NEW AMINOGLYCOSIDE ANTIBIOTICS, SANNAMYCIN*

Sir:

In the course of screening for aminoglycoside antibiotics, new members of the fortimicin[1~4] and sporaricin[5~7] group, sannamycins A and B have been isolated from a culture broth of *Streptomyces* sp. KC-7038 which was classified as *Streptomyces sannanensis* sp. nov.

The strain KC-7038 was cultured in a 500-ml Erlenmeyer flask which contained 100 ml of a medium composed of 4% corn starch, 0.2% soy bean meal, 0.5% corn steep liquor, 0.2% yeast extract and a mixture of a small ammount of inorganic salts including 0.3% NaCl, 0.1% $CaCO_3$ and 0.05% $MgSO_4 \cdot 7H_2O$, with pH adjustment to 7.0 prior to sterilization, on a rotary shaker at 27°C for 4 days.

Fermentation broth (9 liters) was adjusted to pH 2.0 with sulfuric acid. After filter aid was added, the mixture was filtered and readjusted to pH 7.0 with sodium hydroxide. The filtrate was passed through a cation-exchange resin Amberlite IRC-50 (NH_4^+). After washing the resin column with deionized water, the resin was eluted with 1 N ammonium hydroxide. Active fractions were concentrated and lyophilized to give a pale brown powder of the sannamycin complex. The complex was dissolved in deionized water and the solution was charged on a column of CM-Sephadex C-25 (NH_4^+). After washing with deionized water, the column was eluted with aqueous ammonia with a concentration gradient from 0.05 N to 0.5 N. After eluting some minor components, sannamycin A was eluted first, followed by sannamycin B. Each fraction of sannamycins A and B was concentrated and lyophilized to give a colorless crude powder.

They were further purified by column chromatography on a cellulose column. The above crude powder of sannamycin A was charged on a column of cellulose and developed with a lower phase of chloroform - methanol - 17% ammonium hydroxide (2: 1: 1, v/v). The bioactive eluate was collected and concentrated and then diluted with deionized water. The diluted solution was charged on a column of CM-Sephadex C-25 (NH_4^+), followed by elution with 1 N ammonium hydroxide, and the eluate was lyophilized to afford a colorless solid (98 mg) of pure sannamycin A.

The crude powder of sannamycin B was purified in a manner similar to that described above to give a colorless solid (45 mg) of pure sannamycin B.

The physico-chemical properties of sannamycins A and B are listed in Table 1. The molecular ion peak by mass spectometry and the analytical data for sannamycins A and B agreed with the molecular formula of $C_{17}H_{35}N_5O_5$(389) and $C_{15}H_{32}N_4O_4$(332), respectively.

The IR spectra of sannamycins A and B in KBr tablets are demonstrated in Figs. 1 and 2, respectively. The 100 MHz ^1H NMR spectrum

Table 1. Physico-chemical properties of sannamycins A and B.

	Sannamycin A		Sannamycin B	
Nature	Basic, colorless solid		Basic, colorless solid	
$[\alpha]_D^{25}$	+120.5° (c 1, H_2O)		+78° (c 0.5, H_2O)	
Elementary analysis for;	$C_{17}H_{35}N_5O_5 \cdot \frac{1}{2}H_2O$		$C_{15}H_{32}N_4O_4$	
(%)	Found	Calcd.	Found	Calcd.
C	51.21	51.24	53.63	54.19
H	8.82	9.11	9.34	9.70
N	17.37	17.54	16.71	16.85
^1H NMR (D_2O)* ppm				
N-CH_3	2.81		2.81	
N-CH_3	3.58		2.85	
O-CH_3	3.90		3.91	
–NCH_2CO–	4.04		—	
anomeric H	5.37		5.54	
IR (KBr) cm^{-1}	3350, 2930, 1630, 1575		3350, 3140, 2930, 1595	
Mass	390 (M^++1), 389(M^+), 276, 258, 230, 143		333 (M^++1), 332(M^+), 219, 201, 173, 143	
P.P.C. (Rf)**	0.86		0.92	

* TMS as external reference.

