N-METHYLSTREPTOTHRICIN D—A NEW STREPTOTHRICIN-GROUP ANTIBIOTIC FROM A *Streptomyces* spp.

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(Received for publication June 17, 1994)

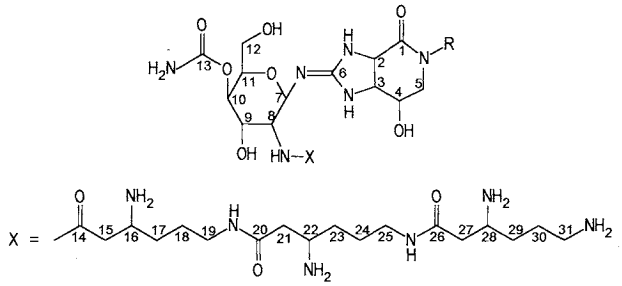
In the course of screening for new bioactive compounds, a new antibiotic in the class of streptothricins has been isolated from a *Streptomyces* spp. cultured from a soil sample collected at the campus of Seoul National University, Seoul, Korea. In the present paper, we wish to describe the production, isolation, physico-chemical and biological properties, and the structure elucidation of compound **2**, as *N*-methylstreptothricin D.

One of the many soil microbes, which have been isolated by the agar-disk screening method[1] and which show strong antifungal as well as antibacterial activities[2], SNU 8810-111 has been identified as a *Streptomyces* spp. The seed-culture of the organism, prepared by incubation at 28°C for 2 days on a reciprocal shaker (180 rpm) in Tryptic Soy Broth (TSB, Sigma, 50 ml) 3.0% is transferred to a medium (500 ml) consisting of TSB 3.0% and $CaCO_3$ 0.6% in a 2-liter Erlenmeyer flask. The broth is cultured under the same conditions as the seed culture for 4 days.

Antibiotics are recovered from the broth (3 liters) by treatment of a cation exchange resin (Diaion WK-20, H^+ form, 250 ml per liter of broth) overnight and by elution with HCl solution (0.005~0.05 N HCl). The antimicrobial fractions are treated with Diaion WA-30 (OH^- form) and evaporated. The residue is dissolved in 95% MeOH (100 ml). After filtration of the solution, it is concentrated and mixed with cellulose powder. The cellulose powder, after the solvent is removed completely, is applied on the top of a column packed with cellulose. The column was developed with *n*-BuOH - EtOH - AcOH - water (10 : 10 : 2 : 5). The active fractions are examined by TLC, and the fractions containing a single component are combined together, then evaporated and washed with diethyl ether. One component, which has been found to be *N*-methylstreptothricin D (**2**) obtained as an amorphous solid (19 mg) shows the physico-chemical properties as summarized in Table 1.

The IR spectrum of **2** shows bands at 1655 and 1555 cm^{-1} which suggest that compound **2** is a peptide. Total hydrolysis (6 N HCl, 100°C, 16 hours) of **2** gives β-lysine which has been identified by comparison of its ^{13}C NMR spectrum with that of the authentic β-lysine[3]. Among antibiotics containing β-lysine as their structural component, streptothricins show very similar spectral characteristics with **2**. The ^1H NMR spectral data of **2** are compared with streptothricin D (**1**)[4] in Table 2. The ^1H NMR spectrum of **2** shows a singlet (3H) at 2.86 ppm in addition to all the signals observed in the ^1H NMR spectrum of streptothricin D. This signal implies the existence of a methyl group attached to a nitrogen atom. The signal of the methyl group is observed at 35.6 ppm in the ^{13}C NMR spectrum of **2** (Table

Fig. 1. The structures of streptothricin D (**1**) and *N*-methylstreptothricin D (**2**).



1 R=H
2 R=CH_3

Table 1. Physico-chemical properties of *N*-methylstreptothricin D.

Appearance	White amorphous powder	
MP	>200°C (dec)	
$[\alpha]_D^{25}$	−34° (*c* 0.1, H_2O)	
UV $\lambda_{max}^{H_2O}$ nm	End absorption	
MW (FAB-MS, $(M+H)^+$)	773	
Color reaction	(+); Ninhydrin, Dragendorff reagent	
	(−); I_2, Anisaldehyde-sulfuric acid reagent, H_2SO_4	
Solubility	Soluble in water and methanol,	
	Insoluble in ethanol, $CHCl_3$, *n*-hexane	
Cellulose TLC (Rf value)	*n*-BuOH-EtOH-0.1 N HCL (1:1:1)	0.21
(Sigma, T-0520)	*n*-BuOH-Py-AcOH-H_2O (15:10:3:12)	0.17

Table 2. Comparison of ^1H NMR chemical shifts of *N*-methylstreptothricin D with those of streptothricin D.

