THE STRUCTURAL ELUCIDATION OF AMINOGLYCOSIDE ANTIBIOTICS, SANNAMYCINS A AND B

Sir:

Sannamycins A and B are new aminoglycoside antibiotics produced by *Streptomyces sannanensis* strain KC-7038 sp. nov.

The production, isolation, physico-chemical and biological properties of sannamycins have been described previously[1]. In this communication, the structural elucidations of these antibiotics are described.

Sannamycins A (**1**) and B (**2**) are obtained as a colorless powder and show no definite melting points. The former (**1**) shows $[\alpha]_D^{25}$ +120.5° (*c* 1, H_2O) and the latter (**2**) shows $[\alpha]_D^{25}$ +78° (*c* 0.5, H_2O). The formulae $C_{17}H_{35}N_5O_5$ and $C_{15}H_{32}N_4O_4$ were established for **1** and **2** by mass spectrometry, ^{13}C NMR spectra and elemental analysis.

The mass spectrometry of **1** or **2** showed a highly intensity peak at *m/e* 143. The ^1H NMR spectra of **1** and **2** in D_2O showed three methyl groups and one anomeric proton signal, as shown in Table 1.

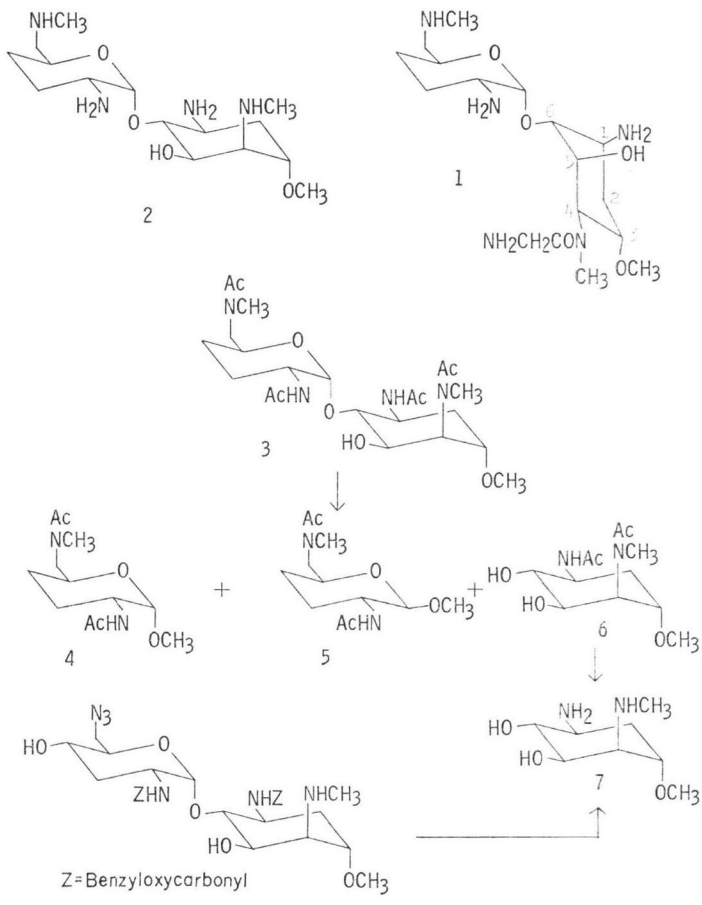
The most significant difference between **1** and **2** is the marked deshielding of one of the N-methyl resonances in **1**. On the basis of their physical properties and their empirical formulae, which coincide with those of sporaricins A and B[2] respectively, we assumed that sannamycins A and B were similar to the sporaricins A and B and fortimicins A and B[3]. Alkaline hydrolysis of **1** (145 mg, 0.37 mmol) with 1 N $Ba(OH)_2$ (20 ml) under reflux for 4 hours afforded **2** (60 mg) and glycine. These facts proved that **1** and **2** were pseudodisaccharides and **1** was the mono glycylated analog of **2**.

