH - 28% NH_4OH	(45: 105: 60)
S-117	$CHCl_3$ - MeOH - 28% NH_4OH	(1: 3: 2)
SG-105	Pyridine - EtOAc - AcOH - H_2O	(15: 5: 1: 3)
M-103	$CHCl_3$ - MeOH	(95: 5)
PL-101	*n*-BuOH - AcOH - H_2O	(4: 1: 1)
SD-101	$CHCl_3$ - MeOH	(3: 1)

N-Diacetyl sorbistin A_1 (**IIa**)

To a solution of **Ia** (200 mg) in 8 ml of absolute methanol was added 0.75 ml of acetic anhydride and the mixture was stirred for 4 hours at room temperature. The solvent was evaporated under reduced pressure to yield a syrupy residue which was crystallized from a mixture of methanol (2 ml) and acetone (10 ml) to deposit colorless needles of **IIa** monohydrate. Yield 163 mg. m.p. 149~150°C. Molecular weight (osmometry), 491. NMR $\delta_{DSS}^{D_2O}$ in ppm: 1.10 (3H, t, J: 7.3 Hz), 1.97 (3H, s), 2.02 (3H, s), 2.27 (2H, quartet, J: 7.3 Hz), 2.7~3.2 (2H, m), 3.4~4.2 (12H, m) and 5.12 (1H, d, J: 3.0 Hz). Anal. Calc'd for $C_{19}H_{35}N_3O_{11}\cdot H_2O$: C 45.68, H 7.47, N 8.41. Found: C 45.73, H 7.49, N 8.19.

N-Diacetyl sorbistin B (**IIc**)

By a procedure analogous to the above, **Ic** (1 g) was reacted with acetic anhydride in methanol to yield colorless prisms of **IIc** monohydrate. Yield 981 mg. m.p. 159~162°C. Molecular weight (osmometry): 484. NMR $\delta_{DSS}^{D_2O}$ in ppm: 1.98~2.03 (9H), 2.7~3.15 (2H, m), 3.4~4.2 (12H, m) and 5.12 (1H, d, J: 3.0 Hz). Anal. Calc'd for $C_{18}H_{33}N_3O_{11}\cdot H_2O$: C 44.53, H 7.27, N 8.66. Found: C 44.96, H 7.44, N 8.53.

N-Diacetyl sorbistin C (**V**)

III (1.19 g) was worked up in the same manner as described above to afford colorless needles of **V**. Yield 1.024 g. m.p. 121~124°C. NMR $\delta_{DSS}^{D_2O}$ in ppm: 2.02 (3H, s), 2.07 (3H, s), 2.8~3.25 (2H, m), 3.4~4.2 (12H, m) and 5.17 (1H, d, J: 3.0 Hz). Anal. Calc'd for $C_{16}H_{30}N_2O_{11}$: C 45.06, H 7.09, N 6.57. Found: C 44.79, H 7.36, N 6.35.

N-Triacetyl sorbistin D (**VI**)

Acetylation of **IV** (1 g) in the same manner as above yielded 780 mg of crystalline N-triacetate **VI**. m.p. 160~162°C. Mixed m.p. with **IIc** 159~163°C. Anal. Calc'd for $C_{18}H_{33}N_3O_{11}\cdot H_2O$: C 44.53, H 7.27, N 8.66. Found: C 44.59, H 7.37, N 8.41.

Alkaline hydrolysis of **Ia**

Ia (550 mg) was dissolved in 20 ml of saturated barium hydroxide solution and heated under reflux for 16 hours. The solution was neutralized with 2 N H_2SO_4 and the resulting precipitate removed by

filtration. The clear solution was concentrated and loaded on a column of Amberlite CG-50 (NH_4^+, 30 ml). The column was washed with water and then developed with 0.1 N NH_4OH. The water wash (100 ml) was concentrated at pH 7 to 1 ml and reacted with *p*-bromophenacyl bromide (281 mg) in 1 ml of ethanol at 100°C for one hour. The mixture was kept in a refrigerator for 18 hours to deposit 41 mg of colorless crystals. m.p. 56.6~58.5°C. Mixed m.p. with *p*-bromophenacyl propionate 57~59°C. Anal. Calc'd for $C_{11}H_{11}O_3Br$: C 48.73, H 4.09. Found: C 48.40, H 3.89.

