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

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The plane structure of the aminopolyol moiety of the aminoglycoside antibiotics P-2563(P) (**1**) and P-2563(A) (**2**) was elucidated to be 1,4-diamino-1,4-dideoxyhexitol (**3**) from physico-chemical and spectroscopic characterizations. The absolute configuration of **3** was determined to be 2*S*, 3*S*, 4*R* and 5*S* on the basis of transformation of **3** into methyl 3,6-diacetamido-2,4-di-O-acetyl-3,6-dideoxy-α-L-gulopyranoside (**12**) by the oxidation of the hydroxymethyl group at C-6.

Keywords——aminoglycoside antibiotics; *Pseudomonas fluorescence*; aglycone; (2*S*,3*S*,4*R*,5*S*)-1,4-diamino-1,4-dideoxyhexitol; methyl 3,6-diacetamido-2,4-di-O-acetyl-3,6-dideoxy-α-L-gulopyranoside (1C); methyl 3,6-diacetamido-2,5-di-O-acetyl-3,6-dideoxy-β-L-gulofranoside

In the previous paper,[1] it was reported that two antibiotics, P-2563(P) (**1**) and P-2563 (A) (**2**), are acylaminoglycosides having the same new aminopolyol[1] (**3**) in their molecules. This report deals with the plane structure and the absolute configuration of **3**.

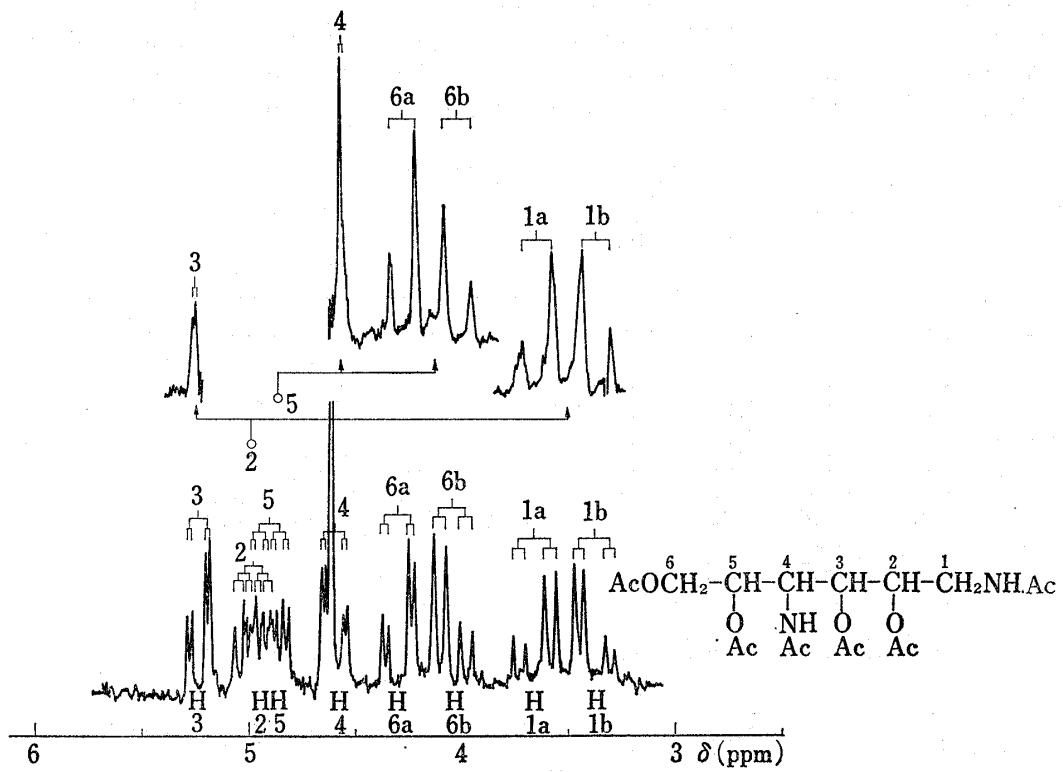

Fig. 1. PMR Spectrum and Spin-decoupling of **5** (in $CDCl_3+D_2O$)

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

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The authors have revealed the structure of **3** by proton magnetic resonance (PMR) and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) studies; the absolute configuration was established by correlating **3** with methyl 3,6-diacetamido-2,4-di-O-acetyl-3,6-dideoxy-α-L-gulopyranoside (**12**).

