THE STRUCTURES OF AMINOGLYCOSIDE ANTIBIOTICS, SU-1, 2, AND 3

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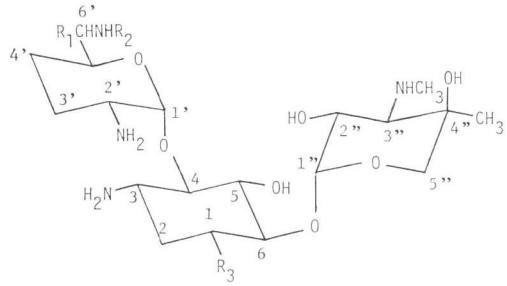
During the course of a study on sagamicin biosynthesis, KASE and his co-workers found that a 2-deoxystreptamine idiotrophic mutant of the sagamicin producer, *Micromonospora sagamiensis* KY 11509[1] produces three new antibiotics named SU-1, 2, and 3 together with a trace amount of sagamicin[2]. Since they showed interesting antimicrobial activity, we elucidated their structures. This paper will describe their structures. The production, isolation, and biological properties will be published in a separate paper[3].

The Structure of SU-2 (1)

SU-2 was obtained as colorless powder, showing $[\alpha]_D+172°$ (c 0.3, water).

Its ^1H NMR spectrum (Fig. 1) closely resembles that of gentamicin C_{1a} (**2**)[4]. The molecular formula was determined as $C_{19}H_{38}N_4O_8$ by high resolution mass spectrometry. The mass spectrum of SU-2 showed extremely intense peaks at m/z 129 and 160, indicating the presence of purpurosamine C and garosamine moieties in the molecule[5]. Absence of the fragment peaks at m/z 191 and 163 derived from 2-deoxystreptamine moiety and appearance of the prominent peaks at m/z 192 and 164 indicate that one of amino group in the 2-deoxystreptamine moiety is replaced by a hydroxyl group (Chart 1). The hydroxyl group was considered to be attached to C-1 because the fragment peak at m/z 290 was observed instead of m/z 289 which was observed in the mass spectrum



1. $R_1=R_2=H$, $R_3=OH$
2. $R_1=R_2=H$, $R_3=NH_2$
3. $R_1=H$, $R_2=CH_3$, $R_3=OH$
4. $R_1=H$, $R_2=CH_3$, $R_3=NH_2$
5. $R_1=CH_3$, $R_2=H$, $R_3=OH$

Fig. 1. ^1H NMR spectrum of SU-2 in DCl-D_2O solution at pD 1.1.

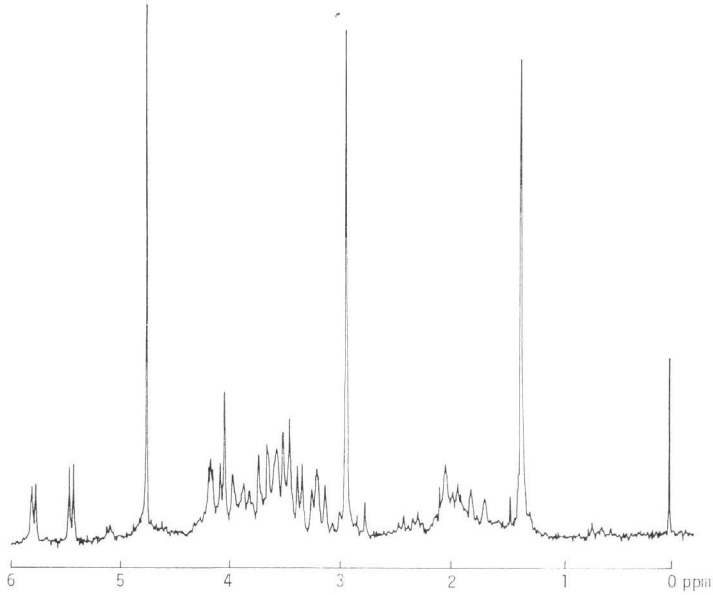


Chart 1. Mass spectral fragmentation of SU-1(R=H) and SU-2(R=CH_3).