The ninhydrin-positive fractions eluted by 0.1 N NH_4OH were pooled, concentrated *in vacuo* and lyophylized to afford 375 mg of **IV** carbonate. TLC: Rf 0.28 (S-117). NMR $\delta_{DSS}^{D_2O+DCl}$: 3.1~3.4 (3H, m), 3.6~4.3 (12H, m) and 5.23 (1H, d, J: 3.0 Hz). Anal. Calc'd for $C_{12}H_{27}N_3O_8 \cdot H_2CO_3$: C 38.70, H 7.25, N 10.42. Found: C 38.45, H 6.91, N 10.07. The physicochemical and spectral properties were identical with those of **IV** isolated from fermentation broth.

Methanolysis of **Ia**

A solution of **Ia** (5.05 g) in 300 ml of absolute methanol was refluxed for 20 hours under continuous bubbling of dry HCl gas. The solution was concentrated *in vacuo* to an oily residue, which was dissolved in water (50 ml), neutralized with Amberlite IR-45 (OH^-) and applied on a column containing 200 ml of Amberlite CG-50 (NH_4^+). The column was developed successively with water, 0.05 N NH_4OH, 0.1 N NH_4OH and 0.25 N NH_4OH. The effluent was collected in fractions and monitored by the ninhydrin reaction. The first ninhydrin-positive fractions eluted by 0.05 N NH_4OH were evaporated *in vacuo* to yield 1.03 g of hygroscopic sugar mixture (**IXa**, **IXb** and **XVI**). Concentration of the second ninhydrin-positive fractions eluted by 0.1 N NH_4OH afforded 1.477 g of crude **IV** as amorphous carbonate. TLC: Rf 0.28 (S-117). Subsequent elution with 0.25 N NH_4OH gave another basic fragment (**X**, 1.332 g) as a carbonate salt. A solution of the carbonate (500 mg) in 3 ml water was acidified to pH 2.0 with dil.H_2SO_4, then diluted with methanol and kept at room temperature to afford colorless prisms of sulfate, 528 mg. m.p. 263~264°C. $[\alpha]_D^{28}$ −9.8° (*c* 0.5, H_2O). TLC: Rf 0.32 (S-117). NMR $\delta_{DSS}^{D_2O}$ in ppm: 3.23 (2H, d) and 3.4~4.2 (6H, m). Anal. Calc'd for $C_6H_{16}N_2O_4 \cdot H_2SO_4$: C 25.90, H 6.52, N 10.07, S 11.52. Found: C 26.10, H 6.47, N 9.93, S 11.29.

The mixture of sugar fragments (1.30 g) was chromatographed again on a column of Amberlite CG-50 (60% NH_4^+ form, 200 ml). After being washed with three resin-volumes of water, the column was developed with 0.05 N NH_4OH. The eluates were monitored by TLC (SG-105, detection by ninhydrin reagent) and the appropriate fractions were combined and concentrated to give the following three components; **IXb** (first fraction, 305 mg), **IXa** (second fractions, 840 mg) and **XVI** (third fractions, 53 mg)

IXb: Hygroscopic white powder, $[\alpha]_D^{25.5}$ −38° (*c* 0.5, H_2O). TLC: Rf 0.74 (S-117) and 0.37 (SG-105). NMR $\delta_{DSS}^{D_2O}$ in ppm: 2.73 (1H, t), 3.60 (3H, s), 3.2~3.85 (5H, m) and 4.37 (1H, d, J: 7.2 Hz).