The PMR spectrum of hexaacetate of **3** (**5**)[1] is shown in Fig. 1. The PMR spectrum of **5** showed two methylene protons and four methine protons. The doublet at δ 6.30 (J=9.7 Hz, Ac–N<u>H</u>–CH–) and the double doublet at δ 6.17 (J=5 and 6 Hz, Ac–N<u>H</u>–C<u>H</u>$_2$–) were diminished on exchange with D_2O. This deuterium exchange also revealed the expected simplification in the double triplet pattern at δ 4.60 and the two octet patterns at δ 3.66 and 3.38, assignable to Ac–NH–C<u>H</u>– proton and Ac–NH–C<u>H</u>$_2$– protons (H-1a, 1b), respectively. In the PMR spectrum after the deuterium exchange, when the N-methine proton at δ 4.60 was irradiated, the double doublet in the lower field (δ 5.24, 1H) was collapsed into a doublet (J=8.5 Hz). On the other hand, when the double doublet centered at δ 4.99 was irradiated, the double doublet at δ 5.24 was collapsed into a doublet (J=2.2 Hz) and, at the same time, the N-methylene octets at δ 3.66 and 3.38 (J=14.8 and 5.7 Hz, J=14.8 and 4.0 Hz) were collapsed into a pair of doublets (J=14.8 Hz). From these evidence, the partial structure as shown in Chart 1 was considered.

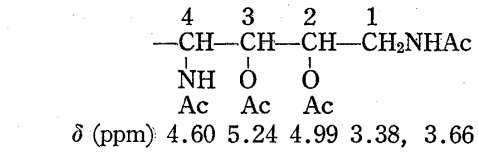


Chart 1

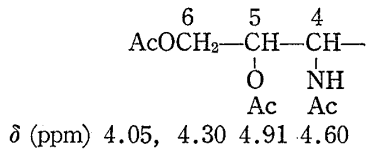


Chart 2

Next, irradiation of the multiplet at δ 4.91 resulted in the decoupling of the downfield O-methylene double doublets (δ 4.05 and 4.30) into a pair of doublets (J=12.5 Hz), and, at the same time, the decoupling of the double doublet of the N-methine at δ 4.60 into a doublet (J=2.2 Hz). This indicated the presence of the partial structure as shown in Chart 2.

Table I. PMR Spectra of **5** (100 MHz in $CDCl_3$+D_2O)

		H 3	H 2	H 5	H 4	H 6a	H 6b	H 1a	H 1b	Ac	NH[a] on C-4	NH[a] on C-1
5	δ (ppm)	5.24	4.99	4.91	4.60	4.30	4.05	3.66	3.38	1.94 2.12	6.30	6.17
		d.d	oct	oct	d.d	d.d	d.d	d.d	d.d	s	d	t like
		1H	1H	1H	1H	1H	1H	1H	1H	3H×6	1H	1H
	J (Hz)	J_{2-3} 8.5	J_{1a-2} 5.7	J_{4-5} 9.7	J_{3-4} 2.2	J_{5-6a} 2.7	J_{5-6b} 5.7	J_{1a-1b} 14.8	J_{1a-1b} 14.8		J_{4-NH} 9.7	J_{1a-NH} 6.0
		J_{3-4} 2.2	J_{1b-2} 4.0	J_{5-6a} 2.7	J_{4-5} 9.7	J_{6a-6b} 12.5	J_{6a-6b} 12.5	J_{1a-2} 5.7	J_{1b-2} 4.0			J_{1b-NH} 5.0
			J_{2-3} 8.5	J_{5-6b} 5.7								

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

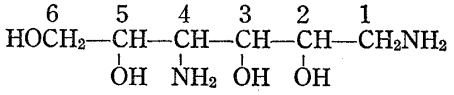


Chart 3

The above decoupling experiment and the measurement of the coupling constants shown in Table I established the plane structure of **3** (Chart 3).

The ^{13}C-NMR spectrum of **3** (Table II) consisted of 6 well-resolved peaks, confirming the presence of six carbon atoms in **3**. The peaks at 44.1 (t), 63.8 (t) and 55.1 (d) ppm were readily assigned to N-methylene, O-methylene and N-methine carbons respectively, while

Table II. ^{13}C-NMR Spectrum of 3

3 (δ) [a]	
44.1 (t) [b]	N-Methylene
55.1 (d)	N-Methine
63.8 (t)	O-Methylene
70.8 (d)	
73.2 (d)	
75.1 (d)	

a) δ: ppm from tetramethylsilane (TMS) using dioxane (δ=67.4 ppm) as the internal reference.
b) (d) indicates doublet, and (t), triplet measured at partially decoupled conditions.

