Notes

3-*N*-METHYLPAROMOMYCIN I PRODUCED BY A *STREPTOMYCES*

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A new member of paromomycin group antibiotics has been isolated from the culture filtrate of *Streptoverticillium* sp. Al-R2827 which was isolated from a soil sample collected in India. In this report, the production, the isolation, characterization and structural elucidation of the antibiotic are reported.

Production and Isolation

Streptoverticillium sp. A1-R2827 was inoculated into a seed medium (50 ml) containing starch 1.0%, glucose 1.0%, Polypeptone 0.5%, meat extract 0.2%, yeast extract 0.3%, soybean meal 0.2% and $CaCO_3$ 0.2% (pH 7.0) in a 250-ml baffled Erlenmeyer flask and cultured for 48 hours at 28°C on a rotatory shaker (250 rpm). Twenty-five milliliters of this inoculum was transferred to the same seed medium (500 ml) in a 2-liter flask and incubated under the similar condition for 48 hours. The seed culture (500 ml) was inoculated into a production medium (30 liters) consisting of soybean cake 5.0%, glucose 6.0%, Na_2SO_4 1.0%, $(NH_4)_2HPO_4$ 0.02%, soybean oil 0.3% and silicone defoamer 0.05% in a 50-liter jar fermentor and cultured for 90 hours at 27°C under agitation (580 rpm) and aeration (15 liters/minute). The fermented broth (60 liters) was filtered (45 liters, 5.5 μg/ml of 3-*N*-methylparomomycin I) at pH 8 using Hyflo Super Cel (Johns-Manville) as the filter aid. The activity of the antibiotics was assayed by the paper disc method using *Bacillus subtilis* PCI 219 as the test organism. The culture filtrate was passed through a column of Amberlite IRC-50 (NH_4^+, 2 liters) and after washing with water, the column was eluted with 1 M NH_4OH. The active eluate was concentrated to dryness and the residue was extracted with 80% aqueous methanol. The extract was concentrated. The antibiotics in the concentrate were adsorbed on a column of Amberlite CG-50 (NH_4^+, 500 ml) and eluted with 0.5 M NH_4OH. The active eluate was concentrated to dryness to give a crude powder (1.86 g, 119 μg/mg). On silica gel TLC with chloroform - methanol - 28% ammonia (1:4:2), the crude powder showed the presence of two antibiotics at Rf 0.23 and Rf 0.16. The latter was identical with that of paromomycin I. The crude powder (760 mg) was dissolved with a small amount of water and the antibiotics were adsorbed on a column of Amberlite CG-50 (NH_4^+, 100 ml). After washing with water (1,000 ml), 0.1 M NH_4OH (500 ml) and 0.15 M NH_4OH (500 ml), the column was eluted with 0.18 M NH_4OH and the eluate was cut into 12.5-ml fractions. Lyophilization of fraction Nos. 9~18 gave a crude powder (57.8 mg, 585 μg/mg) of antibiotic **1** and fraction Nos. 36~66 gave a crude powder (42.7 mg, 536 μg/mg) of paromomycin I (**2**). Further purification of each crude powder by a column of Amberlite CG-50 (NH_4^+, 50 ml) in the same manner mentioned above gave antibiotic **1** (21.5 mg as the hemicarbonate, 1,000 μg/mg) and **2** (20.0 mg as the hemicarbonate, 995 μg/mg) which was identical with paromomycin I[1] on the bases of ^{1}H NMR, ^{13}C NMR, IR, secondary ion mass spectrometry (SI-MS), TLC and high-voltage paper electrophoresis (HVPE).

Characterization and Structural Elucidation

The hemicarbonate of **1** was obtained as a colorless powder melting at 163~167°C with decomposition and showed $[\alpha]_D^{20}$ +67.2° (*c* 1.0, H_2O); SI-MS *m/z* 630 $(M+H)^+$, 652 $(M+Na)^+$; UV (H_2O) end absorption; IR (KBr) 3370, 2910, 1570, 1465, 1365, 1140, 1085, 1025 and 940 cm^{-1} and positive ninhydrin and RYDON-SMITH reac-

Table 1. ^{1}H NMR data of **1** and **2**.