IXa: Solution of crude **IXa** (800 mg) in 10 ml of ethanol containing 0.1 ml of water was kept overnight in a refrigerator to afford 635 mg of colorless prisms. m.p. 170~173°C[3]. $[\alpha]_D^{25}$ +161° (*c* 0.5, H_2O). TLC 0.75 (S-117) and 0.27 (SG-105). NMR $\delta_{DSS}^{D_2O}$ in ppm: 2.72 (1H, m), 3.43 (3H, s), 3.5~3.85 (5H, m) and 4.82 (1H, d, J: 2.5 Hz). Anal. Calc'd for $C_7H_{15}NO_5$: C 43.52, H 7.83, N7.05. Found: C43.54, H 8.03, N 7.00.

XVI: Hygroscopic brown powder. TLC: Rf 0.55 and 0.49 (S-117).

N,O-Tetraacetyl-**IXa** (**XIa**)

IXa (135 mg) was dissolved in a mixture of acetic anhydride (0.8 ml) and pyridine (0.5 ml) and the solution was kept at room temperature for 18 hours. The excess acetic anhydride was decomposed by 5 ml of ice-water and the aqueous solution was concentrated to dryness *in vacuo*. The residue was crystallized from a mixture of acetone, benzene and *n*-hexane to yield colorless plates (**XIa**, 159 mg). m.p. 143~144°C. Mixed m.p. with methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-α-D-glycopyranoside* 143~144°C. NMR $\delta_{TMS}^{ODCl_3}$ in ppm: 1.95~2.12 (12H, 4×CH_3CO), 3.42 (3H, s), 3.7~4.3 (4H, m), 4.8~5.5 (3H, m), 6.18 (1H, d, NH). Anal. Calc'd for $C_{15}H_{23}NO_9$: C 49.86, H 6.42, N 3.88. Found: C 49.61, H. 6.60, N 3.85.

* obtained from 4-trehalosamine[4].

N,O-Tetraacetyl-IXb (XIb)

By essentially the same procedure as above, **IXb** (128 mg) was acetylated to afford colorless needles (**XIb**, 133 mg). m.p. 202~203°C. Anal. Calc'd for $C_{15}H_{23}NO_9$: C 49.86, H 6.42, N 3.88. Found: C 49.65, H 6.51, N 3.86. The NMR and IR spectra were identical with those of methyl 4-acetamido-4-deoxy-2,3,6-tri-O-acetyl-β-D-glucopyranoside[4].

N-Diacetyl-X (XII)

To a solution of **X** (1.1 g) in absolute methanol (52 ml) was added 5.4 ml of acetic anhydride and the solution stirred for 4 hours. The mixture was evaporated to dryness and the oily residue crystallized from a mixture of methanol, acetone and ether to yield colorless needles (XII, 971 mg). m.p. 154~156°C. ν_{max}^{KBr}: 1650, 1550 and 1435 cm^{-1}. TLC: Rf 0.67 (S-117) and 0.10 (SD-101). Anal. Calc'd for $C_{10}H_{20}N_2O_6$: C 45.45, H 7.63, N 10.60. Found: C 45.52, H 7.78, N 10.23.

N,O-Hexaacetyl-X (XIII)

A solution of **XII** (500 mg) in acetic anhydride (4.5 ml) and pyridine (3 ml) was heated at 110°C for 4 hours. The reaction mixture was concentrated under reduced pressure to afford a sticky solid which was applied on a silica gel column (ϕ 1.2×50 cm) and eluted with $CHCl_3$–MeOH (98: 2). The elution was followed by TLC (PL-101) and appropriate fractions were combined. Subsequently to a faster moving by-product (360 mg, TLC: Rf 0.59 by PL-101) the fractions containing **XIII** were eluted, which were concentrated *in vacuo* to afford 390 mg of white solid. The amorphous solid was crystallized from a mixture of chloroform and *n*-hexane. Yield 272 mg. m.p. 116~117°C. TLC: Rf 0.48 (PL-101), 0.95 (S-117). ν_{max}^{KBr}: 1745, 1660, 1550, 1440 and 1380 cm^{-1}. MW: 432 (mass). Anal. Calc'd for $C_{18}H_{28}N_2O_{10}$ C 50.00, H 6.53, N 6.48. Found: C 49.52, H 6.46, N 6.33.