Position	*N*-Methylstreptothricin D[a]		Streptothricin D[b]		$\Delta\delta$[c]
	δ (ppm)	*J* (Hz)	δ (ppm)	*J* (Hz)	
2	4.59 (d)	14	4.63 (d)	14	−0.04
3	4.07 (d)	14	4.08 (d)	14	−0.01
4	4.69	—	4.73 (m)	—	−0.04
5	3.80 (d)	6, 15	3.80 (dd)	6, 15	0.00
	3.47 (d)	15	3.40 (d)	15	+0.07
7	5.05 (d)	10	5.10 (d)	10	−0.05
8	4.22 (dd)	3, 10	4.26 (dd)	3, 10	−0.04
9	4.12 (t)	3	4.16 (t)	3	−0.04
10	4.73	—	4.77 (d)	3	−0.04
11	4.29 (t)	6	4.33 (t)	6	−0.04
12	3.68 (d)	6	3.73 (d)	6	−0.05
	3.68 (d)	6	3.73 (d)	6	−0.05
15	2.74 (dd)	5, 16	2.79 (dd)	5, 17	−0.05
	2.64 (dd)	8, 16	2.69 (dd)	8,17	−0.05
16	3.60 (m)	—	3.66 (m)	—	−0.06
17	1.66 (m)	—	1.72 (m)	—	−0.06
18	1.59 (m)	—	1.65 (m)	—	−0.06
19	3.20 (t)	7	3.26 (t)	7	−0.06
21	2.70 (dd)	5, 16	2.75 (dd)	5, 17	−0.05
	2.59 (dd)	8, 16	2.64 (dd)	8, 17	−0.05
22	3.60 (m)	—	3.66 (m)	—	−0.06
23	1.66 (m)	—	1.72 (m)	—	−0.06
24	1.59 (m)	—	1.65 (m)	—	−0.06
25	3.20 (t)	7	3.26 (t)	7	−0.06
27	2.67 (dd)	5, 16	2.71 (dd)	5, 16	−0.04
	2.57 (dd)	8, 16	2.62 (dd)	9, 16	−0.05
28	3.60 (m)	—	3.66 (m)	—	−0.06
29	1.76 (m)	—	1.81 (m)	—	−0.05
30	1.76 (m)	—	1.81 (m)	—	−0.05
31	3.01 (m)	—	3.06 (t)	5	−0.05
N-CH_3	2.86 (s)	—	—	—	—

[a] Spectrum recorded at 500 MHz in D_2O with the TMS as the internal reference.
[b] Values from ref 4.
[c] $\Delta\delta = \delta$ (*N*-methylstreptothricin D) − δ (streptothricin D).

3). The signal is completely absent in the ^{13}C NMR spectrum of streptothricin D. Comparison of the ^{13}C NMR chemical shifts of **2** with those of streptothricin D suggests that the methyl is attached to the amide nitrogen atom in streptolidine moiety. As shown in the last column in Table 3, the carbons

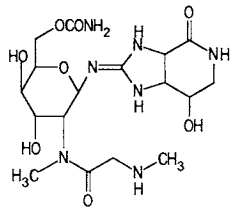
Table 3. Comparison of ^{13}C NMR[a] chemical shifts of *N*-methylstreptothricin D with those of streptothricin D.

Position	Chemical shifts δ (ppm)		$\Delta\delta$[c]
	N-Methylstreptothricin D[a]	Streptothricin D[b]	
1	170.1	172.5	−2.4
2	57.0	57.0	0.0
3	63.8	63.5	+0.3
4	65.1	63.5	+1.6
5	60.0	51.9	+8.1
6	160.4	160.5	−0.1
7	81.3	81.5	−0.2
8	51.4	51.5	−0.1
9	69.0	69.1	−0.1
10	72.6	72.6	0.0
11	76.0	76.1	−0.1
12	62.8	62.9	−0.1
13	165.3	165.3	0.0
14	174.6	174.8	−0.2
15	39.0	39.2	−0.2
16	51.0	51.1	−0.1
17	32.0	32.1	−0.1
18	26.8	26.9	−0.1
19	41.3	41.4	−0.1
20	174.3	174.4	−0.1
21	39.1	39.2	−0.1
22	51.0	51.1	−0.1
23	31.9	32.1	−0.2
24	26.8	26.9	−0.1
25	41.3	41.4	−0.1
26	174.1	174.2	−0.1
27	39.3	39.4	−0.1
28	51.22	51.3	−0.1
29	31.6	31.7	−0.1
30	25.4	25.5	−0.1
31	41.5	41.5	0.0
N-CH_3	35.6	—	0.0