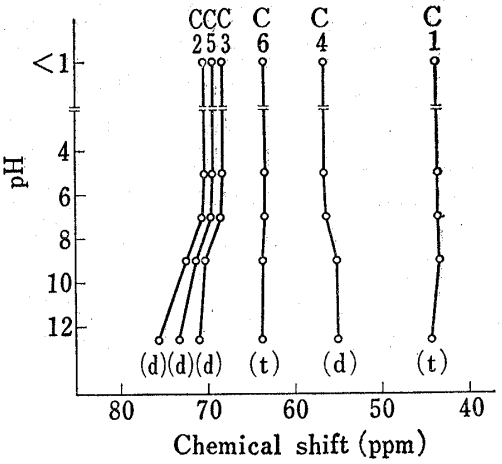


Fig. 2. pH-dependency of ^{13}C Chemical Shift of 3

(d) indicates doublet, (t), triplet measured at partially decoupled conditions.

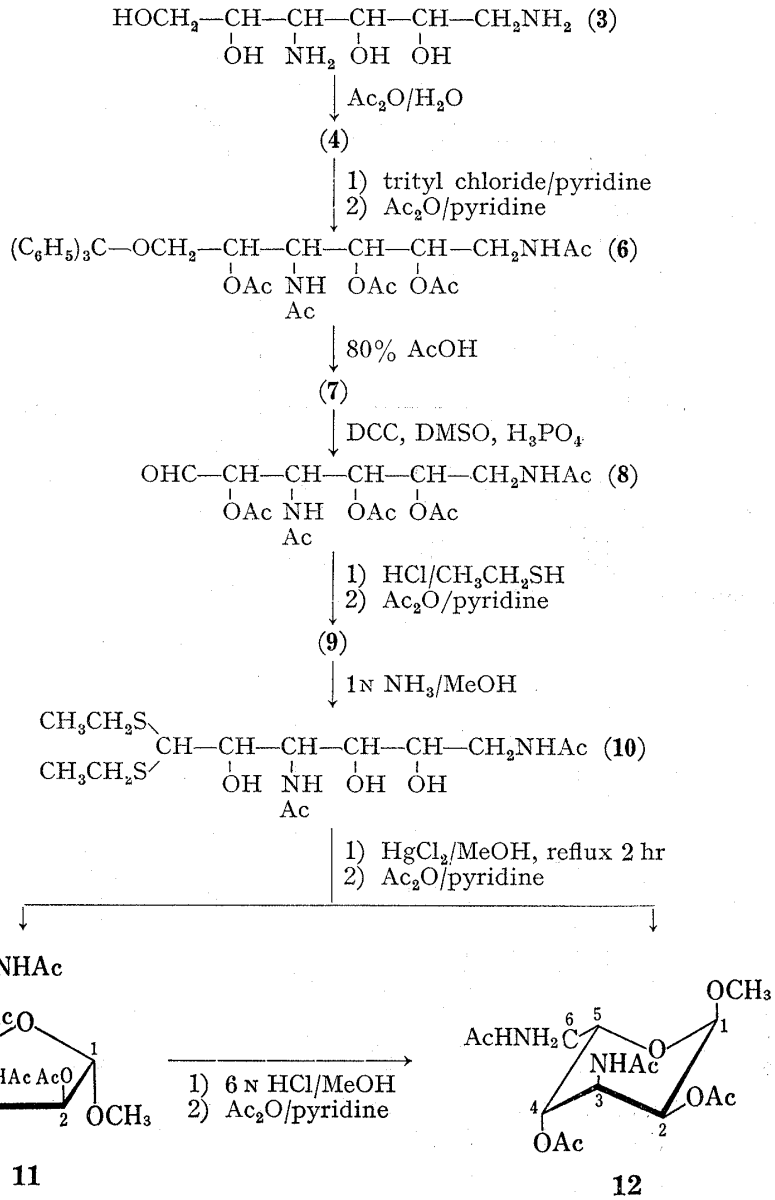


Chart 4

peaks at 70.8 (d), 73.2 (d) and 75.1 (d) ppm seemed likely to represent resonances of O-methine carbons, suggesting the straight-chain carbon skeleton of **3**.

The pH-dependency of ^{13}C-NMR chemical shift of **3** is shown in Fig. 2.

It was observed that all three O-methine carbons showed an upfield shift approximately to the same extent. Koch, *et al.*[3] have reported that the difference of α-, γ-, and δ-carbon shifts between a primary amine and its protic salt is small (0.5—1.5 ppm), while, the $\Delta\delta$ value for the β-carbon ($\Delta\delta^{\beta}$) is large. This $\Delta\delta^{\beta}$ effect is recognized to be valuable in structure analysis.