N-Diacetyl-diisopropylidene-X (XIV)

To a solution of **XII** (264 mg) in 10 ml of dry dimethylformamide (DMF) was added dimethoxypropane (0.7 ml) and *p*-toluenesulfonic acid (15 mg), and the mixture was kept overnight at room temperature[13]. The solution was concentrated *in vacuo* to an oily residue which was applied on a column of silica gel and developed with EtOAc-MeOH (98: 2). **XIV** was obtained as a hygroscopic white powder. Yield 230 mg. TLC: Rf 0.66 (SD-101). Mass spectrum: *m/e* 329 (M−15), 285, 272, 243, 186, 172, 106, 72.

4-Amino-4-deoxy-D-glucose (XVI)

A solution of **IXa** (50 mg) in 10% H_2SO_4 (1 ml) was heated on a boiling water bath for 4 hours. The cooled solution was adjusted to pH 7.5 with saturated $Ba(OH)_2$ solution and white precipitate which deposited was filtered off. The clear solution was evaporated to dryness to yield a hygroscopic brown powder (**XVI**, 43 mg). TLC: Rf 0.55 and 0.49 (S-117), NMR $\delta_{DSS}^{D_2O+DCl}$ in ppm: 2.7~3.9 (6H, m), 4.31 (0.6H, J: 7.5 Hz) and 4.89 (0.4H, J:3.1 Hz).

Synthesis of 1,4-diamino-1,4-dideoxy-D-sorbitol

A mixture of **XVI** (HCl salt, 700 mg), sodium acetate (1 g) and hydroxylamine hydrochloride (280 mg) in 100 ml of 40% ethanol was heated under reflux for 3 hours. The solution was adjusted to pH 2.3~2.5 with dil.HCl and then filtered to remove insoluble materials. The oxime solution thus obtained was hydrogenated over 10% palladium on charcoal (360 mg) at room temperature for 18 hours. After the catalyst was removed by filtration the solution was concentrated to 5 ml and charged on a column containing 80 ml of Amberlite CG-50 (NH_4^+). The column was developed successively with water, 0.2 N NH_4OH and 0.5 N NH_4OH. The elution was monitored by ninhydrin test and the ninhydrin-positive fractions eluted with 0.5 N NH_4OH were evaporated to afford the desired product as a white hygroscopic powder (165 mg, carbonate salt). A portion of the solid (116 mg) was crystallized as a sulfate by the procedure aforementioned. Yield 124 mg. m.p. 262~263°C. Mixed m.p. with the sulfate of **X** 261.5~263°C. $[\alpha]_D^{28}$ −10.3° (*c* 0.38, H_2O).

Methylation of **VI** and isolation of **XVIII**

Sodium hydride (3.9 g, 50% dispersion in oil) washed with dry petroleum ether (200 ml) was added a dry DMF solution (35 ml) of **VI** (900 mg). The mixture was stirred for one hour at room temperature and then cooled below 10°C. Methyl iodide (15 ml) was added dropwise into the solution under vigorous

stirring and the stirring was continued for 18 hours at 20~25°C. The insoluble material was removed by filtration and the filtrate was concentrated *in vacuo* with an addition of toluene to expedite evaporation. The residue was dissolved in a mixture of $CHCl_3$ (50 ml) and water (50 ml). The $CHCl_3$ layer was separated, washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent gave the per-methylation product (**XVII**, 1.030 g) as a sticky solid.

A solution of **XVII** (970 mg) in 6 N HCl (50 ml) was heated under reflux for 3 hours and then concentrated to dryness. The residue was charged on a column of Amberlite CG-50 (NH_4^+, 200 ml), and after being washed with 300 ml of water the column was developed with 0.05 N NH_4OH. Evaporation of the eluate gave **XVIII** (149 mg) as a colorless oil. TLC: Rf 0.64 (S-114), 0.03 (SD-101). NMR $\delta_{DSS}^{D_2O}$ in ppm: 2.27 (6H, s), 2.55 (2H, m), 3.22 (3H, s), 3.27 (3H, s), 3.32 (3H, s) and 3.3~3.7 (6H, m).

