Communication to the editors

POLYMYXIN P, NEW ANTIBIOTICS OF POLYMYXIN GROUP

Sir:

In our screening studies of peptide antibiotics produced by soil bacteria, a new antibiotic complex, polymyxin P was isolated from a culture of strain T-39, obtained from soil collected at Suma-ku, Kobe City, Japan. The strain showed the following characters:

Rods, 0.5~1.0 by 2.0~7.0 μ, not in chains. Motile, aerobic, gram-variable. Spores, 1.2 ~1.5 by 1.5~2.5 μ, ellipsoidal, central to subterminal. Sporangia definitely bulged.

Glucose agar slant: Growth usually very thick and raised on agar, glistening, gummy, with production of gas.

NaCl broth: no growth in 5 % NaCl.

B.C.P.-milk: coagulated with production of gas.

Indole not produced. Starch hydrolyzed. VOGES-PROSKAUER reaction positive. Butyleneglycol, acetone and a small amount of ethanol are also produced. Nitrite produced from nitrates. As a result, T-39 was identified as *Bacillus polymyxa*.

General properties of the antibiotic complex resemble those of the known polymyxins, but obvious differences were found by chromatography and in the amino acid content of the hydrolyzate.

Table 1. Thin-layer chromatography (Silica gel)

Polymyxin	A	B	E (=colistin)	New antibiotic complex
Rf value	0.34	0.48	0.45	0.36

Solvent system: Upper layer of *n*-BuOH : AcOH : H_2O = 4 : 1 : 3 and 1/20 volume of pyridine.

Table 2. Amino acid constituents of polymyxins

Polymyxin	Phe	Leu	Thr	DAB	Literature
A	0	1	3	6	(1)
B	1	1	2	6	(2)
E (=colistin)	0	2	2	6	(3)
New antibiotic complex	1	0	3	6	

Table 3. Antimicrobial activity of polymyxin P acetate

Test organisms	M. I. C. (mcg/ml)
Escherichia coli NIHJ	0.78
Salmonella typhosa	0.78
Shigella flexneri 2a	0.78
Shigella sonnei I	0.78
Vibrio cholerae Inaba	1.56
Pseudomonas aeruginosa	3.12
Klebsiella pneumoniae	0.78
Proteus vulgaris	>100
Staphylococcus aureus 209P	>100
Streptococcus pyogenes	>100
Diplococcus pneumoniae I	>100
Corynebacterium diphtheriae	50
Bacillus subtilis	100
Candida albicans	>100

For antibiotic production, the following medium was used: Corn meal 5 %, starch 1 %, yeast extract 0.1 %, ammonium sulfate 0.5 % and calcium carbonate 1 %. Antimicrobial activity attained a maximum after cultivation for 60 hours in a jar fermentor.

The antibiotics in the broth were adsorbed on a column of Amberlite IRC-50(H^+). After washing with water, the columun was eluted by 0.05 N hydrochloric acid. The active eluate was neutralized with Amberlite IR-45(OH^-) and the solution concentrated under reduced pressure to a small volume. The concentrate was subjected to gel-filtration on Sephadex G-25 using 30 % acetic acid solution as developing solvent. The acetate was obtained as a white powder. It gave a single spot on thin-layer chromatogram and high-voltage paper electrophoresis.

Physical and chemical properties of the acetate were as follows: m. p. 215~218°C (decomp.), $[\alpha]_D^{20}$ −37.6° (*c* 1, H_2O). Amino acid analysis of the acetate gave Phe 0.57, Thr 1.71, DAB (α, γ-diaminobutyric acid) 3.52 μmole/mg (Phe : Thr : DAB = 1 : 3 : 6), and molecular weight by gel-filtration method on fine Sephadex G-25 was about 1,400. It was soluble in water and methanol, slightly soluble in ethanol and insoluble in acetone, ethylacetate, chloroform, benzene and ether. The free base was soluble in water. It gave a positive ninhydrin test, but negative MOLISCH, SAKAGUCHI, PAULY and

HOPKINS-COLE tests. The relative mobility on high-voltage paper electrophoresis was almost the same as polymyxin A (kindly supplied by Dr. WILKINSON), but the antibiotics hydrochloride was distinguished from polymyxins A, B and E (colistin) hydrochlorides by thin-layer chromatography (Table 1). Comparison of its amino acid constituents with those of the known polymyxin group antibiotics is shown in Table 2.

Thus, the antibiotics were concluded to be new members of the polymyxin group and named as polymyxin P. Abbreviation P is based on the isolation of 1 mole D-phenylalanine from the acid hydrolyzate. The antimicrobial activity of the polymyxin P acetate is summarized in Table 3.

Further purification of the sample was accomplished by countercurrent distribution (solvent system, *n*-BuOH : *sec*-BuOH : 0.1 N HCl=6 : 30 : 40). After 2,100 transfers, it was separated into two active components, P_1 (Kd=0.056) and P_2 (Kd=0.041). The amino acid constituents of P_1 and P_2 were the same to polymyxin P, but the former contained 6-methyloctanoic acid in acid hydrolyzate and the latter isooctanoic acid. Hydrochloride of P_1 and P_2 gave m.p. 220~221°C (decomp.), $[\alpha]_D^{20}$ −37.4° (*c* 1, H_2O) and m.p. 210~211°C (decomp.), $[\alpha]_D^{20}$ −43.3° (*c* 1, H_2O).

The present study revealed that the fatty acid of polymyxin P_1 is identical with that of polymyxins A_1, B_1 and E_1, and that the fatty acid of polymyxin P_2 is identical with that of polymyxins A_2, B_2 and E_2.

YUKIO KIMURA
EMIKO MURAI
MICHIKO FUJISAWA
TSURU TATSUKI
FUMIKO NOBUE

Laboratory of Biochemistry
Faculty of Pharmaceutical Sciences,
Mukogawa Women's University
Nishinomiya, Japan

(Received June 30, 1969)

References

1) WILKINSON, S. & L. A. LOWE : Structure of the polymyxins A and the question of identity with the polymyxins M. Nature 212 : 311, 1966
2) HAUSMANN, W. & L. C. CRAIG : Polymyxin B_1. Fractionation, molecular weight determination, amino acid and fatty acid composition. J. Am. Chem. Soc. 76 : 4892~4896, 1954
3) SUZUKI, T.; H. INOUYE, K. FUJIKAWA & Y. SUKETA : Studies on the chemical structure of colistin. 1. Fractionation, molecular weight determination, amino acid and fatty acid composition. J. Biochem. 54 : 25~33, 1961