D-β-LYSYLMETHANEDIAMINE, A NEW BIOGENETIC AMINE PRODUCED BY A *STREPTOMYCES*

Sir:

D-β-Lysylmethanediamine, a new amine which has weak antibacterial activity has been isolated from the culture filtrate of *Streptomyces nashvillensis* MD743-GF4. It has a unique open-chain aldoaminal structure. It also enhances both delayed-type hypersensitivity and antibody-formation, although effect was not marked. In this communication, the isolation, characterization, structural elucidation and synthesis of D-β-lysylmethanediamine are reported.

The strain MD743-GF4 was cultured at 28°C for 3 days on a rotatory shaker (180 rpm) in a medium (110 ml) containing galactose 2.0%, dextrin 2.0%, peptone (Bacto-soytone, Difco) 1.0%, corn steep liquor (Ajinomoto) 0.5%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% in a 500-ml baffled Erlenmeyer flask. The vegetative growth (2%) grown for 2 days in the same medium was inoculated.

The culture broth in 90 flasks was collected and filtered (pH 6.0, 9.0 liters, 80 μg/ml of the antibiotic assayed by the cylinder-plate method using *Bacillus subtilis* PCI219 as the test organism). The antibiotic in the filtrate was adsorbed on a column of Amberlite IRC-50 (70% NH_4^+ form, 550 ml) and eluted with 1.2 M NH_4OH. The active eluate (1,370 ml) was concentrated to dryness, yielding a crude powder (636 mg, 644 μg/mg). It was dissolved in H_2O (10 ml) and chromatographed on a column of Amberlite CG-50 (NH_4^+, 60 ml). After washing the column with H_2O (120 ml) and 0.6 M NH_4OH (300 ml), the antibiotic was eluted with 1.2 M NH_4OH (300 ml). The active eluate (114 ml) was concentrated to dryness, yielding 370 mg of pure D-β-lysylmethanediamine (51% yield from the culture filtrate).

The picrate was obtained as yellow crystals, mp 181~183°C (dec). *Anal* Calcd for $C_7H_{18}N_4O \cdot 3C_6H_3N_3O_7$: C 34.85, H 3.16, N 21.13, O 40.85. Found: C 34.74, H 3.21, N 21.40, O 40.95. The free base was obtained as a colorless hygroscopic powder; $[\alpha]_D^{26}$ −7.4° (*c* 0.5, H_2O), $[\alpha]_D^{26}$ −21.3° (*c* 0.46, 0.08 N HCl), SI-MS *m/z* 175 (MH^+), UV (H_2O) no characteristic absorption, IR (KBr) 3350, 3080, 2950, 1640, 1565, 1475, 1390, 1325, 1150, 1100, 1040, 950 and 820 cm^{-1}, positive ninhydrin and RYDON-SMITH reactions. The ^1H and ^{13}C NMR chemical shifts are shown in Table 1. By high-voltage paper electrophoresis with 3,300 V for 15 minutes in HCOOH - CH_3COOH - H_2O (1: 3: 36), the antibiotic moved toward the cathode with Rm (relative mobility to alanine) 2.50. The thin-layer chromatogram on Avicel SF (Funakoshi) with PrOH - pyridine - AcOH - H_2O (15: 10: 3: 12) as a developing solvent showed Rf 0.38.

The antibiotic had weak antibacterial activity

Table 1. The ^1H and ^{13}C NMR chemical shifts of D-β-lysylmethanediamine.

Position	Chemical shift (δ ppm)	
	^1H	^{13}C
1	4.56 s 2H	46.6 t
3		174.3 s
4	2.76 dd 1H	37.4 t
	2.90 dd 1H	
5	3.77 br 1H	49.3 d
6	1.83 m 4H	30.2 t
7		24.0 t
8	3.09 br 2H	40.2 t

NMR spectra were measured in D_2O at pD 6.

Table 2. Effect of D-β-lysylmethanediamine on immune responses to SRBC in mice.

D-β-Lysyl-methane-diamine[a] (μg/mouse, ip)	DTH,[b] increase of footpad thickness ×0.1 mm	Antibody formation[c] PFC (×10^3)/ spleen
0	11.6±0.56	269.2±26.1
0.001	12.7±0.90	—
0.01	16.1±0.61***	—
0.1	14.8±1.3**	436.7±46.8***
1	14.1±0.06*	374.5±36.6**
10	12.8±0.03	353.3±41.5**
100	11.9±0.05	260.6±78.6
1,000	—	222.2±43.0

[a] D-β-Lysylmethanediamine was given once at the time of immunization.