** Whatman No. 1 filter paper
Solvent: Lower phase of $CHCl_3$ - MeOH - 17% NH_4OH (2: 1: 1, v/v)

* This antibiotic was initially designated as KA-7038.

Fig. 1. IR spectrum of sannamycin A.

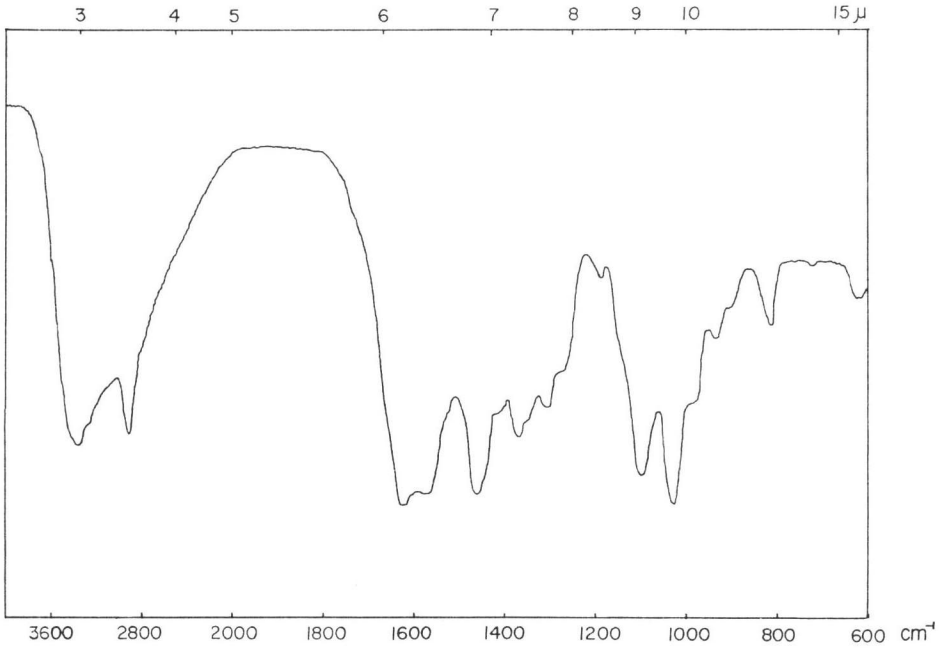


Fig. 2. IR spectrum of sannamycin B.

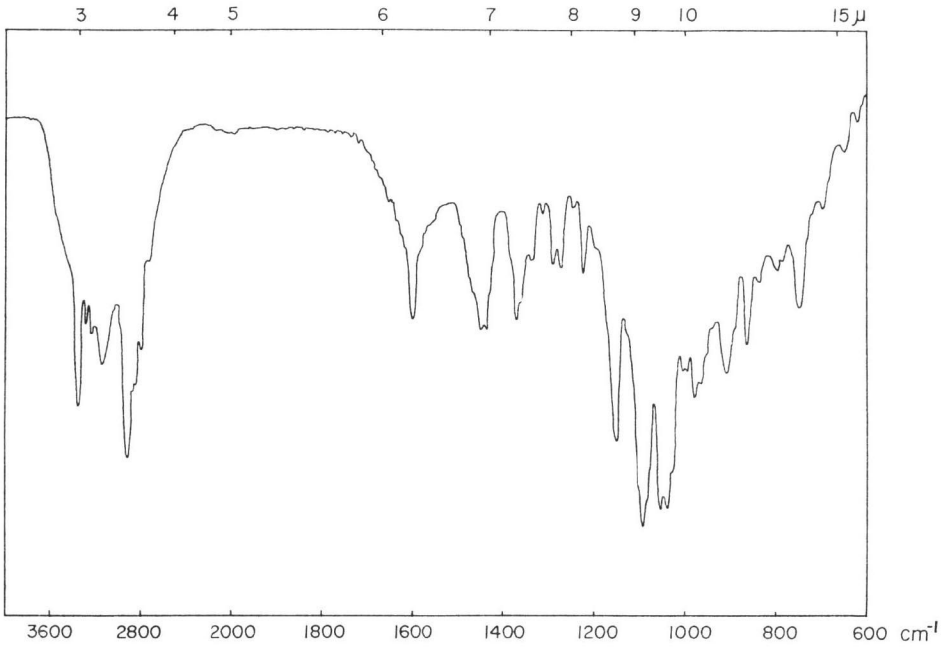


Table 2. Antimicrobial spectra of sannamycins A, B and sporaricin A.