N,O-Triacetyl-**XVIII** (**XIX**)

A mixture of **XVIII** (79 mg), acetic anhydride (0.6 ml) and pyridine (0.5 ml) was stirred in a water bath (50°C) for 4 hours. The solution was evaporated and the residue was applied on a column of silica gel (ϕ 1.0×40 cm). The column was developed with $CHCl_3$ and then with $CHCl_3$–MeOH (98: 2). Eluates with the latter mixed-solvent afforded **XIX** (82 mg) as a colorless oil. GLC (OV-17 3%, column temp. 240°C): Rt 6.1 min. TLC: Rf 0.41 (SD-101). Anal. Calc'd for $C_{17}H_{32}N_2O_7$: C 54.24, H 8.57, N 7.44. Found: C 54.01, H 8.69, N 7.13.

N-Diacetyl-**XVIII** (**XX**)

A solution of **XVIII** (58 mg) in acetic anhydride (0.5 ml) and absolute methanol (3 ml) was kept at room temperature for 5 hours. The solution was evaporated and the residue was chromatographed on a silica gel column which was eluted by $CHCl_3$–MeOH (98: 2). **XX** was obtained as a colorless oil. Yield, 43 mg. TLC: Rf 0.33 (SD-101), 0.36 (PL-101). IR ν_{max}^{liq}: 3300~3500, 2850~3000, 1640, 1105.

N-Diacetyl-3-oxo-**XVIII** (**XXI**)

A solution of **XX** (42 mg) in dry pyridine (0.5 ml) was added dropwise at 10°C to a solution of chrominium trioxide (35 mg) in pyridine (0.5 ml). The mixture was stirred at 10°C for 2 hours and then kept at room temperature for 42 hours. After the inorganic precipitate was removed by filtration, the reaction mixture was diluted with 5 ml of water and concentrated to dryness under reduced pressure. The residue was extracted with 5 ml of benzene and the extract was concentrated to yield 35 mg of crude product, which was chromatographed on a silica gel column using $CHCl_3$–MeOH (99: 1) as an elution solvent. The appropriate fractions monitored by TLC (PL-101) were combined and evaporation of the solvent afforded the desired product (**XXI**, 26.5 mg) as a colorless oil. TLC: Rf 0.45 (PL-101). GLC (OV-17 3%, column temp. 230°C): Rt 10.9 min. IR ν_{max}^{liq}: 2850~3000, 1730, 1645, 1100. Anal. Calc'd for $C_{15}H_{28}N_2O_6$: C 54.20, H 8.49, N 8.43. Found: C 53.73, H 8.40, N 8.04.

Methanolysis of **III**

A solution of **III** (300 mg) in 2 N methanolic hydrogen chloride (25 ml) was heated under reflux for 5 hours. The solvent was evaporated and the residue loaded on a column of Amberlite CG-50 (NH_4^+, 15 ml). After being washed with water the column was eluted with 0.1 N NH_4OH, followed by 0.2 N NH_4OH. A small amount of **III** (16 mg) was recovered from the 0.1 N NH_4OH eluate and then 106 mg of aglycone **X** was isolated from 0.2 N NH_4OH eluate.

The anthrone-positive fractions of the water washing were combined and evaporated to afford 151 mg of white powder. Identical TLC and GLC (TMS-derivative) with those of methyl D-glucoside. TLC: Rf 0.63 (S-117). GLC (OV-17 3%, column temp. 180°C): Rt 10.0 min. $[\alpha]_D^{24.5}$ +102° (*c* 2.0, H_2O).