[b] CDF_1 mice (female, 10 weeks old) were immunized with 10^5 SRBC to the hind footpad iv; 4 days thereafter, the same number of SRBC was given sc to elicit response in the other footpad; 24 hours thereafter, the resulting edema was measured.[1,2]

[c] CDF_1 mice were immunized with 10^5 SRBC iv; 4 days thereafter, plaque-forming cells (PFC) were counted.[3,4]

***; $P<0.001$, **; $P<0.01$, *; $P<0.05$.

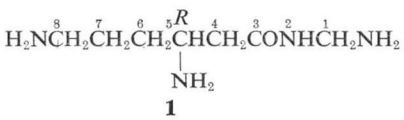
against Gram-positive bacteria; the minimum inhibitory concentrations (μg/ml on 0.5% peptone agar) were as follows: *Staphylococcus aureus* Smith, 50; *S. aureus* MS8710, 100; *Bacillus anthracis*, 100; *B. subtilis* NRRL B-558, 50; *B. subtilis* PCI219, 50. Intraperitoneal injection of 0.01, 0.1 or 1 μg/mouse of D-β-lysylmethanediamine enhanced delayed-type hypersensitivity (DTH) to sheep red blood cells (SRBC)[1] and the number of antibody-forming cells in the mouse spleen was increased by doses of 0.1 to 10 μg/mouse,[2] as shown in Table 2. D-β-Lysylmethanediamine did not inhibit aminopeptidase B, alkaline phosphatase and esterase at a concentration of 100 μg/ml. A single intravenous injection of 250 mg/kg did not cause death in mice.

Acetylation of the antibiotic (**1**) with acetic anhydride in a mixture of pyridine and H_2O gave the crystalline triacetate, mp 245°C (dec), EI-MS *m/z* 300 (M^+). Hydrolysis of **1** in 4 N HCl at 100°C for 3 hours followed by column chromatography on Amberlite CG-50 (NH_4^+) eluted with 0.6 M NH_4OH gave D-β-lysine (**2**), $[\alpha]_D^{22}$ −24.5° (*c* 0.8, 1 N HCl) [ref[3]) $[\alpha]_D^{25}$ −22.5° (*c* 0.8, 1 N HCl)]. The ^1H and ^{13}C NMR spectra of **1** (Table 1) suggested that the antibiotic had the methanediamine moiety as shown in structure **1**.

This structure, (5*R*)-1,5,8-triamino-2-azaoctan-3-one was confirmed by synthesis starting from D-β-lysine (**2**). Treatment of **2** with benzyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate (Kokusan Chemical Works) in 50% aqueous MeOH in the presence of triethylamine overnight at room temperature afforded 3,6-bis(*N*-benzyloxycarbonyl)-D-β-lysine (**3**), mp 148~151°C in 77% yield. Compound **3** was coupled with glycine by the method using *N*-hydroxy-5-norbornene 2,3-dicarboximide and dicyclohexylcarbodiimide in dioxane overnight at room temperature to yield 3,6-bis(*N*-benzyloxycarbonyl)-D-β-lysylglycine (**4**), mp 166~168°C in 93% yield. The conversion of the carboxylic acid of **4** into the *N*-acylamine (**6**) through the acid azide (**5**) was achieved by a modified CURTIUS procedure.[4] To a solution of **4** and *N*,*N*-diisopropylethylamine in THF a solution of ethyl chloroformate in THF was added at 0°C. After stirring for 3.5 hours, a solution of NaN_3 in H_2O was added at 0°C. The mixture was stirred for 1 hour and poured into ice water to yield a precipitate of **5**. A solution of **5** in toluene was refluxed overnight in the presence of benzyl alcohol, 4-*tert*-butylcatecol and pyridine to afford **6**, mp 204~214°C in 14% yield according to the method of OVERMAN *et al.*[4] Catalytic hydrogenation of **6** with 10% Pd-carbon in 50% aqueous acetic acid overnight in a Parr apparatus (3.2 kg/cm^2) gave **1** (68% yield) which was identical with the natural D-β-lysylmethanediamine in all respects including antibacterial activity.

Coupling of 3,6-bis(*N*-*tert*-butoxycarbonyl)-D-β-lysine with methanediamine dihydrochloride using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide in anhydrous THF in the presence of triethylamine at room temperature for 45 minutes was not successful for the synthesis of **1** and gave 3,6-bis(*N*-*tert*-butoxycarbonyl)-D-β-lysine amide in 53% yield.

The L-β-lysyl, $[\alpha]_D^{22}$ +19° (*c* 0.1, 0.08 N HCl), D-lysyl, $[\alpha]_D^{20}$ −39° (*c* 0.7, 0.06 N HCl) and L-lysyl, $[\alpha]_D^{21}$ +39° (*c* 0.37, 0.06 N HCl) analogs were synthesized by similar methodology as described above. They showed no antibacterial activity.



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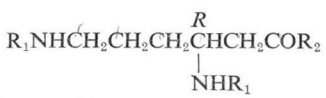

2 R_1=H, R_2=OH
3 R_1=Cbz, R_2=OH
4 R_1=Cbz, R_2=$NHCH_2COOH$
5 R_1=Cbz, R_2=$NHCH_2CON_3$
6 R_1=Cbz, R_2=$NHCH_2NHCbz$
Cbz=$PhCH_2OCO$

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