Communications to the Editor

A NEW AMINOGLYCOSIDE ANTIBIOTIC, SUBSTANCE AC4437

Sir:

In the course of a screening program for aminosugar antibiotics from microorganisms, a new antibiotic AC4437 has been isolated from a culture broth. The producing organism was obtained from a soil sample collected at Chiba Prefecture, Japan. It was identified as *Streptomyces* sp. AC4437 (FERM P-7240). Fermentation was carried out in a 30-liter jar fermentor under agitation at 30°C for 4 days. The medium consisted of starch 4.0%, soybean meal 2.0%, corn steep liquor 1.0%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05% and $CoCl_2 \cdot 6H_2O$ 0.0013% (pH 7.0). The maximum titer, 30 μg/ml, was estimated by the paper disc method using *Bacillus subtilis* ATCC 6633 as a test organism.

The fermented broth was filtered at pH 2.0, the filtrate (19 liters) was adjusted to pH 7.0 and passed through a column of Amberlite IRC 50 (NH_4^+, 1 liter). The adsorbed antibiotic was eluted with 0.5 N HCl (5 liters). The eluate was adjusted to pH 8.0 and passed through a column of active carbon (0.5 liter). The acidic 50% aq Me_2CO eluate was concd and lyophilized. The residue was subjected to column chromatography over silica gel (200 ml), previously equilibrated with propanol - 0.5 M AcOH (4: 1). The antibiotic was eluted with the same solvent. The eluate was concd, desalted with active carbon and further concd to give a solution of AC4437 hydrochloride. Passage through a column of Amberlite IRA 400 (OH^-, 100 ml) and lyophilization of the aq effluent afforded AC4437 free base (150 mg).

The antibiotic thus obtained was a white powder, melting at 168~172°C. It was soluble in H_2O, less soluble in MeOH, and almost insoluble in Me_2CO and EtOAc. It gave positive Sakaguchi and Molisch reactions, but a negative

Fig. 1. Structure of AC4437.

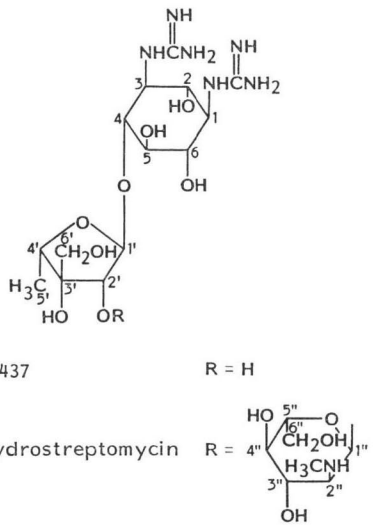

Table 1. ^{13}C NMR chemical shifts (δ) of AC4437 and dihydrostreptomycin.

Carbon	AC4437	Dihydrostreptomycin
1	59.8 d	59.8 d
2	71.0 d	71.5 d
3	58.9 d	59.1 d
4	79.0 d	78.7 d
5	74.0 d	74.2 d
6	72.1 d	72.4 d
1′	108.8 d	106.7 d
2′	80.4 d	84.9 d
3′	80.2 s	81.7 s
4′	78.6 d	78.5 d
5′	13.7 q	13.5 q
6′	63.7 t	64.2 t
1″		94.4 d
2″		62.1 d
3″		70.3 d
4″		70.3 d
5″		73.6 d
6″		61.3 t
NCH_3		32.8 q
1-$NHCNH_2$ ‖ NH	158.8 s	158.6 s
3-$NHCNH_2$ ‖ NH	158.8 s	159.2 s

δ: ppm from TMS in D_2O using dioxane (δ 67.4 ppm) as the internal reference. The free bases of antibiotics were measured (>pD 9). Chemical shifts of dihydrostreptomycin were assigned according to refs 3 and 5.

Table 2. Antimicrobial spectra of AC4437 and dihydrostreptomycin.

Test microorganism	MIC (μg/ml)	
	AC4437	Dihydrostreptomycin
Staphylococcus aureus ATCC 6538P	12.5	6.3
S. epidermidis sp-al-1	6.3	3.1
Streptococcus pyogenes N.Y. 5	12.5	3.1
Bacillus subtilis ATCC 6633	1.6	0.8
Escherichia coli NIHJ-JC2	6.3	1.6
Klebsiella pneumoniae ATCC 10031	6.3	3.1
Salmonella enteritidis Gaertner	6.3	3.1
Shigella sonnei E33	12.5	3.1
Morganella morganii 0239	50	50
Citrobacter freundii GN346	>100	>100
Pseudomonas aeruginosa IAM 1095	>100	50

Elson-Morgan reaction. The antibiotic showed $[\alpha]_D^{23}$ −32.4° (*c* 1, H_2O); UV (H_2O) end absorption; IR (KBr) 3360, 1670, 1400, 1110, 1010 cm^{-1}; ^1H NMR (D_2O) 1.81 (3H, d, C–CH_3), 4.96 (1H, d, anomeric H) and ^{13}C NMR data listed in Table 1. The molecular formula, $C_{14}H_{28}N_6O_8 \cdot 1\frac{1}{2}H_2O$, was determined on the basis of the number of carbon atoms in the ^{13}C NMR spectrum and the elemental analysis. *Anal* Found: C 38.75, H 7.32, N 19.54. Calcd: C 38.62, H 7.13, N 19.31, and FAB mass spectrum (MH^+ 409). From the physico-chemical properties, substance AC4437 is clearly a member of the streptomycin[1] group which includes antibiotics such as dihydrostreptomycin[2,3], hydroxystreptomycin[4], bluensomycin[5], *N*-demethylstreptomycin[6], mannosidostreptomycin[7] and mannosidohydroxystreptomycin[8]. AC4437 showed Rf 0.36 on silica gel TLC developed with propanol - pyridine - AcOH - H_2O (15:10:3:12), and Rf 0.27 on silica gel TLC developed with butanol - AcOH - H_2O (2:1:1). Dihydrostreptomycin, which is closely related to AC4437, showed Rf values of 0.23 and 0.16, respectively.

The ^{13}C NMR chemical shifts of AC4437 were assigned by comparing with those of dihydrostreptomycin as shown in Table 1. The ^{13}C NMR spectrum of AC4437 revealed the absence of an *N*-methyl-L-glucosamine moiety in its structure. We assigned that C-1′ the only anomeric carbon, C-2′ the methine carbon and C-3′ the only quaternary carbon of AC4437 are at δ 108.8, δ 80.4 and δ 80.2, respectively. The chemical shifts of C-1′, C-2′ and C-3′ were different from those of dihydrostreptomycin. The upfield shift of C-2′ in AC4437 can be explained by the absence of an *N*-methyl-L-glucosamine.

MUNRO *et al.*[5] reported in the model compound benzyl α-L-dihydrostreptoside, C-3′ the only quaternary carbon is at δ 80.4.

Based on the data presented, we propose the structure shown in Fig. 1 for antibiotic AC4437 which is therefore the de-*N*-methyl-L-glucosamine analog of dihydrostreptomycin.

AC4437 showed moderate activity against a wide range of Gram-positive and Gram-negative bacteria. The antibacterial spectrum of AC4437 was similar to that of dihydrostreptomycin, although the potency was slightly less (Table 2).

No toxicity was observed with AC4437 when it was administered ip to mice at a dose of 50 mg/kg.

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(Received January 10, 1986)

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