Acetylation of **2** (664 mg, 2 mmol) with acetic anhydride in methanol by standing overnight at room temperature gave the tetra-N-acetyl derivative (**3**) quantitatively, *m/e* 500 M^+, $[\alpha]_D^{23}$ +189° (*c* 1, H_2O).

Methanolysis of **3** (500 mg, 1 mmol) with 6 N hydrogen chloride in anhydrous methanol (20 ml, 80°C, 8 hours in a sealed tube) followed by re-N-acetylation afforded three spots except for unreacted **3** on silica-gel thin-layer chromatography developed with chloroform - methanol (8: 1). The reaction mixture was evaporated under reduced pressure and chromatographed on a column of silica gel. The column was developed with chloroform - acetone (1: 2) to give a methyl N-acetyl-α-glycoside (**4**, 120 mg, $[\alpha]_D^{24}$ +142° (*c* 1, H_2O), *m/e* 258 M^+, Anal. $C_{12}H_{22}N_2O_4$) and the anomer (**5**, 52 mg, $[\alpha]_D^{24}$ 0° (*c* 1, H_2O), *m/e* 258 M^+) as colorless syrups and a colorless solid of an N-acetylaminocyclitol (**6**, 201 mg, $[\alpha]_D^{24}$ +108° (*c* 1, H_2O), *m/e* 274 M^+) as shown in Fig. 1.

Comparison of the ^{13}C NMR spectra data of

Fig. 1.



1 and **2** with those of purpurosamine-containing antibiotics suggests the sugar moieties of sannamycins A and B to be 6-N-methylpurpurosamine C, known as a component of sagamicin[4] (gentamicin C_{2b}). Then we synthesized methyl 2,6-di-N-acetyl-6-N-methylpurpurosamine C ($[\alpha]_D^{23}$ +135° (*c* 1, H_2O)) from methyl N-benzyloxycarbonyl-α-D-glucosaminide using the TIPSON COHEN method[5] as shown in Scheme 1 in an overall yield 36%. This synthetic compound was found to be identical with **4** obtained from sannamycin B in specific rotation, IR and ^1H NMR spectra.

Treatment of **6** (200 mg, 0.78 mmol) with 4 N sodium hydroxide (12 ml) at 110°C for 5 hours afforded the aminocyclitol (**7**, 117 mg), $[\alpha]_D^{24}$ −44° (*c* 1, H_2O), *m/e* 190 M^+, Anal. $C_8H_{18}N_2O_3 \cdot H_2O$. As shown in Tables 1, 2 and 3, the structure of **7** was shown to be 2-deoxyfortamine or its mirror image by the ^1H and ^{13}C NMR spectra of **7**.

In order to prove the structure and absolute configuration of **7**, 2-deoxyfortamine was afforded by the methanolysis of 6-O-(6-azido-2-N-benzyloxycarbonyl-6-deoxy-α-lividosaminyl)-1-N-benzyloxycarbonyl-2-deoxyfortamine[6] (**8**) prepared starting from lividamine. They exhibited identical mobilities on thin-layer chromatograms virtually, superimposable IR and NMR spectra and similar optical rotations.

The position of linkage of the aminosugar to the aminocyclitol was decided by the ^{13}C NMR spectra of **2** and **7** (Table 3). The significant change in assignment for C_6 in **2** (85.3 ppm) and

Table 1. Chemical shifts of ^1H NMR spectra.

Proton	Chemical shifts (ppm)		
	Sannamycin A (**1**)	Sannamycin B (**2**)	Aminocyclitol (**7**)
1′	5.37	5.54	
2′	3.28	~3.4	
3′, 4′	1.8~2.4	1.8~2.4	
5′	~3.95	~4.5	
6′	3.10	3.10	
1	~3.95	3.51	3.27
2ax.	2.39	~2.1	2.05
2eq.	2.90	2.55	2.54
3	4.68	4.23	4.23
4	5.10	~3.4	3.48
5	4.65	4.51	4.26
6	4.31	4.03	3.83
6′ N-CH_3	2.81	2.81	
4 N-CH_3	3.58	2.85	2.83
O-CH_3	3.90	3.91	3.87
Gly. CH_2	4.04		

Chemical shifts of ^1H NMR spectra were measured in D_2O using TMS as the external reference.