Proton	**1**			**2**		
	ppm	*J* (Hz)		ppm	*J* (Hz)	
1	3.39	12.6 (2_{ax})	3.9 (2_{eq})	3.40	12.8 (2_{ax})	4.3 (2_{eq})
		9.2 (6)			9.0 (6)	
2_{ax}	1.87	12.6 (1)	12.6 (2_{ax})	1.89	12.8 (1)	12.8 (2_{eq})
		12.8 (3)			12.8 (3)	
2_{eq}	2.54	3.9 (1)	12.6 (2_{ax})	2.51	4.3 (1)	12.8 (2_{ax})
		4.1 (3)			3.9 (3)	
3	3.63	12.8 (2_{ax})	4.1 (2_{eq})	3.62	12.8 (2_{ax})	3.9 (2_{eq})
		10.0 (4)			10.2 (4)	
4	4.20	10.0 (3)	9.0 (5)	4.08	10.2 (3)	9.0 (5)
5	3.98	9.0 (4)	9.0 (6)	3.96	9.0 (4)	9.0 (6)
6	3.76	9.2 (1)	9.0 (5)	3.75	9.0 (1)	9.0 (5)
3-NCH_3	2.81			—		
1′	5.87	4.1 (2′)		5.81	4.1 (2′)	
2′	3.44	4.1 (1′)	10.8 (3′)	3.44	4.1 (1′)	11.0 (3′)
3′	3.97	10.8 (2′)	9.2 (4′)	3.94	11.0 (2′)	9.2 (4′)
4′	3.51	9.2 (3′)	9.2 (5′)	3.51	9.2 (3′)	9.5 (5′)
5′	3.74～3.80			3.72～3.80		
6′a	3.74～3.80			3.72～3.80		
6′b	3.87～3.92			3.89～3.93		
1″	5.42	2.1 (2″)		5.40	2.3 (2″)	
2″	4.44	2.1 (1″)	4.6 (3″)	4.43	2.3 (1″)	4.9 (3″)
3″	4.58	4.6 (2″)	7.1 (4″)	4.57	4.9 (2″)	6.9 (4″)
4″	4.21	7.1 (3″)	4.4 (5″a)	4.22	6.9 (3″)	4.4 (5″a)
		2.8 (5″b)			3.1 (5″b)	
5″a	3.78	4.4 (4″)	12.3 (5″b)	3.78	4.4 (4″)	12.6 (5″b)
5″b	3.92	2.8 (4″)	12.3 (5″a)	3.92	3.1 (4″)	12.6 (5″a)
1‴	5.31	1.8 (2‴)		5.31	1.8 (2‴)	
2‴	3.60	1.8 (1‴)	3.1 (3‴)	3.60	1.8 (1‴)	3.1 (3‴)
		1.3 (4‴)			1.3 (4‴)	
3‴	4.23	3.1 (2‴)	3.1 (4‴)	4.24	3.1 (2‴)	3.3 (4‴)
4‴	3.82	1.3 (2‴)	3.1 (3‴)	3.83	1.3 (2‴)	3.3 (3‴)
		1.3 (5‴)			1.3 (5‴)	
5‴	4.34	1.3 (4‴)	4.1 (6‴a)	4.35	1.3 (4‴)	4.1 (6‴a)
		6.4 (6‴b)			6.4 (6‴b)	
6‴a	3.38	4.1 (5‴)	14.1 (6‴b)	3.38	4.1 (5‴)	13.3 (6‴b)
6‴b	3.43	6.4 (5‴)	14.1 (6‴a)	3.43	6.4 (5‴)	13.3 (6‴a)

δ: ppm from TSP (δ 0 ppm) in D_2O as the internal reference. These antibiotics were measured at pD 1.

tions.