Test organisms	M.I.C. (mcg/ml)		
	Sannamycin A	Sannamycin B	Sporaricin A
Staphylococcus aureus FDA 209P	0.39	>100	0.20
Bacillus anthracis	0.20	50	<0.1
Bacillus cereus	1.56	>100	0.78
Bacillus subtilis ATCC 6633	0.20	50	0.20
Streptococcus faecalis	50	>100	25
Escherichia coli NIHJ	3.13	>100	1.56
Escherichia coli K-12 ML 1410	1.56	>100	1.56
Escherichia coli K-12 ML 1410 R- 81[I]	6.25	>100	3.13
Escherichia coli K-12 ML 1410 R- 83[II]	6.25	>100	3.13
Escherichia coli K-12 ML 1410 R-101[III]	6.25	>100	1.56
Proteus vulgalis OX-19	3.13	>100	1.56
Proteus inconstans[IV]	6.25	>100	1.56
Klebsiella pneumoniae PCI 602	1.56	>100	0.78
Pseudomonas aeruginosa Shibata	6.25	>100	3.13
Pseudomonas aeruginosa 99[V]	>100	>100	>100
Pseudomonas aeruginosa GN 315[VI]	25	>100	12.5
Serratia sp.	1.56	>100	1.56

Medium: Nutrient agar pH 7.0 (Eiken Chemical Co., Ltd., Japan)
I) APH(3′)-I II) APH(3′)-II III) AAD(2″) IV) AAC(2′) V) AAC(3)-I VI) AAC(6′)-IV

Fig. 3. 100 MHz ^1H NMR spectrum of sannamycin A in D_2O.

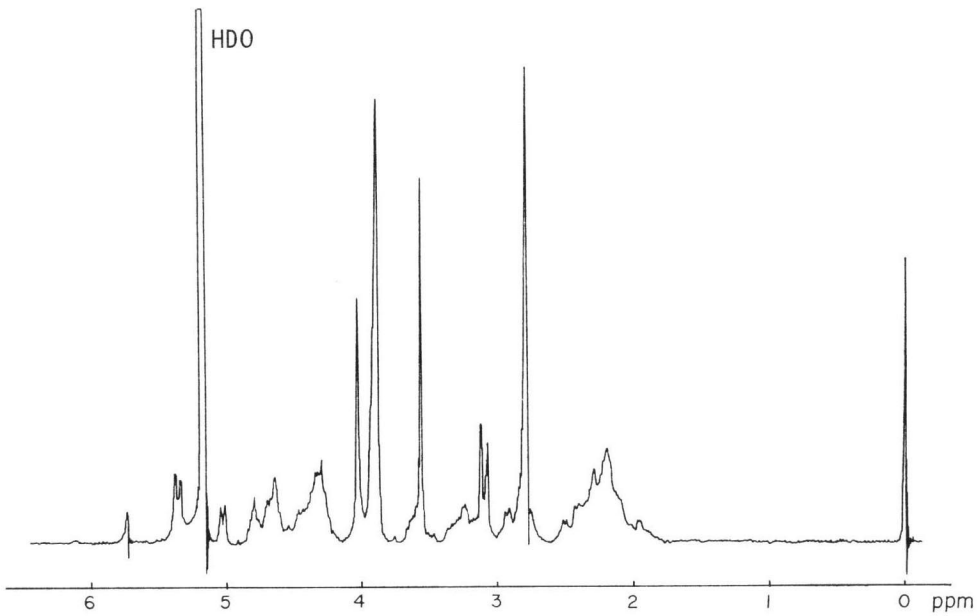


Fig. 4. 100 MHz ^1H NMR spectrum of sannamycin B in D_2O.