Here authors try to apply this new analytical method to the determination of the position of the N-methine in **3**. When C-1 position is taken as the N-methylene of **3**, only C-4

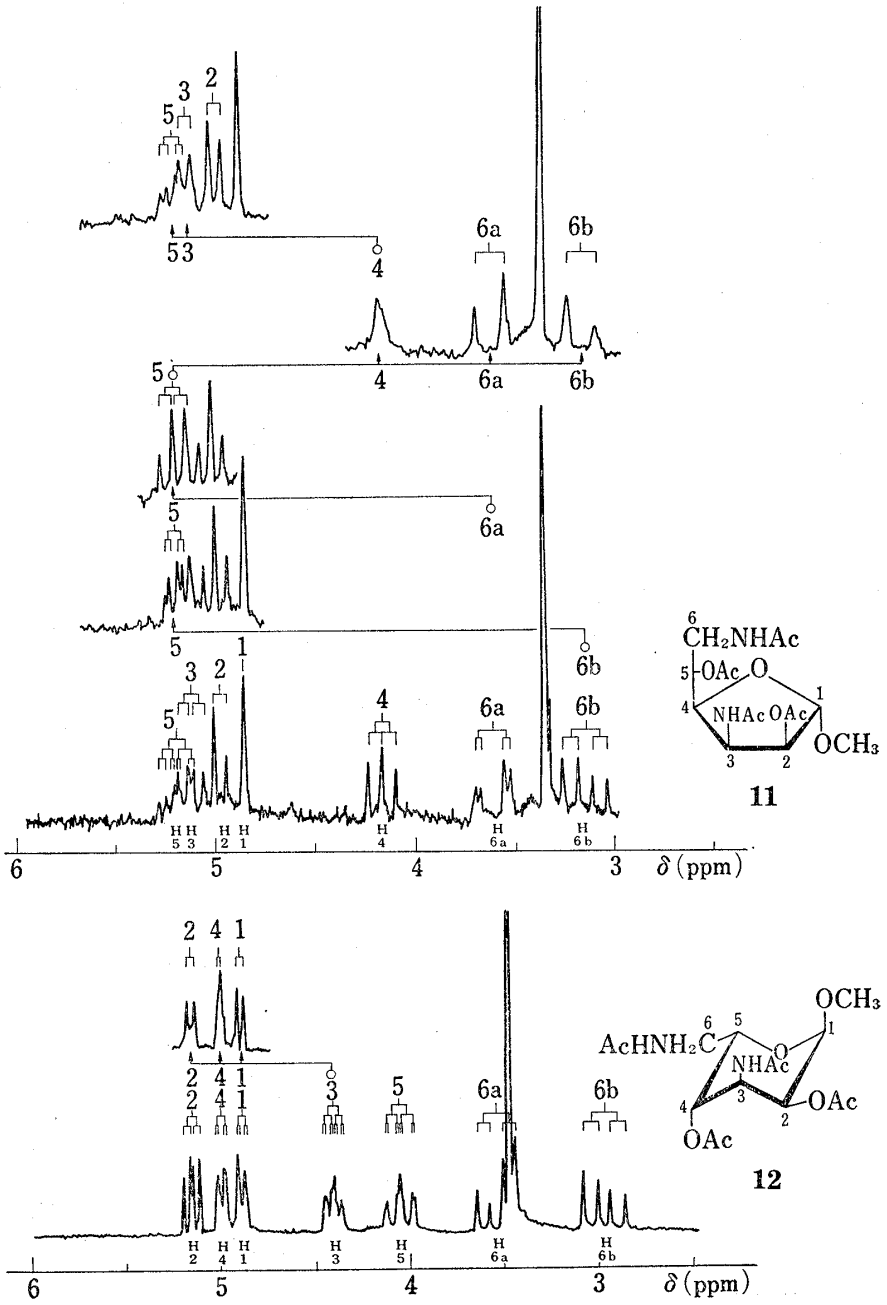


Fig. 3. PMR Spectra and Spin-decoupling of **11** and **12** (100 MHz in $CDCl_3+D_2O$)

3) K.F. Koch, J.A. Rhoades, E.W. Hagaman, and E. Winkert, *J. Am. Chem. Soc.*, **96**, 300 (1974).

position is chosen for the N-methine to satisfy this $\Delta\delta^{8}$ effect. This agrees well with the assigned structure.

An attempt to convert **3** into **12** (**11**) through oxidation of the hydroxy methyl group and subsequent thioacetalization was successful as shown in Chart 4. The conversion provided crucial evidence for the stereochemistry of **3**.