Table 2. Coupling constants of ^1H NMR spectra.

Coupling	Coupling constants (Hz)		
	Sannamycin A (**1**)	Sannamycin B (**2**)	Aminocyclitol (**7**)
$J_{1',2'}$	3.5	3.7	
$J_{5',6'}$	6.0	5.5	
$J_{1,2ax.}$	4.0	10.0	12.0
$J_{1,2eq.}$	3.0	4.2	4.5
$J_{2eq.,2ax.}$	13.5	14.5	14.0
$J_{2ax.,3}$	11.0	4.0	3.3
$J_{2eq.,3}$	4.2	4.5	4.0
$J_{3,4}$	11.5	4.0	3.3
$J_{4,5}$	3.0	4.2	4.5
$J_{5,6}$	3.0	8.5	9.5
$J_{1,6}$	3.0	8.5	9.5

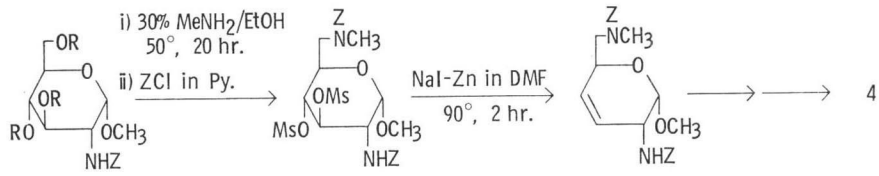
Table 3. Chemical shifts of ^{13}C NMR spectra.

Carbon	Chemical shifts (ppm)	
	Sannamycin B (**2**)	Aminocyclitol (**7**)
1′	102.4	
2′	50.9	
3′	27.5	
4′	29.3	
5′	69.5	
6′	56.1	
1	49.6	50.4
2	31.6	31.3
3	76.7	77.0
4	63.7	63.8
5	71.7	72.2
6	85.3	76.4
6′-N-CH_3	36.2	
4-N-CH_3	35.3	35.8
O-CH_3	57.3	57.3

The ^{13}C-FT NMR spectra were taken with a JNM-FX-100 spectrometer.

Samples were dissolved in D_2O containing dioxane as the internal reference (67.4 ppm).

Scheme 1.



7 (76.4 ppm) was explained by the deshielding effect of glycosidation. From this result it was concluded that the position of linkage of 6-N-methylpurpurosamine C to the aminocyclitol was C-6 in **2**.

The axial-equatorial coupling ($J=3.7$ Hz) of the C-1′ proton in the ^{1}H NMR of **2** established the α configuration of the glycoside.

Thus, the absolute structure of **2** was determined to be 6-O-(2-amino-2,3,4,6-tetradeoxy-6-methylamino-α-D-*erythro*-hexopyranosyl)-2-deoxyfortamine as shown in Fig. 1.

The structure of **1** was determined by comparison of the ^{1}H NMR spectra in **1** and **2** (Table 1). Down-field shifts were observed at the C-4 and N-methyl protons. The structure of sannamycin A is thus established in all stereochemical details and is shown in Fig. 1.

Sannamycin is the first N-methylpurpurosamine C containing compound produced by *Streptomyces* sp.

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ISAMU WATANABE
TAKEO DEUSHI
TAKASHI YAMAGUCHI
KAZUHIRO KAMIYA
MASAHITO NAKAYAMA
TOSHIHITO MORI

Tokyo Research Laboratories,
Kowa Co., Ltd.,
Higashimurayama, Tokyo 189,
Japan

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