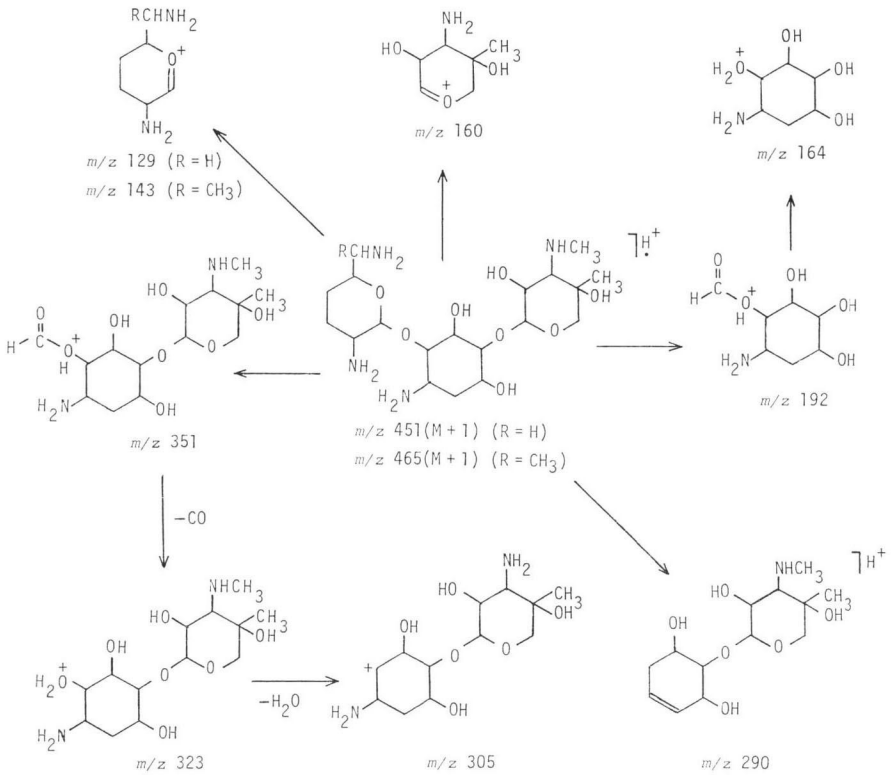


Table 1. ^{13}C Chemical shifts of SU-2 (**1**), gentamicin C_{1a} (**2**), and SU-3 (**3**) (δc ppm down field shift from tetramethylsilane in D_2O).

	1	**1** H^+	**2**	**3**	**3** H^+
C-1′	101.8	95.6	102.2	101.8	95.5
C-2′	50.6	49.6	51.0	50.7	49.6
C-3′	26.7	21.3	27.1	26.9	21.3
C-4′	28.2	26.3	28.5	28.8	26.4
C-5′	71.0	66.7	71.5	69.0	66.4
C-6′	45.7	43.5	46.1	55.7	53.0
6′-*N*-Me				35.9	34.4
C-1	70.9	69.7	51.7	70.9	69.7
C-2	36.9	33.5	36.7	36.9	33.5
C-3	49.2	49.1	50.6	49.3	49.1
C-4	87.1	78.3	88.3	87.3	78.1
C-5	74.8	74.5	75.4	74.8	74.4
C-6	84.9	83.3	87.8	85.0	83.3
C-1″	100.3	99.1	101.3	100.4	99.1
C-2″	70.2	67.2	70.2	70.3	67.2
C-3″	64.0	65.0	64.4	64.1	65.0
C-4″	73.2	70.8	73.3	73.2	70.8
C-5″	68.5	67.7	68.7	68.6	67.7
3″-*N*-Me	37.7	36.0	38.0	37.8	36.0
4″-*C*-Me	22.4	21.8	23.0	22.5	21.9

of gentamicin C_{1a}[5]. ^{13}C NMR spectral data (Table 1) support the structure, that is, the chemical shifts of purpurosamine C and garosamine parts in SU-2 are very close to those of gentamicin C_{1a}[6]. Carbons of the aminocyclitol part, however, showed different chemical shifts from those of gentamicin C_{1a}. The C-1 of gentamicin C_{1a} free base was observed at 51.7 ppm, but the corresponding signal of SU-2 free base was observed at 70.9 ppm.

