Communications to the Editor

A NEW STREPTOMYCIN GROUP ANTIBIOTIC PRODUCED BY *STREPTOMYCES SIOYAENSIS*

Sir:

A new antibiotic, 6‴-*O*-α-D-mannopyranosyl mannosidostreptomycin has been isolated from the culture filtrate of strain MD753-C2, together with streptomycin and mannosidostreptomycin. This strain has been classified as *Streptomyces sioyaensis*[1] which is a new streptomycin-producer. In this communication, the isolation, characterization and structural elucidation of the antibiotic are reported.

S. sioyaensis MD753-C2 on an asparagine-glucose agar slant was inoculated into a medium (110 ml) containing starch 2.0%, glucose 0.05%, soybean meal 2.5%, Peptone 0.1%, NaCl 0.3%, $CaCl_2$ 0.1%, K_2HPO_4 0.1% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 6.2) in a 500-ml baffled Erlenmeyer flask and cultured for 3 days at 37°C on a rotatory shaker (180 rpm). The culture broth in 37 flasks was collected and filtered at pH 6.4 to give 3.2 liters of the filtrate (22 μg/ml assayed by the cylinder plate method using *Bacillus subtilis* PCI219 as the test organism and the pure new antibiotic hydrochloride, 890 μg/mg, as the assay standard). Siomycin produced by *S. sioyaensis*[1] and its related peptide antibiotics could not be found in the filtrate.

The antibiotics in the filtrate were adsorbed on a column of Amberlite IRC-50 ($Na^+ : H^+$, 7:3, 240 ml) and eluted with 1 N HCl. The active eluate (226 ml) was neutralized to pH 5.6 with Amberlite IR-45 (OH^-) and chromatographed on a carbon column (4 g) eluted with 0.1 N HCl - MeOH (1:1). After neutralization with Amberlite IR-45 (OH^-), the active eluate (65 ml) was concentrated to give a crude powder (214 mg, 246 μg/mg). The high-voltage paper electrophoresis[2] (HCOOH - CH_3COOH - H_2O, 1:3:36; 3,500 V; detected by bioautography using *B. subtilis* PCI219; Rm was calculated taking the mobility of alanine as 1.0) showed that the crude powder contained a new antibiotic (Rm 1.02), mannosidostreptomycin (Rm 1.19) and a small amout of streptomycin (Rm 1.33). Separation of the individual antibiotics was achieved by column chromatography on CM-Sephadex C-25 (25 ml, equilibrated with 0.4 M NaCl) developed with 0.4 M NaCl (90 ml) and 0.6 M NaCl (350 ml). Approximately 5-ml fractions were collected. The new antibiotic was eluted in fractions 27~35 (45 ml) and mannosidostreptomycin in fractions 36~40 (25 ml). The antibiotic in the former eluate was further purified by carbon chromatography (500 mg) eluted with 0.1 N HCl - MeOH (1:1) followed by column chromatography on Sephadex LH-20 (100 ml) developed with MeOH to give a pure antibiotic hydrochloride (17 mg, 890 μg/mg) as a colorless hygroscopic powder. Mannosidostreptomycin in the latter eluate was purified by carbon chromatography (300 mg) eluted with

Scheme 1.

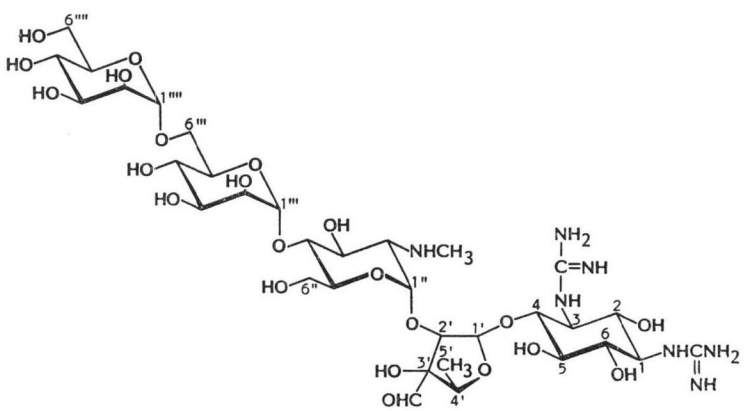

Table 1. ^{13}C NMR chemical shifts (δ).

Carbon	Streptomycin	Mannosidostreptomycin	The antibiotic	Carbon	Streptomycin	Mannosidostreptomycin	The antibiotic
1	59.6 d	59.6 d	59.4 d	4″	70.1 d	78.0 d	76.9 d
2	71.7 d	71.7 d	71.3*2 d	5″	73.8 d	73.8 d	73.8 d
3	59.0 d	59.0 d	59.2 d	6″	61.4 t	61.6*1 t	61.6*1 t
4	78.6 d	78.8 d	78.7 d	NCH_3 (2″)	33.1 q	33.2 q	33.1 q
5	73.9 d	74.2 d	74.2 d	1‴		102.0 d	102.0*3 d
6	72.3 d	72.4 d	72.2 d	2‴		71.1 d	71.3*2 d
C=NH (1)	159.1 s	159.1 s	159.0 s	3‴		71.1 d	71.3*2 d
C=NH (3)	158.9 s	158.9 s	158.9 s	4‴		67.5 d	67.4*4 d
1′	106.5 d	106.1 d	105.6 d	5‴		72.7 d	72.6*5 d
2′	85.6 d	86.2 d	83.4 d	6‴		61.0*1 t	66.1 t
3′	83.0 s	82.9 s	82.9 s	1⁗			100.5*3 d
4′	78.3 d	78.0 d	78.2 d	2⁗			71.1*2 d
5′	13.3 q	13.3 q	13.5 q	3⁗			70.9*2 d
CHO (3′)	90.4 d	90.5 d	90.2 d	4⁗			67.6*4 d
1″	95.5 d	95.7 d	93.9 d	5⁗			72.9*5 d
2″	62.4 d	62.5 d	62.4 d	6⁗			61.9*1 t
3″	70.3 d	69.0 d	69.1 d				

δ: ppm from TMS in D_2O using dioxane (δ 67.4 ppm) as the internal reference. The hydrochlorides of antibiotics were measured. Chemical shifts of streptomycin were assigned according to ref 3. Assignments * within any vertical column may be reversed.

