Communications to the editor

A NEW AMINOGLYCOSIDE ANTIBIOTIC, KA-5685

Sir:

A new aminoglycoside antibiotic belonging to the apramycin group, KA-5685 has been isolated from the culture broth of *Saccharopolyspora hirsuta* ATCC 27875[1]. This strain also co-produced apramycin[2]. In this communication, the isolation, characterization and structural elucidation of the antibiotic KA-5685 are reported.

S. hirsuta ATCC 27875 was cultured at 27°C for 5 days in 500-ml Erlenmeyer flasks containing 50 ml of a medium, composed of 2% glycerol, 1.5% dry yeast, 0.3% NaCl, 0.3% $CaCO_3$ and 1.0% cotton seed oil (pH 7.0). The culture broth (8.2 liters) was filtered at pH 2.0, and the filtrate was filtered again at pH 7.0. The antibiotic was purified with the following succesive chromatographies: (1) Amberlite IRC-50 (NH_4^+, 4×40 cm, 1 N aqueous ammonia), (2) CM-Sephadex C-25 (NH_4^+, 2.2×60 cm, water - 0.4 N aqueous ammonia), (3) Dowex 1X2 (OH^-, 1.2×150 cm, water). Lyophilization of active fractions of the last column gave a crude powder (24 mg). Further purification of the powder was accomplished by a column chromatography on silica gel (1.75×45 cm, 10% aqueous ammonium acetate - methanol, 1:1). The fractions were monitored by bioactivity against *Bacillus subtilis* ATCC 6633 and thin-layer chromatography (silica gel, Wako gel B-5; 10% aqueous ammonium acetate - methanol, 1:1). KA-5685 (Rf 0.46) was eluted first, followed by apramycin (Rf 0.31). Final purification of KA-5685 was accomplished by Amberlite IRC-50 (NH_4^+, 2×10 cm) column chromatography developed with 1 N aqueous ammonia followed by lyophilization to give pure KA-5685 as a colorless solid, 7.7 mg.

KA-5685 shows $[\alpha]_D^{25}+124°$ (c 0.5, H_2O). The molecular formula $C_{21}H_{40}N_4O_{12}$ for KA-5685 is

Fig. 1. Comparison of 200 MHz ^1H NMR spectrum of KA-5685 with apramycin.

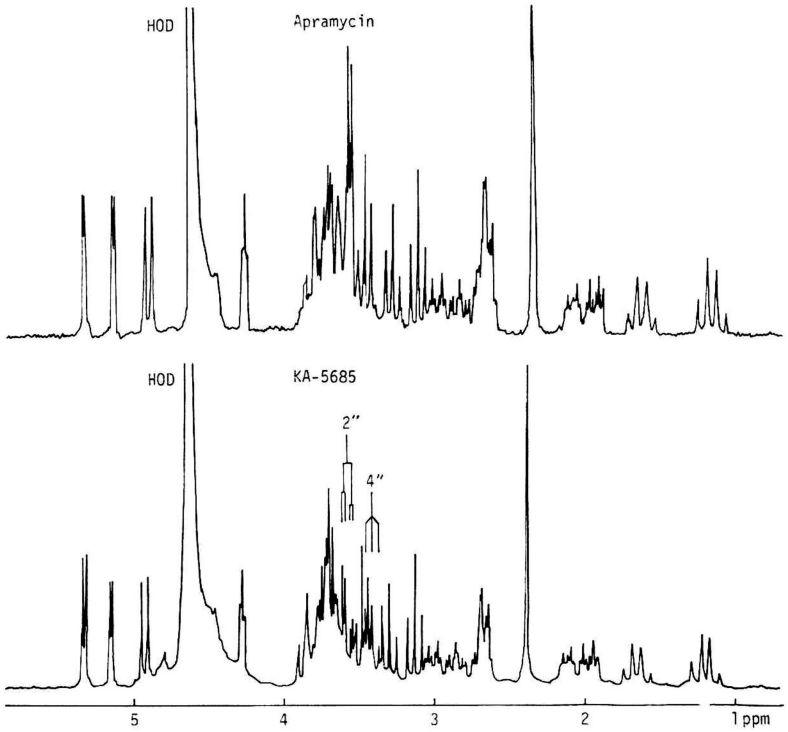


Fig. 2. IR spectrum of KA-5685.