N-Acetylation, tritylation and O-acetylation of **3** afforded 2,3,5-tri-O-acetyl-6-O-trityl-1,4-diacetamido-1,4-dideoxyhexitol (**6**). Detritylation of **6** with 80% AcOH gave 2,3,5-tri-O-acetyl-1,4-diacetamido-1,4-dideoxyhexitol (**7**). Oxidation of **7** with dimethyl sulfoxide (DMSO)–dicyclohexylcarbodiimide (DCC) method[4] yielded 2,4,5-tri-O-acetyl-3,6-diacetamido-3,6-dideoxyhexose (**8**). Thioacetalization with ethanethiol of **8** and reacetylation of partially hydrolyzed OH groups gave 2,4,5-tri-O-acetyl-3,6-diacetamido-3,6-dideoxyhexose diethyl dithioacetal (**9**). Treatment of **9** with 1 N NH_3 in MeOH afforded O-deacetyl-derivative (**10**). Methanolysis of **10** with $HgCl_2$ in MeOH, followed by acetylation gave methyl glycoside tetra-acetates (**11**), $C_{15}H_{24}N_2O_8$, and **12**, $C_{15}H_{24}N_2O_8$.

The structures of **11** and **12** have also been clarified by PMR studies (Fig. 3). The chemical shifts and coupling constants in PMR spectra of **11** and **12** are shown in Table III.

TABLE III. PMR Spectra of **11** and **12** (100 MHz in $CDCl_3+D_2O$)

		H 2	H 4	H 1	H 3	H 5	H 6a	H 6b	Ac	OCH_3
11[a]	δ (ppm)	4.99	4.17	4.86	5.14	5.21	3.62	3.16	2.18 2.16 2.00	3.36
	J (Hz)	J_{1-2} <0.5 J_{2-3} 6.0	J_{3-4} 7.0 J_{4-5} 7.0	J_{1-2} <0.5	J_{2-3} 6.0 J_{3-4} 7.0	J_{4-5} 7.0 J_{5-6a} 3.5 J_{5-6b} 8.0	J_{5-6a} 3.5 J_{6a-6b} 15.0	J_{5-6b} 8.0 J_{6a-6b} 15.0		
12	δ (ppm)	5.16	5.00	4.89	4.40	4.05	3.54	2.97	2.18 2.10 2.04 2.00	3.48
	J (Hz)	J_{1-2} 3.7 J_{2-3} 5.0	J_{3-4} 4.0 J_{4-5} 1.5	J_{1-2} 3.7 J_{1-3} 1.5	J_{2-3} 5.0 J_{3-4} 4.0 J_{1-3} 1.5	J_{4-5} 1.5 J_{5-6a} 6.0 J_{5-6b} 8.0	J_{5-6a} 6.0 J_{6a-6b} 14.0	J_{5-6b} 8.0 J_{6a-6b} 14.0		

a) The assignments and description of **11** in the previous paper,*) which had been done on the assumption that **11** was a methyl glycopyranoside were incorrect, because it was found that **11** was a methyl glycofranoside, an unexpected product under this experimental conditions. *) K. Nara, K. Katamoto, S. Suzuki, S. Akiyama, and E. Mizuta, *Chem. Lett.*, **1977**, 229.

It was found from these data that, in **12**, (1) a relatively broad long-range coupling of about 1.5 Hz between H-1 and H-3, which is ascribed to a "W" letter arrangement of bonds,[5] is present, (2) the chemical shift of methoxy protons is 3.48 ppm, indicating that the methoxy group is axial,[6] and (3) coupling-constants of ring-protons are relatively small. These findings and consideration of the stability of conformers suggested only six possible structures [*i.e.* β-D-talopyranoside (1C), β-L-talopyranoside (1C), α-D-idopyranoside (C 1), α-L-idopyrano-

4) K.E. Pfitzner and J.G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5661, 5670 (1965).
5) L.D. Hall and L. Hough, *Proc. Chem. Soc.*, **1962**, 382.
6) A. Konowat and A. Zamojski, *Ann. Soc. Chim. Polonorum.*, **44**, 1607 (1970).

side (1C), α-D-gulopyranoside (C 1) and α-L-gulopyranoside (1C)] for **12** among the probable sixty four structures.

On the other hand, **11** was assumed to be a methyl glycofranoside on the basis of the fact that the chemical shift of H-5 (5.21 ppm) is in the lowerfield than that of H-4 (4.17 ppm). From the table of coupling constants of pentofranose derivatives reported by Stevens, *et al.*,[7] the presence of only eight structures [*i.e.* α-D (or L)-mannofranoside, α-D(or L)-talofranoside, β-D(or L)-allofranoside, β-D(or L)-gulofranoside] remained possible among the probable thirty two structures, because the coupling constants between H-1, H-2, H-3 and H-4 showed small (<0.5 Hz), large (6.0 Hz) and large (7.0 Hz), respectively. Further, taking into account the fact[7] that, for pairs of compounds isomeric at C-4, when the C-4 substituent (–CH_2–O-acyl) is on the same side of the ring as H-3 (H-3 and H-4: *trans*), this hydrogen (H-3) is much more shielded than the isomer, it could be assumed that the C-4 substituent in **11** is on the other side of the ring as H-3 (H-3 and H-4: *cis*), because H-3 (5.14 ppm) in **11** is remarkably deshielded in spite of the presence of an N-methine. The deshielding effect for H-3 in **11** may be attributable to the diamagnetic anisotropic effect[8] of the carbonyl group on C-5.