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

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA209P	>100
S. aureus Smith	100
Micrococcus luteus PCI1001	100
Bacillus anthracis	25
B. subtilis PCI219	25
B. subtilis NRRL B-558	100
Escherichia coli NIHJ	50
E. coli K-12	12.5
E. coli K-12 R5	50
E. coli K-12 ML1629	50
E. coli K-12 ML1630	100
Klebsiella pneumoniae PCI602	50
Shigella dysenteriae JS11910	100
S. flexneri 4b JS11811	100
S. sonnei JS11746	50
Salmonella typhi T-63	25
S. enteritidis 1891	50
Proteus vulgaris OX19	25
Serratia marcescens	50
Pseudomonas aeruginosa A3	3.13
P. aeruginosa No. 12	>100

0.1 N HCl - MeOH (1:1) to give its hydrochloride (9 mg, 1,009 μg/mg).

The trihydrochloride of the new antibiotic shows mp 190~195°C (dec); $[\alpha]_D^{17}$ −24.5° (*c* 1.0, H_2O); IR (KBr) 3370, 2940, 1730, 1670, 1650, 1470, 1400, 1140, 1100 and 1060 cm^{-1}; SI-MS *m/z* 906 (MH^+), and positive SAKAGUCHI and maltol reactions. *Anal* Calcd for $C_{33}H_{59}N_7O_{22}\cdot 3HCl$: C 39.04, H 6.16, N 9.66, Cl 10.48. Found: C 38.53, H 5.72, N 8.54, Cl 10.31.

From the above-mentioned properties, ^1H NMR [(400 MHz, D_2O) δ 5.60 (1H, d, 1″-H), 5.35 (1H, d, 1′-H), 5.06 (1H, s, CHO), 4.96, 4.92 (each 1H, d, 1-H of mannose), 4.49 (1H, d, 2′-H), 4.42 (1H, q, 4′-H), 3.30 (1H, dd, 2″-H), 2.85 (3H, s, NCH_3), 1.26 (3H, d, 5′-H)] and ^{13}C NMR (chemical shifts were assigned by comparing with those of streptomycin and mannosidostreptomycin as shown in Table 1), the structure of the new antibiotic was suggested to be 6″- or 6‴-*O*-α-D-mannopyranosyl mannosidostreptomycin.

In order to determine the position of the glycosidic linkage of the mannosidostreptomycin molecule with mannose, the antibiotic was modified as follows. After reduction of the antibiotic with PtO_2 in H_2O overnight in a Parr apparatus (2.8 kg/cm^2), *N*-benzyloxycarbonylation with benzyloxycarbonyl chloride in the presence of Na_2CO_3 in a mixture of acetone and H_2O (1:2) at pH 9.4, followed by acetalation with *p*-tolualdehyde dimethyl acetal and camphorsulfonic

acid in anhydrous dimethyl sulfoxide[4] gave the 2″-*N*-(benzyloxycarbonyl)dihydro-tetrakis(*O*-*p*-methylbenzylidene) derivative (SI-MS: *m/z* 1,451, MH^+), but did not give the pentakis(*O*-*p*-methylbenzylidene) derivative. While, the similar modifications of streptomycin and mannosidostreptomycin gave 2″-*N*-(benzyloxycarbonyl)dihydro-bis(*O*-*p*-methylbenzylidene) (SI-MS: *m/z* 922, MH^+) and -tris(*O*-*p*-methylbenzylidene) (SI-MS: *m/z* 1,187, MH^+) derivatives, respectively. From these results, the structure of the antibiotic was determined to be 6‴-*O*-α-D-mannopyranosyl mannosidostreptomycin.

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

Yoko Ikeda
Shuichi Gomi
Kazuteru Yokose
Hiroshi Naganawa
Takako Ikeda
Mayumi Manabe
Masa Hamada
Shinichi Kondo
Hamao Umezawa

Institute of Microbial Chemistry
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

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References

1) Nishimura, H.; S. Okamoto, M. Mayama, H. Ohtsuka, K. Nakajima, K. Tawara, M. Shimohira & N. Shimaoka: Siomycin, a new thiostrepton-like antibiotic. J. Antibiotics, Ser. A 14: 255~263, 1961
2) Maeda, K.; A. Yagi, H. Naganawa, S. Kondo & H. Umezawa: Application of high-voltage paper electrophoresis for separation and identification of antibiotics. J. Antibiotics 22: 635~636, 1969
3) Munro, M. H. G.; R. M. Stroshane & K. L. Rinehart, Jr.: Location of guanidino and ureido groups in bluensomycin from ^{13}C NMR spectra of streptomycin and related compounds. J. Antibiotics 35: 1331~1337, 1982
4) Usui, T.; T. Tsuchiya, H. Umezawa & S. Umezawa: Transformation of dihydrostreptomycin into 3″-deoxydihydrostreptomycin. Bull. Chem. Soc. Jpn. 54: 781~786, 1981