

PEPTHIOMYCIN, A NEW PEPTIDE ANTIBIOTIC MIXTURE

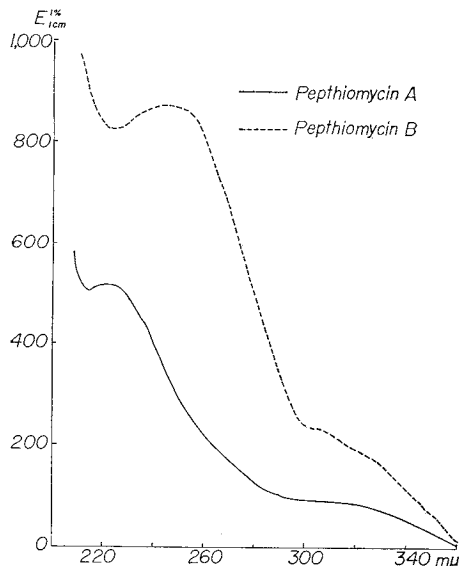
Sir:

A new peptide antibiotic mixture, named pepthiomycin, has been isolated from the culture broth of an actinomycete designated as *Streptomyces roseospinus*¹⁾. Pepthiomycin consists of two related peptides referred to as A and B, which have similar antimicrobial spectra.

The antibiotics were produced by submerged culture of the organism in a medium containing 1.0 % starch, 1.0 % glucose, 1.5 % soybean meal, 0.3 % NaCl, 0.1 % MgSO₄·7H₂O, and 0.1 % K₂HPO₄. Whole broth culture was adjusted to pH 3.5 with hydrochloric acid and filtered. The wet precipitate was extracted with methanol. The extract was concentrated *in vacuo* to remove the methanol, and the aqueous solution was extracted with *n*-butanol. The extract was washed with water, and was again concentrated *in vacuo* to an oily syrup. Upon addition of petroleum ether a brownish precipitate of pepthiomycin mixture was formed.

In order to separate the mixture, the active powder was chromatographed on a column of aluminum oxide (Woelm neutral, activity grade 1), which was developed with methanol followed by 80 % methanol containing 0.028 % ammonia. The first active eluate was evaporated to dryness yielding a

Fig. 1. Ultraviolet absorption spectra of pepthiomycin A and B in methanol.

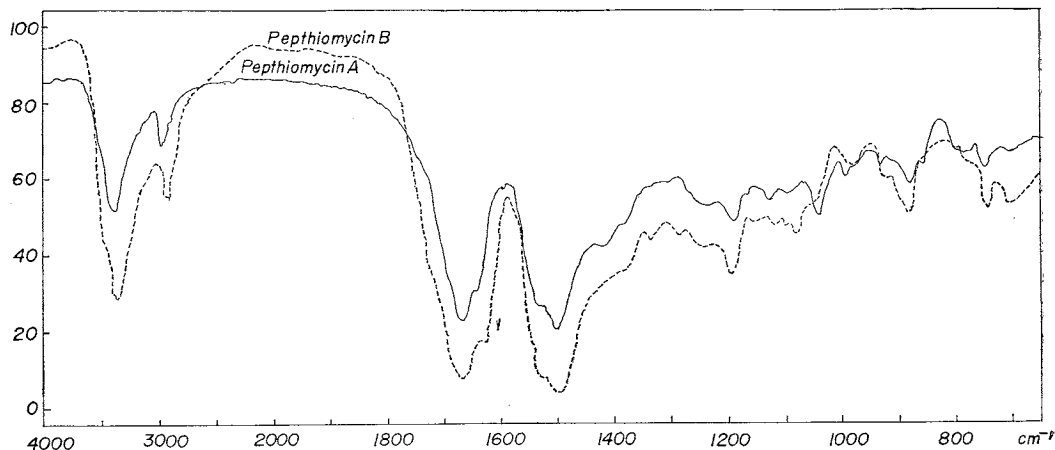


yellowish powder designated as pepthiomycin A, the next active eluate contained pepthiomycin B.

Each antibiotic was purified by silica gel column chromatography using the following developers: ethyl acetate-methanol (20:1) for pepthiomycin A, and ethyl acetate-*n*-butanol (4:1) saturated with water for pepthiomycin B. Both pepthiomycin A and B were obtained as pale yellow powders.

