Communications to the Editor

5′-HYDROXY-2″-*N*-DEMETHYL-DIHYDROSTREPTOMYCIN PRODUCED BY A *STREPTOMYCES*

Sir:

A new member of streptomycin group antibiotics has been found in the culture filtrate of a *Streptomyces* strain MG571-fF6, isolated from a soil sample collected at Hayama, Kanagawa Prefecture, Japan. In this communication, the isolation, characterization and structural elucidation of the antibiotic are reported.

The strain MG571-fF6 was cultured at 27°C for 4 days on a rotatory shaker (180 rpm) in a medium containing 1.5% glycerol, 1.5% soluble starch, 1.5% fish meal, 0.5% soybean meal and 0.2% $CaCO_3$ (adjusted to pH 7.4). Vegetative inoculum, 2% by volume grown for 48 hours in the same medium was used.

The culture broth in 45 flasks was collected and filtered (4.2 liters, 47.5 μg/ml of the antibiotic assayed by the cylinder plate method using *Bacillus subtilis* PCI219 as the test organism).

The antibiotic in the filtrate (filtered at pH 2.0 and adjusted to pH 7.0) was adsorbed on a column of Amberlite IRC-50 (Na^+ H^+, 7: 3, 200 ml) and

Table 1. ^{13}C NMR chemical shifts (δ).

Carbon	The antibiotic	Dihydro-streptomycin
1	59.7	59.7
2	71.4	71.5
3	59.1	59.1
4	79.1	78.8
5	74.3	74.2
6	72.3	72.4
C=NH (1)	159.0	159.1
C=NH (3)	158.5	158.5
1′	106.8	106.8
2′	85.3	84.9
3′	81.5	81.7
4′	82.4	78.5
5′	61.4**	13.4
CH_2OH (3′)	65.1	64.2
1″	97.2	94.4
2″	54.9	62.0
3″	70.3*	70.3*
4″	70.2*	70.1*
5″	74.0	73.7
6″	61.1**	61.2
NCH_3(2″)		32.7

δ: ppm from TMS in D_2O (pD 5.5) using dioxane (δ 67.4 ppm) as the internal reference.
*,** Assignments within any vertical column may be reversed.

Fig. 1. The IR spectrum of 5′-hydroxy-2″-*N*-demethyldihydrostreptomycin (KBr).

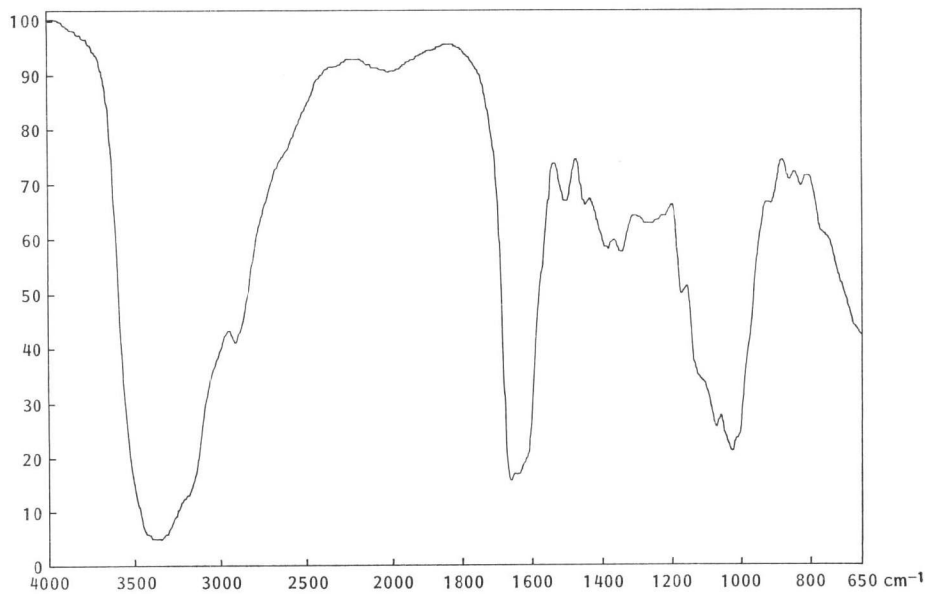
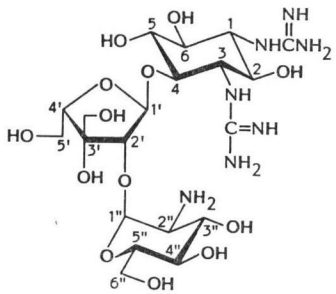


Table 2. The antibacterial spectrum on Mueller-Hinton agar.