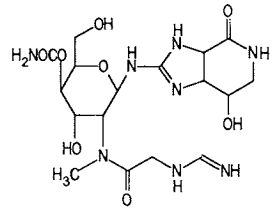
[a] Spectrum recorded at 125 MHz in D_2O with TMS as the internal reference.
[b] Values from ref 4.
[c] $\Delta\delta = \delta$ (*N*-methylstreptothricin D) $- \delta$ (streptothricin D).

at 1, 3, 4 and 5 positions show differences of more than 0.1 ppm in chemical shifts between **2** and streptothricin D, whereas the rest carbon atoms existing in the gulosamine and β-lysine portions show virtually identical chemical shifts. Especially, the difference of the chemical shift of the carbon atom at 5 position is exceptionally high ($\Delta\delta = +8.1$ ppm) between compound **2** and streptothricin D. The carbon atom at 5 has been assigned to the methylene group attached to the lactam nitrogen atom in the streptolidine moiety in streptothricin D[4]. Thus, we conclude that the methyl group is attached to the nitrogen atom of the lactam group in the streptolidine moiety. Existence of a methyl group in compound **2** has been further confirmed

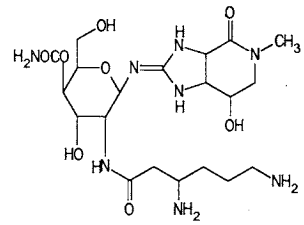
Fig. 2. Streptothricin-like antibiotics containing *N*-methyl group.



A-269A′



LL-AC 541



N-Methylstreptothricin F

Table 4. *In vitro* antimicrobial activities of *N*-methylstreptothricin D.

Organism[a]	MIC (μg/ml)
Bacillus cereus ATCC 11778	32
Bacillus megaterium ATCC 9885	0.13
Micrococcus luteus ATCC 9341	2
Staphylococcus aureus ATCC 6538p	0.25
Staphylococcus aureus ATCC 10537	1
Staphylococcus epidermidis ATCC 12228	0.5
Streptococcus faecalis ATCC 29212	>128
Acinetobacter calcoaceticus ATCC 15473	4
Citrobacter freundii ATCC 8090	4
Enterobacter aerogenes ATCC 29751	4
Enterobacter cloacae ATCC 27508	4
Escherichia coli ATCC 10536	4
Escherichia coli ATCC 25922	2
Klebsiella pneumoniae ATCC 10031	1
Morganella morganii ATCC 8076h	4
Proteus mirabilis ATCC 25933	4
Proteus vulgaris ATCC 6059	8
Providencia rettgeri ATCC 9250	0.5
Salmonella typhimurium ATCC 14028	4
Serratia marcescens ATCC 27117	16
Shigella flexneri ATCC 11836	8
Shigella sonnei ATCC 11060	4
Pseudomonas aeruginosa ATCC 25619	128
Pseudomonas aeruginosa ATCC 27853	>128
Pseudomonas aeruginosa ATCC 10145	>128
Pyricularia oryzae IFO 5994[b]	16

Medium: [a], Mueller-Hinton agar, [b], Potato dextrose broth agar.

by its FAB-mass spectrum in which a protonated molecular ion is observed at *m/z* 773, the value of which is higher than that of streptothricin D by 14. From these data, we propose that compound **2** isolated from SNU 8810-111 has the structure **2**, which we call *N*-methylstreptothricin D.

Methyl groups at nitrogen atoms have been found in several streptothricin-like antibiotics, such as A-269A'[5] and LL-AC 541[6], which have glycine derivatives at the nitrogen atom of the gulosamine moieties (Fig. 2). Both compounds have methyl groups at the nitrogen atoms in the gulosamine moieties. Typical streptothricins have β-lysine or poly-β-lysine group attached by an amide bond at the nitrogen atom of the gulosamine moiety. Among these compounds, *N*-methylstreptothricin F (A 37812)[7] has been the only known compound bearing a methyl group at the amide-nitrogen atom in the streptolidine moiety. Thus, compound **2**, *N*-methylstreptothricin D is another example which has a *N*-methyl amidic group at the streptolidine moiety. Compound **2** shows strong antimicrobial activities against fungi as well as Gram-positive and Gram-negative bacteria (Table 4).

Acknowledgment

This work was partly supported by The Basic Science Research Institute Program, Ministry of Education (BSRI-94-3417) and partly by The Korea Science and Engineering Foundation.

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