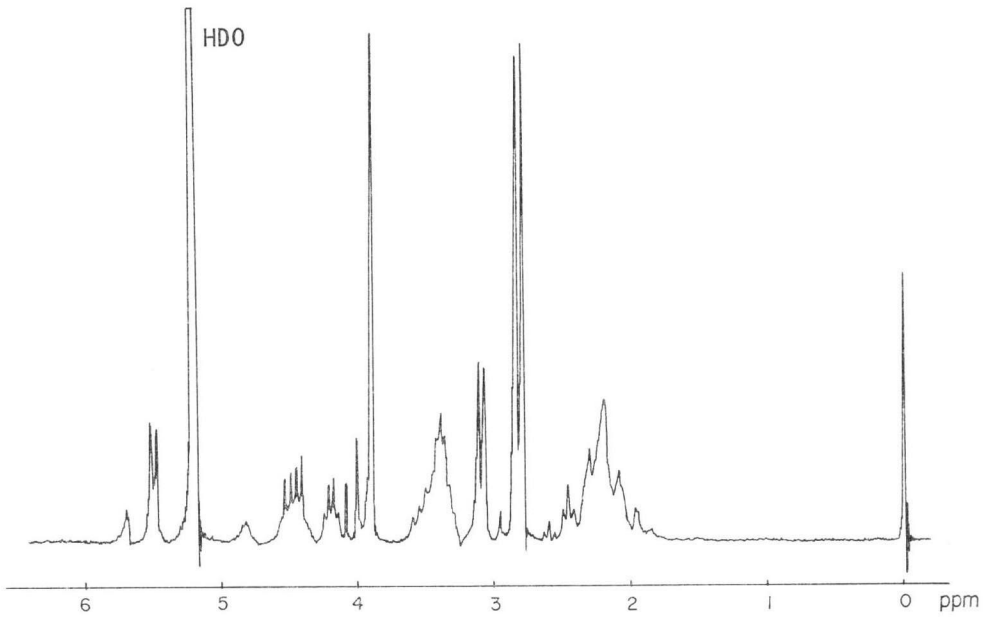
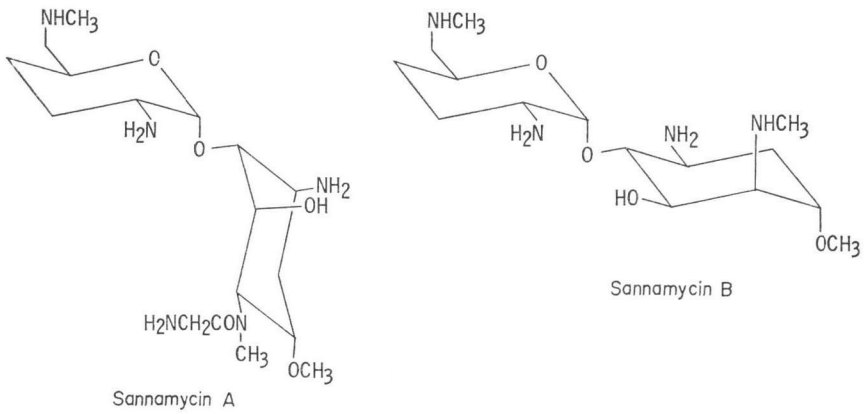


Fig. 5.



of sannamycin A indicated one anomeric proton (5.37 ppm) and three methyl groups assigned to N-CH_3 (2.81 and 3.58 ppm) and O-CH_3 (3.90 ppm).

Sannamycins A and B were clearly differentiated from known aminoglycoside antibiotics by the physico-chemical characteristics (Table 1).

Sannamycin A is highly active against Gram-positive and Gram-negative organisms including various aminoglycoside-resistant strains (Table 2). The antibacterial activity of sannamycin A was slightly weaker than that of sporaricin A.

Intravenous acute LD_{50} of sannamycins A and B in mice were 100~200 mg/kg and >400 mg/kg, respectively.

The structures of sannamycins A and B are shown in Fig. 5. A detailed account of the structure elucidation will be presented in a separate paper.

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TAKEO DEUSHI
AKIO IWASAKI
KAZUHIRO KAMIYA
TOSHIMI MIZOGUCHI
MASAHITO NAKAYAMA
HISAKATSU ITOH
TOSHIHITO MORI

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

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