Test organism	MIC (μg/ml)	Test organism	MIC (μg/ml)
Staphylococcus aureus FDA209P	25	*E. coli* K-12 LA290R55	25
S. aureus Smith	25	*E. coli* K-12 LA290R56	12.5
S. aureus Ap01	50	*E. coli* K-12 LA290R64	12.5
S. epidermidis 109	>100	*E. coli* W677	12.5
Micrococcus flavus FDA16	50	*E. coli* JR66/W677	>100
M. luteus PCI1001	25	*E. coli* K-12 C600 R135	12.5
Bacillus anthracis	12.5	*E. coli* JR225	12.5
B. subtilis PCI219	6.25	*Klebsiella pneumoniae* PCI602	12.5
B. subtilis NRRL B-558	25	*K. pneumoniae* 22#3038	>100
B. cereus ATCC 10702	50	*Shigella dysenteriae* JS11910	50
Corynebacterium bovis 1810	12.5	*S. flexneri* 4b JS11811	25
Mycobacterium smegmatis ATCC 607	6.25	*S. sonnei* JS11746	25
Escherichia coli NIHJ	100	*Salmonella typhi* T-63	50
E. coli K-12	6.25	*S. enteritidis* 1891	25
E. coli K-12 R5	>100	*Proteus vulgaris* OX19	6.25
E. coli K-12 R388	12.5	*P. rettgeri* GN311	6.25
E. coli K-12 J5R11-2	12.5	*P. rettgeri* GN466	3.13
E. coli K-12 ML1629	50	*Serratia marcescens*	50
E. coli K-12 ML1630	50	*Pseudomonas aeruginosa* A3	12.5
E. coli K-12 ML1410	25	*P. aeruginosa* No. 12	>100
E. coli K-12 ML1410 R81	>100		



eluted with 1 N HCl. On thin-layer chromatography using Avicel (Funakoshi) with 1-propanol - pyridine - acetic acid - water (15: 10: 3: 12), the eluate showed a single biologically active spot at Rf 0.24, while dihydrostreptomycin showed Rf 0.35. The active eluate (420 ml) was concentrated to dryness and extracted with anhydrous methanol (30 ml). The extract was concentrated to dryness, yielding a crude powder (17 g, 11.5 μg/mg). It was purified by carbon chromatography (12 g) developed with water to yield a colorless powder (283 mg, 575 μg/mg). Further purification by column chromatography on CM-Sephadex C-25 (180 ml, equilibrated with 0.2 M NaCl) developed with 0.2 M (350 ml), 0.6 M (900 ml) and 0.8 M NaCl (900 ml), followed by carbon chromatography (800 mg) eluted with water gave a purified powder of the hydrochloride (99 mg, 748 μg/mg, 41% yield from the culture filtrate).

The trihydrochloride of the antibiotic was obtained as a colorless hygroscopic powder decomposing gradually at 105~130°C. It shows $[\alpha]_D^{23}$ −84.6° (*c* 1.0, water) and positive SAKAGUCHI reaction. *Anal* Calcd for $C_{20}H_{39}N_7O_{13}\cdot 3HCl\cdot 5H_2O$: C 30.60, H 6.68, N 12.49, Cl 13.55. Found: C 30.36, H 6.27, N 11.62, Cl 13.38. The IR spectrum is shown in Fig. 1.

The ^{13}C NMR spectrum (Table 1) is very similar to that of dihydrostreptomycin[1] except the lack of an *N*-methyl signal at C-2″ and the presence of a hydroxymethyl signal instead of a *C*-methyl at C-4′ in the latter. The optical rotation is also similar to that of dihydrostreptomycin trihydrochloride ($[\alpha]_D^{25}$ −89.5°).[2] From these results, the structure of the antibiotic was determined to be 5′-hydroxy-2″-*N*-demethyldihydrostreptomycin.

The antibiotic has weak antibacterial activity as shown in Table 2.

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2) Peck, R. L.; C. E. Hoffhine, Jr. & K. Folkers: Streptomyces antibiotics. IX. Dihydrostreptomycin. J. Am. Chem. Soc. 68: 1390~1391, 1946