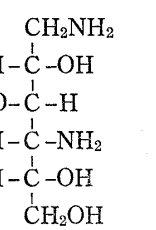
From these, α-D(or L)-mannofranoside or β-D(or L)-gulofranoside was assigned to **11**.

Treatment of **11** with 6 N HCl in MeOH under reflux, followed by reacetylation, afforded **12**, indicating that the configurations of C 2, 3, 4 and 5 in **11** and **12** are the same.

Separately, the authors synthesized 3,6-diacetamido-3,6-dideoxy-D-gulose from D-glucose by the procedures reported by Weidmann.[9] The methyl-glycosidation and subsequent O-acetylation afforded methyl 3,6-diacetamido-2,4-di-O-acetyl-3,6-dideoxy-α-D-gulopyranoside (**13**), $C_{15}H_{24}N_2O_8$, $[\alpha]_D^{21}$ +21° (c=1.0, $CHCl_3$). The infrared (IR) and PMR spectra of **12** were identical with those of **13**, while specific rotations of **12** and **13** were −25.1° and +21°, respectively.

Thus, the structure of **12** was confirmed to be the mirror image of **13** and the structure of **11** was assumed to be methyl 3,6-diacetamido-2,5-di-O-acetyl-3,6-dideoxy-β-L-gulofranoside.

From these facts, the absolute configuration of **3** was concluded to be(2*S*, 3*S*, 4*R*, 5*S*)-1,4-diamino-1,4-dideoxyhexitol (Chart 5).



3

Chart 5

Experimental

The following instruments were used for obtaining physical data. Melting point, Yanagimoto's micro-scope 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.

2,3,5-Tri-O-acetyl-6-O-trityl-1,4-diacetamido-1,4-dideoxyhexitol (6)——3 (20 g) in water (200 ml) was acetylated with Ac_2O (60 ml) for 8 hr at room temperature. The reaction mixture was evaporated *in vacuo* to dryness. After drying over P_2O_5 *in vacuo*, the product was dissolved in pyridine (300 ml) and tritylated with trityl chloride (310 g) for 16 hr at room temperature with stirring. The reaction mixture was acetylated with Ac_2O (60 ml) for 16 hr, poured into ice-water and the solution was evaporated *in vacuo* to dryness to afford crude powder of **6** (75 g). The crude powder was dissolved in EtOAc (750 ml) and the solution was charged in a silica gel column (1 l). The column was eluted with EtOAc. The eluates were monitored by thin-layer chromatography (TLC) [silica gel (Merck), EtOAc; detected by Rydon-Smith reagent[10]]. The fractions showing an *Rf* 0.16 were evaporated to dryness to give a crude powder (50 g). The crude powder was crystallized from EtOAc as the prisms (**6**), mp 105—106°, $[\alpha]_D^{25}$ +17.4° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{40}N_2O_9$: C, 66.46; H, 6.33; N, 4.43. Found: C, 66.40; H, 6.34; N, 4.33.

7) J.D. Stevens and H.G. Fletcher, Jr., *J. Org. Chem.*, 33, 1799 (1968).
8) L.M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Ltd., London, 1959.
9) von H. Weidmann, *Liebigs Ann. Chem.*, **687**, 250 (1965).
10) H.N. Rydon and P.W.G. Smith, *Nature* (London), **169**, 922 (1952).

2,3,5-Tri-O-acetyl-1,4-diacetamido-1,4-dideoxyhexitol (7)——6 (44 g) in 80% aqueous AcOH (450 ml) was detritylated in a boiling water bath for 10 min. The reaction mixture was cooled and the white crystals which separated were removed by filtration and the filtrate was passed through a silica gel column (1 l). The column was eluted with EtOAc–MeOH (10: 1). The effluents were monitored by TLC [silica gel (Merck), EtOAc–MeOH=10: 1; detected by Rydon–Smith reagent]. The fractions showing an *Rf* 0.5 were evaporated to a crude powder (26 g). The crude **7** was crystallized from EtOAc to white crystals (22 g), mp 175—180°, $[\alpha]_D^{24}$ +41.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{16}H_{26}N_2O_9$: C, 49.23; H, 6.67; N, 7.18. Found: C, 49.26; H, 6.80; N, 7.08.