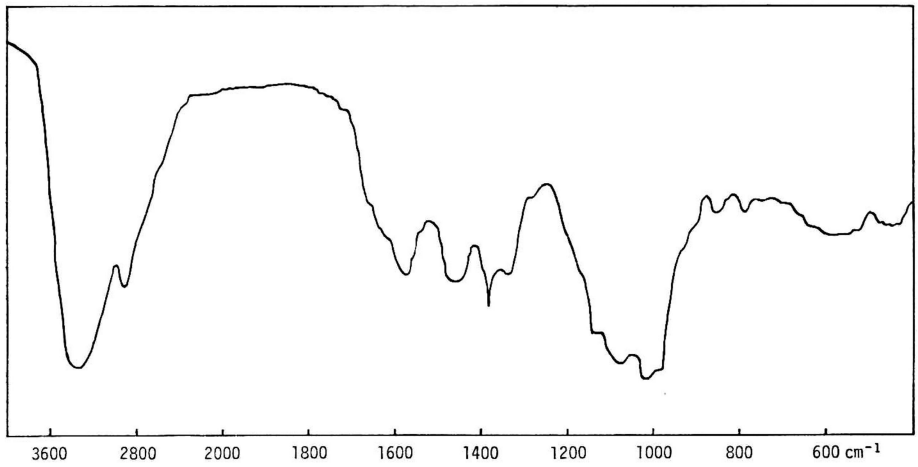


Table 1. Comparison of thin-layer chromatography of KA-5685 with apramycin.

Solvent systems	Rf	
	KA-5685	Apramycin
1	0.68	0.66
2	0.13	0.14
3	0.38	0.36
4	0.46	0.31

Sovent system 1: $CHCl_3$ - MeOH - 17% NH_4OH (2:1:1, v/v) lower layer. 2: *n*-BuOH - EtOH - $CHCl_3$ - 28% NH_4OH (4:5:2:5). 3: *n*-BuOH - AcOH - H_2O (1:2:2). 4: 10% aq. CH_3COONH_4-MeOH (1:1).

Thin-layer chromatography using TLC aluminum sheets silica gel 60 F_{254} pre-coated in the case of solvent systems 1~3 and Wako gel B-5 in the case of solvent system 4.

Detection: ninhydrin.

Table 2. Chemical shifts of ^{13}C NMR spectra of KA-5685 and apramycin.

Carbons	Chemical shifts (ppm) in D_2O (pD>12)	
	KA-5685	Apramycin
1	51.2	51.1
2	36.9	36.6
3	50.3	50.3
4	88.2	87.8
5	76.9	76.8
6	78.6	78.4
1′	101.7	101.6
2′	49.8	49.8
3′	33.0	32.9
4′	67.9	67.9
5′	71.2	71.0
6′	66.5	66.2
7′	62.6	62.3
8′	96.7	96.4
N-CH_3	33.2	32.9
1″	95.4	95.3
2″	71.7	71.7
3″	74.0*	74.2
4″	70.7	53.2
5″	73.8*	73.4
6″	61.6	61.6

* These chemical shifts may be interchangeable.

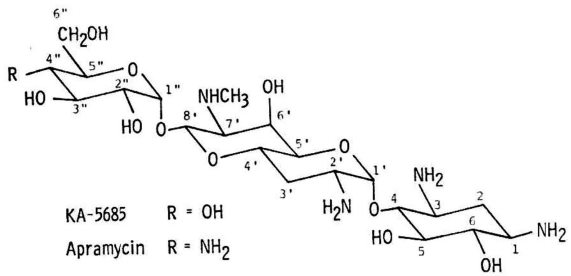
derived from mass spectrum (*m/z* 541.2699, Calcd. MH^+ 541.2718). The 200 MHz ^1H NMR spectrum of KA-5685 (Fig. 1) indicates three anomeric protons (δ 4.91, 5.14, and 5.34), a methyl group assigned to N-CH_3 (δ 2.40) and two methylene groups (δ 1.21, 1.67, 1.98 and 2.12). TLC data of this antibiotic are shown in Table 1 compared with apramycin.