In addition, although a significant protonation shift was observed at C-4 in the titration experiments on SU-2, no protonation shift was observed at C-6. These observations lead the hydroxyl group in SU-2 is at the 1-position. The stereochemistry of the hydroxyl group was assigned to be equatrial (1*R*) because of the absence of a signal at 4.24 ppm which is expected for H-1 equatorial[7] and by comparison of its ^{13}C NMR chemical shifts with those of 1-deamino-1-hydroxysagamicin (**3**) which has been obtained by chemical transformation from sagamicin (**4**) in our laboratory.

Recently, 1-deamino-1-hydroxygentamicin C_{1a} was chemically synthesized from gentamicin C_{1a} by the Schering group[7]. The ^{13}C-chemical shifts of SU-2 are almost identical with those reported by them.

The Structures of SU-1 (5) and SU-3 (3)

SU-1 was obtained as colorless powder. The molecular formula, $C_{20}H_{40}N_4O_8$ was determined by high resolution mass spectrometry. Its ^1H NMR spectrum was very similar to that of SU-2 except for the presence of a secondary methyl signal. The characteristic signals observed at pD 0.8 were assigned as follows: 1.33 (3H, d, J=6.6 Hz, 6′-CH_3), 1.34 (3H, s, 4″-CH_3), 2.92 (3H, s, 3″-NCH_3), 5.45 (1H, d, J=3.9 Hz, H-1″), and 5.84 (1H, d, J=3.7 Hz, H-1′).

Again the absence of a signal at 4.24 ppm proved that the hydroxyl group at the 1-position is equatorial. SU-1, thus, was considered to be 6′-*C*-methyl SU-2. The structure was firmly established by its mass spectrum as shown in Chart 1.

SU-3 was also obtained as colorless powder. Its mass spectral fragmentation pattern, including the protonated molecular ion at m/z 465 ($C_{20}H_{40}N_4O_8$), closely resembled that of SU-1. In its ^1H NMR spectrum measured at pD 0.6 an additional *N*-methyl signal was observed at 2.76 ppm by comparison with that of SU-2. All twenty carbons were observed in its ^{13}C NMR spectrum and assigned easily by comparison with those of SU-2 and sagamicin (**4**) (Table 1). SU-3 was, therefore, determined to be 6′-*N*-methyl SU-2. All the spectral data obtained for SU-3 in this study were consistent with those of synthetic 1-deamino-1-hydroxysagamicin.

Experimental

Low and high resolution mass spectra were obtained on a JEOL JMS 01SG-2 spectrometer at 75 eV. ^1H NMR spectra were measured on a JEOL PFT-100A spectrometer in deuterium oxide and chemical shifts are reported in ppm downfield from internal DSS. ^{13}C NMR spectra were determined on a JEOL JNM FX-100 spectrometer in deuterium oxide. pD of solutions were adjusted by addition of deuteriochloric acid. Reported pD values are uncorrected pH meter readings of deuterated solutions with an Okura model AH 21 pH meter.

High resolution mass measurements.

SU-2, Found; $M^+ = m/z$ 451.2736
Calcd. for $C_{19}H_{38}N_4O_8$=451.2767

SU-1, Found; $M^+ - NH_3 = m/z$ 447.2604
Calcd. for $C_{20}H_{37}N_3O_8$=447.2580

^{13}C-Chemical shift-titration.

For ^{13}C NMR chemical shift-titration, ^{13}C NMR spectra of SU-2 were determined at pD 10.8, 10.0, 9.2, 8.5, 7.3, 6.5, and 1.5.

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