Trimethylsilylation (TMS)

All trimethylsilyl derivatives used for mass spectrometry and gas chromatography were prepared by the usual method. An example for **XXIIa** is shown below: To a suspension of **IIa** (20 mg) in dry pyridine (1.5 ml) was added hexamethyldisilazane (0.4 ml) and trimethylchlorosilane (0.2 ml), and the mixture was vigorously shaken for 5 minutes. The solution was diluted with 10 ml of $CHCl_3$ and washed three times with 5-ml portions of water. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and evaporated to yield a white solid of pertrimethylsilyl-**IIa** (**XXIIa**, 21 mg).

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References

1) TSUKIURA, H.; M. HANADA, K. SAITO, K. FUJISAWA, T. MIYAKI, H. KOSHIYAMA & H. KAWAGUCHI: Sorbistin, a new aminoglycoside antibiotic complex of bacterial origin. I. Production, isolation and properties. J. Antibiotics 29: 1137~1146, 1976
2) ERICKSON, J. L. E.; J. M. DECHARY & M. R. KESLING: Diazoketones as reagents for the identification of organic acids. J. Am. Chem. Soc. 73: 5301~5302, 1951
3) REIST, E. J.; R. R. SPENCER, D. F. CALKINS, B. R. BAKER & L. GOODMAN: Derivatives of 4-amino-4-deoxy-D-glucose. J. Org. Chem. 30: 2312~2317, 1965
4) NAGANAWA, H.; N. USUI, T. TAKITA, M. HAMADA, K. MAEDA & H. UMEZAWA: 4-Amino-4-deoxy-α,α-trehalose, a new metabolite of a *Streptomyces*. J. Antibiotics 27: 145~146, 1974
5) PAULSEN, H.; K. STEINERT & K. HEYNS: Darstellung freier 4-Amino-4-desoxy-D-glukose und 4-Amino-4-desoxy-D-galaktose. Dimerisierung zu Bis-pyrrolidinzuckern. Chem. Ber. 103: 1599~1620, 1970
6) WOLFROM, M. L.; F. SHAFIZADEH, J. D. WEHRMULLER & R. K. ARMSTRONG: Synthesis of amino compounds in the sugar series by phenylhydrazone reduction. J. Org. Chem. 23: 571~575, 1958
7) PAULSEN, H.; K. PROPP & K. HEYNS: Monosaccharide mit stickstoffhaltigem Ring. XXI. 4-Amino-4-desoxy-D-glukose und 4-Amino-4-desoxy-D-galaktose. Tetrahedron Letters 1969-9: 683~686, 1969
8) CHARLSON, A. J.; P. A. J. GORIN & A. S. PERLIN: Determination of the configuration of glycosidic linkages in oligosaccharides. Method in Carbohydrate Chemistry. I. pp. 419~426, 1963
9) REIMAN, H.; D. J. COOPER, A. K. MALLAMS, R. S. JARET, A. YEHASKELL, M. KUGELMAN, H. F. VERNAY & D. SCHUMACHER: The structure of sisomicin, a novel aminocyclitol antibiotic from *Micromonospora inyoensis*. J. Org. Chem. 39: 1451~1457, 1974
10) POOS, G. I.; G. E. ARTH, R. E. BEYLER & L. H. SARETH: Approach to the total synthesis of adrenal steroids. V. J. Am. Chem. Soc. 75: 422~429, 1953
11) KAMERLING, J. P.; J. F. G. VLIEGENTHART, J. VINK & J. J. DE RIDDER: Mass spectrometry of pertrimethylsilyl aldosyl oligosaccharides. Tetrahedron 27: 4275~4288, 1971
12) BENVENISTE, R. & J. DAVIES: Structure-activity relationship among the aminoglycoside antibiotics: Role of hydroxyl and amino groups. Antimicr. Agents & Chemoth. 4: 402~409, 1973
13) UMEZAWA, H.; T. TSUCHIYA, R. MUTO & S. UMEZAWA: Studies on aminosugars. XXIX. The synthesis of 3'-O-methylkanamycin. Bull. Chem. Soc. Japan 45: 2842~2847, 1972