The above physico-chemical characteristics indicated that KA-5685 is a new aminoglycoside antibiotic belonging to the apramycin group and it was that an amino group in apramycin was replaced by a hydroxyl group in KA-5685. The ^1H NMR spectrum of KA-5685 was similar to that of apramycin, the most significant difference being a movement of the H-4″ signal from δ 2.7 in the latter to δ 3.44 (t, J=9.5 Hz) in the former. The splitting patterns of H-2″ and H-4″ (Fig. 1) indicated that the configuration of the sugar moiety was identical with apramycin. Moreover,

Table 3. Antimicrobial spectra of KA-5685 and apramycin.

Organisms	MIC (μg/ml)		Organisms	MIC (μg/ml)	
	KA-5685	Apramycin		KA-5685	Apramycin
Staphylococcus aureus 209P JC-1	0.78	0.2	*P. cepacia* IID 1340	100	100
Streptococcus faecalis Imanari	6.25	12.5	*P. maltophilia* IID 1275	6.25	12.5
Bacillus subtilis ATCC 6633	6.25	0.78	*P. putida* IID 5121	1.56	1.56
Escherichia coli NIHJ JC-2	3.13	3.13	*E. coli* ML 1410	6.25	6.25
Klebsiella pneumoniae PCI 602	1.56	0.78	*E. coli* ML 1410 R-81[a]	6.25	6.25
Enterobacter cloacae IID 977	12.5	3.13	*E. coli* ML 1410 R-83[b]	6.25	3.13
Serratia marcescens NHL	3.13	6.25	*E. coli* ML 1410 R-102[c]	3.13	1.56
Proteus inconstans 93	3.13	1.56	*E. coli* ML 1410 R-82[b,d]	6.25	3.13
P. vulgaris IID 874	6.25	12.5	*P. aeruginosa* GN 315[e]	12.5	3.13
Pseudomonas aeruginosa NCTC 10490	0.78	0.78	*E. coli* JR 88[f]	12.5	3.13
			E. coli R 176[g]	12.5	6.25
			P. aeruginosa PST-1[h]	25	25
			E. coli JR 225/W 677[i]	100	100

Tests were conducted in Heart Infusion agar.
Organisms contained the following aminoglycoside-modifying enzymes[4,5]:
[a] APH (3′)-I, [b] APH (3′)-II, [c] AAD (2″), [d] AAD (3″), [e] AAC (6′)-IV, [f] AAC (3)-I, [g] AAC (3)-II, [h] AAC (3)-III, [i] AAC (3)-IV.



in the comparison of ^{13}C NMR spectrum of KA-5685 with apramycin[3], a significant change in assignment for C-4″ in apramycin (δ 53.2) and KA-5685 (δ 70.7) was observed as shown in Table 2. From these results, the structure of KA-5685 was determined to be 4″-deamino-4″-hydroxyapramycin. This compound is a second apramycin analogue preceded by 3′-α-hydroxy-apramycin (nebramycin factor 7)[8].

KA-5685 is highly active against Gram-positive and Gram-negative organisms including aminoglycoside resistant strains with the single exception of *Escherichia coli* JR 225/W 677 which contains an AAC (3)-IV modifying enzyme as shown in Table 3.*

* The antimicrobial data were obtained by Mr. T. Koshi.

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References

1) Lacey, J. & M. Goodfellow: A novel actinomycete from sugar-cane bagasse, *Saccharopolyspora hirsuta* gen. et sp. nov. J. Gen. Microbiol. 88: 75~85, 1975
2) O'Connor, S.; L. K. T. Lam, N. D. Jones & M. O. Chaney: Apramycin, a unique aminocyclitol antibiotic. J. Org. Chem. 41: 2087~2092, 1976

3) KOCH, K. F.; K. E. MERKEL, S. C. O'CONNOR, J. L. OCCOLOWITZ, J. W. PASCHAL & D. E. DORMAN: Structure of some of the minor aminoglycoside factors of the nebramycin fermentation. J. Org. Chem. 43: 1430~1434, 1978
4) MITSUHASHI, S.: R-Factor. *In* Drug Resistance Plasmid. pp. 195~251, University Tokyo Press, Tokyo, 1977
5) DAVIES, J. & S. O'CONNOR: Enzymatic modification of aminoglycoside antibiotic: 3-N-Acetyltransferase with broad specificity that determines resistance to the novel aminoglycoside apramycin. Antimicrob. Agents Chemother. 14: 69~72, 1978