Anal Calcd for $C_{24}H_{47}N_5O_{14}\cdot\frac{1}{2}H_2CO_3$:
C 44.54, H 7.32, N 10.60.
Found: C 45.02, H 7.54, N 10.36.

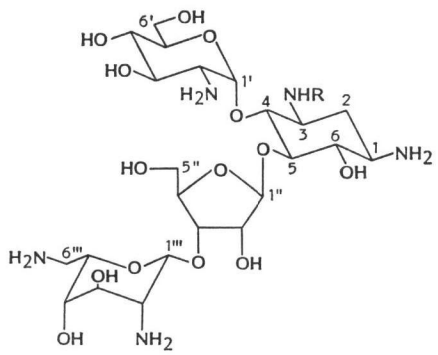
By HVPE under 3,500 V for 15 minutes in formic acid - acetic acid - water (1: 3: 36), **1** moved to the cathode with Rm 1.86 (relative mobility to alanine as 1.0), while **2** showed Rm 1.92 at the same time. The ^{1}H and ^{13}C NMR data of **1** and **2** are represented in Tables 1 and 2, respectively. All assignments of the NMR data of **1** and **2** were determined by proton selective decoupling experiments, proton-proton shift correlation spectroscopy and carbon-proton shift correlation spectroscopy. NMR spectra of both antibiotics were closely similar to each other but the remarkable differences were observed. *N*-Methyl signal at 2.81 ppm in the ^{1}H NMR spectrum of **1** and at 31.0 ppm in the ^{13}C NMR spectrum of **1** which was absent from those of **2**. Existence of an *N*-methyl group was also supported by the result of the SI-MS spectrum. Molecular ion peak at *m/z* 630 $(M+H)^+$ of **1** was 14 mass units larger than molecular ion peak at *m/z* 616 $(M+H)^+$ of **2**. The carbon-13 chemical shifts of **1** were nearly con-

Table 2. ^{13}C NMR data of **1** and **2**.

Carbon	1 ppm	1 m	2 ppm	2 m
1	50.8	d	50.7	d
2	26.2	t	29.2	t
3	56.2	d	50.0	d
4	76.6	d	78.4	d
5	85.0	d	85.0	d
6	73.1	d	73.1	d
3-NCH_3	31.0	q	—	
1′	96.8	d	96.8	d
2′	54.7	d	54.8	d
3′	69.6	d	69.8	d
4′	70.0	d	70.2	d
5′	75.2	d	74.9	d
6′	61.3	t	61.3	t
1″	110.5	d	110.7	d
2″	74.4	d	74.3	d
3″	76.0	d	76.1	d
4″	82.2	d	82.3	d
5″	61.1	t	61.2	t
1‴	96.1	d	96.1	d
2‴	51.9	d	51.9	d
3‴	68.6	d	68.6	d
4‴	68.2	d	68.2	d
5‴	71.1	d	71.1	d
6‴	41.5	t	41.5	t

δ: ppm from TMS in D_2O using dioxane (δ 67.4 ppm) as the internal reference. These antibiotics were measured at pD 1.

m: Multiplicity.



1 R = CH_3

2 R = H

Table 3. The antibacterial spectra of **1** and **2**.