2,4,5-Tri-O-acetyl-3,6-diacetamido-3,6-dideoxyhexose (8)——A solution of anhydrous crystalline orthophosphoric acid (3 g) in DMSO (6 ml) was added to a solution of **7** (20 g), pyridine (2 ml) and DCC (35 g) in DMSO (100 ml). The mixture was kept at 20—25° for 3 hr with occasional ice-cooling. The reaction mixture was diluted with EtOAc (250 ml) and a solution of oxalic acid dihydrate (12.9 g) in MeOH (25 ml). Deposited crystals were removed by filtration and the filtrate was evaporated *in vacuo* to a syrup (110 ml). Dilution with ethyl ether (1 l) precipitated a powder. The supernatant was discarded and the precipitate was dissolved in EtOAC (1 l). The solution was passed through a silica gel column (1 l). The column was eluted with EtOAc–MeOH (10: 1). The fractions were monitored by TLC [silica gel (Merck). EtOAc–MeOH (10: 1); detected by Molish–Udransky reagent]. The fractions showing an *Rf* 0.42 were combined and evaporated *in vacuo* to a crude **8** (6.5 g), which was crystallized from EtOAc to white crystals (5.2 g), mp 131—133° (dec.), $[\alpha]_D^{24}$ +23.7° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3325, 1750, 1660, 1560, 1380, 1230, 1100—1030. PMR ($CDCl_3$) δ: 1.96—2.10 (3H×5, s, $COC\underline{H}_3$), 3.48 (2H, m), 4.46—5.44 (4H, m), 6.30—6.76 (2H, m), 9.46 (1H, s like, –C<u>H</u>O). *Anal.* Calcd. for $C_{16}H_{24}N_2O_9$: C, 49.48; H, 6.19; N, 7.22. Found: C, 49.35; H, 6.30; N, 7.02.

2,4,6-Tri-O-acetyl-3,6-diacetamido-3,6-dideoxyhexose Diethyl Dithioacetal (9)——8 (1 g) was dissolved in 3 ml of conc. HCl (d_4^{25} 1.18) in an ice bath and 25 g of ethanethiol was added. The mixture was stirred for 6 hr, neutralized in the cold with conc. NH_4OH and evaporated to dryness *in vacuo* at 30°. After drying over P_2O_5 *in vacuo* at room temperature, the residue was acetylated with 21 ml of a mixture of pyridine and Ac_2O (2: 1 v/v) by allowing the mixture to stand overnight at room temperature. The mixture was then poured into 200 ml of ice-water. The crude product obtained was dissolved with EtOAc–MeOH (10: 1) and the solution was passed through a silica gel column (200 ml). The column was eluted with EtOAc–MeOH (20 :1). The eluates were monitored by TLC [silica gel (Merck), EtOAc–MeOH=5: 1; detected by Molish–Udransky reagent]. The fractions showing an *Rf* 0.72 were combined and evaporated to dryness. The product was crystallized with MeOH–EtOAc–hexane to white crystals (**9**, 1.02 g), mp 183—185°, $[\alpha]_D^{24}$ +26.6° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3280, 2970, 2930, 1750, 1660, 1540, 1375, 1230, 1040, 600. PMR ($CDCl_3$) δ: 1.26 (3H×2, t, J=7 Hz, S–CH_2–C<u>H</u>$_3$), 1.96—2.12 (3H×5, s, $COC\underline{H}_3$), 2.52—2.90 (4H, m, S–C<u>H</u>$_2$–CH_3), 3.36—3.72 (2H; m), 3.95 (1H, d, J=4 Hz), 3.90 (1H, m), 3.98 (1H, m), 4.10 (1H, dd, J=4 and 8 Hz), 4.31 (1H, dd, J=2 and 9 Hz), 5.91—6.26 (2H, m). Mass Spectrum (MS) m/e: 495 (M^++1). *Anal.* Calcd. for $C_{20}H_{34}N_2O_8S_2$: C, 48.58; H, 6.88; N, 5.67; S, 12.96. Found: C, 48.57; H, 7.03; N, 5.35; S, 13.03.