Pepthiomycin A melts at 204~210°C (decomp.), $[\alpha]_D^{20} +35^\circ$ (c 1.0, DMF). It is soluble in acetic acid, pyridine, DMSO and DMF, moderately soluble in lower alcohols

Fig. 2. Infrared spectra of pepthiomycin A and B



and esters, but insoluble in water and nonpolar solvents. The ultraviolet absorption spectrum in methanol exhibits a maximum at 230 $m\mu$ ($E_{1\text{cm}}^{1\%}$ 510) with inflection at 310 $m\mu$ ($E_{1\text{cm}}^{1\%}$ 87) as shown in Fig. 1. The infrared absorption spectrum is shown in Fig. 2. Elementary analysis data is as follows: C 58.73 %, H 6.23 %, N 11.23 %, O 20.27 %, S 4.22 %. It gives a positive permanganate test, but negative SAKAGUCHI, FeCl_3 , TOLLENS, MOLISCH and EHRLICH reactions, and doubtful ninhydrin and biuret reactions. After hydrolysis with 6 N hydrochloric acid for 20 hours at 105°C in a sealed tube, the amino acid composition of peptiomycin A was determined using an amino acid analyzer. The results were as follows: threonine, glycine, alanine, valine, isoleucine and three unknown ninhydrin-positive substances.

Peptiomycin B melts at 280~290°C (decomp.), $[\alpha]_D^{20} -30^\circ$ (c 0.5, DMF), and similar in solubility as peptiomycin A. The ultraviolet spectrum in methanol exhibits a maximum at 246 $m\mu$ ($E_{1\text{cm}}^{1\%}$ 872) with inflection at 310 $m\mu$ ($E_{1\text{cm}}^{1\%}$ 220). The infrared absorption spectrum is shown in Fig. 2. Elementary analysis data is as follows: C 51.43 %, H 5.26 %, N 14.81 %, O 23.12 %, S 4.73 %. It gives positive permanganate test, but negative SAKAGUCHI, FeCl_3 , TOLLENS, and MOLISCH reactions, and doubtful ninhydrin, biuret and EHRLICH reactions. The results of amino acid analysis under the same conditions as described above, were as follows: threonine, glycine, alanine, valine, leucine, isoleucine, phenylalanine, and three unknown ninhydrin-positive substances.

As shown in Table 1, peptiomycin A and B are both principally active against Gram positive bacteria including various antibiotic-resistant strains, but inactive against bryamycin-resistant strains of *Staphylococcus aureus*, Gram negative bacteria and fungi except *Torula utilis* und *Xanthomonas oryzae*. Curative effect on mice infected with *Staphylococcus aureus* SMITH

Table 1. Antimicrobial activity of peptiomycin A and B

Test organism	Minimal inhibitory concentration (mcg/ml)	
	A	B
<i>Staphylococcus aureus</i> FDA 209P	3.12	0.39
" " R1	3.12	0.39
" " R2	3.12	0.39
" " R3	3.12	0.39
" " R4	12.50	>50.00
" 52-34	3.12	0.39
" " R5	3.12	0.39
" 193	3.12	0.39
" " R6	3.12	0.39
" 308A-1	6.25	0.78
" " R7	6.25	0.78
<i>Sarcina lutea</i> PCI 1001	0.78	0.20
<i>Bacillus subtilis</i> PCI 219	3.12	0.39
<i>Escherichia coli</i> NIHJ	>100	>50
<i>Shigella flexneri</i> 1a Ew 8	>100	>50
<i>Pseudomonas aeruginosa</i> A3	>100	>50
<i>Klebsiella pneumoniae</i> PCI 602	>100	>50
<i>Mycobacterium</i> 607	>100	>50
<i>Candida albicans</i> 3147	>100	>100
<i>Saccharomyces cerevisiae</i>	>100	>100
<i>Torula utilis</i>	3.12	0.78
<i>Xanthomonas oryzae</i>	3.12~25.0	12.5
<i>Trichophyton mentagrophytes</i>	>100	>100
<i>Aspergillus niger</i>	>100	>100

R1 : Streptomycin resistant, R2 : Novobiocin resistant, R3 : Actinomycin resistant, R4 : Bryamycin resistant, R5 : Tetracycline and erythromycin resistant, R6 : Erythromycin resistant, R7 : Enduracidin resistant

strain was observed after intraperitoneal injection of doses comparable to minimal inhibitory concentration. The intraperitoneal injection of 5 mg of peptiomycin A or B to mice was not toxic. The above properties are sufficient to differentiate peptiomycin A and B from other known sulfur-containing peptide antibiotics such as bryamycin²⁾, thioestrepton³⁾, taitomycin⁴⁾, siomycin⁵⁾ and A-59⁶⁾ in respect to their ultraviolet absorption maxima, specific rotations and amino acid composition.

Acknowledgement

The authors wish to express their sincere thanks to Prof. YUTAKA NAGAI, Tokyo Medical and Dental University, for amino acid analysis.

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(Received April 23, 1968)

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