Test organism	MIC (μg/ml)* 1	2
Staphylococcus aureus FDA 209P	1.56	0.39
S. aureus Smith	1.56	0.39
S. aureus Ap01	6.25	1.56
S. epidermidis 109	>100	>100
Micrococcus flavus FDA 16	>100	50
M. luteus PCI 1001	100	3.13
Bacillus anthracis	6.25	1.56
B. subtilis PCI 219	0.39	0.20
B. subtilis NRRL B-558	1.56	1.56
B. cereus ATCC 10702	6.25	6.25
Corynebacterium bovis 1810	>100	1.56
Escherichia coli NIHJ	12.5	3.13
E. coli K-12 R5	6.25	1.56
E. coli K-12 R388	6.25	3.13
E. coli K-12 J5R11-2	>100	>100
E. coli K-12 ML1629	>100	>100
E. coli K-12 ML1630	>100	>100
E. coli K-12 ML1410	12.5	3.13
E. coli K-12 ML1410 R81	>100	>100
E. coli K-12 LA290 R55	12.5	6.25
E. coli K-12 LA290 R56	6.25	3.13
E. coli K-12 LA290 R64	6.25	1.56
E. coli W677	12.5	3.13
E. coli JR66/W677	>100	>100
E. coli K-12 C600 R135	6.25	1.56
E. coli JR225	6.25	3.13
Klebsiella pneumoniae PCI 602	6.25	1.56
K. pneumoniae 22#3038	>100	>100
Shigella dysenteriae JS11910	25	6.25
S. flexneri 4b JS11811	12.5	6.25
S. sonnei JS11746	12.5	3.13
Salmonella typhi T-63	3.13	0.78
S. enteritidis 1891	6.25	3.13
Proteus vulgaris OX19	6.25	1.56
Providencia rettgeri GN311	1.56	0.39
P. rettgeri GN466	12.5	6.25
Serratia marcescens	12.5	3.13
Pseudomonas aeruginosa A3	50	12.5
P. aeruginosa No. 12	>100	50

* The MICs were determined on Mueller-Hinton agar.

sistent with the corresponding chemical shifts of **2** except for the four carbon signals (C-2, C-3, C-4 and 3-*N*-methyl). These differences firmly point out that the amino group at C-3 of **2** is replaced by a methylamino group in **1**. As a result the C-3 signal of **1** appeared lower field at 56.2 ppm than that of **2** (50.0 ppm) owing to the deshielding effect of *N*-methylation. Simultaneously the C-2 and C-4, at the β-position for *N*-methyl group, signals of **1** were found at 26.2 ppm and 76.6 ppm, respectively, higher field than those of **2** (29.2 ppm and 78.4 ppm, respectively) due to the shielding effect of *N*-methyla-

tion. Furthermore, the W-type long range coupling observed between 2‴-H and 4‴-H (J=1.3 Hz) indicates the 2,6-diamino-2,6-dideoxy-β-L-idopyranosyl moiety approaching a $1C$ conformation. This is also clarified by the fact that the signals due to C-2‴, C-3‴ and C-4‴ of β-L-*ido* unit were at higher field compared to their C-2′, C-3′ and C-4′ of α-D-*gluco* unit.

Acid hydrolysis of hygromycin B and destomycin A gave a dextrorotatory *N*-methyldeoxystreptamine ($[\alpha]_D^{26}$ +39.8°, *c* 2.0, H_2O)[2] and a levorotatory *N*-methyldeoxystreptamine ($[\alpha]_D^{22}$ −17.8°, *c* 2.0, H_2O)[3], respectively, and absolute configuration of the latter was determined by KONDO *et al.*[4] Acid hydrolysis of **1** with 6 N hydrochloric acid at 110°C for 15 hours, followed by adsorption on a column of Amberlite CG-50 (NH_4^+ - H^+, 7 : 3) and elution with 0.1 M NH_4OH, afforded a dextrorotatory *N*-methyldeoxystreptamine: $[\alpha]_D^{20}$ +23.5° (*c* 0.4, H_2O); electron impact MS *m/z* 176; ^1H NMR (400 MHz, D_2O, pD 1) δ 1.81 (1H, ddd, 2_{ax}-H), 2.55 (1H, ddd, 2_{eq}-H), 2.80 (3H, s, 3-NCH_3), 3.30 (1H, ddd, 1-H or 3-H), 3.34 (1H, ddd, 1-H or 3-H), 3.44 (1H, dd, 5-H), 3.56 (1H, dd, 4-H or 6-H), 3.62 (1H, dd, 4-H or 6-H). This aminocyclitol was identical with hyosamine.[2] Thus, **1** was determined to be 3-*N*-methylparomomycin I.

The antibacterial activity of **1** is a little weaker than that of paromomycin I (**2**) as shown in Table 3.

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