3,6-Diacetamido-3,6-dideoxyhexose Diethyl Dithioacetal (10)——9 (1 g) in 1 N NH_3/MeOH (50 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 EtOAc–MeOH (10: 1) (20 ml), passed through a silica gel column (200 ml). The column was eluted with EtOAc–MeOH (10: 1). The fractions were monitored by TLC [silica gel (Merck), EtOAc–MeOH=5: 1; detected by Molish–Udransky reagent]. The fractions showing an *Rf* 0.3 were combined and evaporated to give a powder (**10**, 452 mg), $[\alpha]_D^{24}$ +20.0° (c=0.5, H_2O). PMR (D_2O) δ: 1.28 (3H×2, t, J= 8 Hz, S–CH_2–C<u>H</u>$_3$), 2.02, 2.04 (3H×2, s, $COC\underline{H}_3$), 2.71 (2H×2, q, J=8 Hz), 3.30—4.80 (7H, m), 6.80—7.04 (2H, m, N<u>H</u>$COCH_3$).

Methyl 3,6-Diacetamido-2,5-di-O-acetyl-3,6-dideoxy-β-L-gulofranoside (11) and Methyl 3,6-Diacetamido-2,4-di-O-acetyl-3,6-dideoxy-α-L-gulopyranoside (12)——10 (450 mg) was dissolved in boiling MeOH (10 ml), and to the solution mercuric chloride (2 g) in hot MeOH (5 ml) was added. After refluxing 2 hr, the solution was filtered from the precipitate. By passing hydrogen sulfide through the filtrate, the excess of mercuric chloride was removed as mercuric sulfide, and the colorless filtrate was evaporated *in vacuo* to a sirup. The sirup was dissolved with water (20 ml), passed through a Dowex 1×2 (OH) column (50 ml). The effluent was evaporated *in vacuo* to leave a crude powder (350 mg). The crude powder was acetylated with 7.5 ml of a mixture of pyridine and Ac_2O (2: 1 v/v) by allowing the mixture to stand overnight at room temperature. The reaction mixture was treated with ice-water to yield a precipitate (360 mg). A solution of the precipitate in EtOAc was applied to silica gel plates and developed with EtOAc–MeOH (10: 1) (detected by Molish–Udransky reagent). A band having an *Rf* 0.41 was extracted with MeOH, the extract was evaporated to dryness and dissolved in EtOAc. The solution was evaporated *in vacuo* to dryness to afford a white powder of **11** (300 mg).

11: $[\alpha]_D^{27}$ +59.5° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3275, 2925, 1740, 1660, 1540, 1370, 1240, 1050, 600. The PMR spectrum is shown in Fig. 3. MS m/e: 361 (M^++1), 329 (M^+−OCH_3). *Anal.* Calcd. for $C_{15}H_{24}N_2O_8$: C, 50.0; H, 6.67; N, 7.78. Found: C, 49.93; H, 6.70; N, 7.69.

A band having an *Rf* 0.31 was extracted with MeOH; the extract was evaporated *in vacuo* to dryness to a white powder of **12** (15 mg).

12: $[\alpha]_D^{27}$ −25.1° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1750, 1670, 1520, 1380, 1240, 1065, 1045,

600. The PMR spectrum is shown in Fig. 3. MS *m/e*: 361 ($M^+ + 1$), 329 ($M^+ - OCH_3$). *Anal.* Calcd. for $C_{15}H_{24}N_2O_8$: C, 50.0; H, 6.67; N, 7.78. Found: C, 50.2; H, 6.70; N, 7.70.

Methyl 3,6-Diacetamido-2,4-di-O-acetyl-3,6-dideoxy-α-D-gulopyranoside (13)——3,6-Diacetamido-3,6-dideoxy-D-gulose (200 mg) synthesized from D-glucose by the procedures reported by Weidmann[9] was methylglycosidated with 6 N HCl/MeOH (10 ml) for 18 hr under reflux. The reaction mixture was evaporated *in vacuo* to dryness. The product was dissolved in water (5 ml), passed through a Dowex 1×2 (OH) column. The effluent was evaporated *in vacuo* to dryness to afford a crude powder. The crude powder was acetylated with 3 ml of a mixture of pyridine and Ac_2O (2: 1 v/v) by allowing the mixture to stand overnight at room temperature. The reaction mixture was diluted with ice-water to deposit a precipitate. A solution of this crude product in EtOAc was applied to preparative TLC with EtOAc–MeOH (10: 1) as the solvent (detected by Molish–Udransky reagent). A band having an *Rf* 0.31 was extracted with MeOH, the extract was evaporated to dryness and the residue was taken up in EtOAc. The solution was evaporated *in vacuo* to dryness to afford a white powder of **13** (150 mg), $[\alpha]_D^{21}$ +21° (c=1.0, $CHCl_3$). The IR, PMR and Mass spectra were identical with those of **12**. *Anal.* Calcd. for $C_{15}H_{24}N_2O_8$: C, 50.0; H, 6.67; N, 7.78. Found: C, 49.9; H, 6.70; N, 7.65.

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