POLYMYXIN F, A NEW PEPTIDE ANTIBIOTIC

Sir:

Polymyxin F is a new antibiotic produced by *Bacillus circulans* ATCC No. 31228. Application of the isolation procedure outlined in Chart 1 to 20 liters of fermentation broth gave 0.45 g of the pure antibiotic hydrochloride.

Polymyxin F hydrochloride is a white powder that melts *in vacuo* at 213~219°C. It is freely soluble in water and methanol but insoluble in acetone, benzene, *etc.* Chromatographic mobilities of polymyxin F in a number of systems are listed in Table 1. The antibiotic is detectable by bioautography against *Escherichia coli* or by reagents that give color reactions with primary amines. The optical rotations, $[\alpha]^{22}(\lambda)$, of the hydrochloride in 0.5 M HCl (*c* 0.5) are −43° (589), −47° (578), −54° (546), −97° (436) and −160° (365 nm). The infrared spectrum in KBr is shown in Fig. 1. The UV spectrum in water has end absorption but no maximum above 200 nm.

Hydrolysis in 6 N HCl at 110°C for 16 hours and amino acid analysis of the hydrolysate indicate that the antibiotic is comprised of 2,4-diaminobutyric acid (Dab), Thr, Ser, Ile, and Leu in a ratio of 5:1:1:1:2. The hydrolysate also contains three extractable fatty acids with relative abundances of 78: 19: 3. These were identified as 6-methyloctanoic acid, isooctanoic acid and octanoic acid, respectively, by gas-chromatographic comparison of the methyl esters with those obtained from polymyxin B. These hydrolysis products and their relative abundances indicate that the antibiotic is a polymyxin-type molecule and justify naming it as such, even though it is produced by a strain of *Bacillus circulans* rather than a strain of *Bacillus polymyxa*. To the best of our knowledge, no polymyxin has been previously reported with the particular amino acid composition listed above. The antibiotic is presumed to be a mixture of three components, designated polymyxin F_1, F_2, and F_3 corresponding to the three fatty acids in order of decreasing abundance. The elemental composition calculated for polymyxin F_1 hydrochloride on the basis of the observed hydrolysis products is in satisfactory agreement with that found for the antibiotic.

Anal. Calcd. for $C_{54}H_{105}N_{15}O_{13}Cl_4$: C, 49.34; H, 8.05; N, 15.99; Cl, 10.79.

Found: C, 49.68; H, 8.05; N, 16.13; Cl (ionic) 10.96%.

Chart 1. Isolation and purification of polymyxin F

Fermented broth

↓ adjust to pH 2 with HCl and centrifuge.

Supernate

↓ extract antibiotic into butanol at pH 2 and concentrate *in vacuo*.

Extract concentrate

↓ dilute with methanol and precipitate antibiotic with acetone.

Acetone-insoluble powder

↓ adsorb antibiotic on IRC-50 (Na^+) resin at pH 7 and elute it with MeOH–H_2O, 1: 1, at pH 1.5 (HCl).

Eluate

↓ concentrate *in vacuo* to remove MeOH, adjust to pH 10.5 (NaOH), extract into butanol, wash butanol phase with 1 N HCl and remove butanol *in vacuo*

Polymyxin F, *ca.* 50% pure

↓ chromatograph on Whatman CM52 carboxymethyl cellulose, eluting with a NaCl gradient and isolating active material as in the previous step.

Polymyxin F hydrochloride

Fig. 1. Infrared spectrum of polymyxin F hydrochloride in KBr

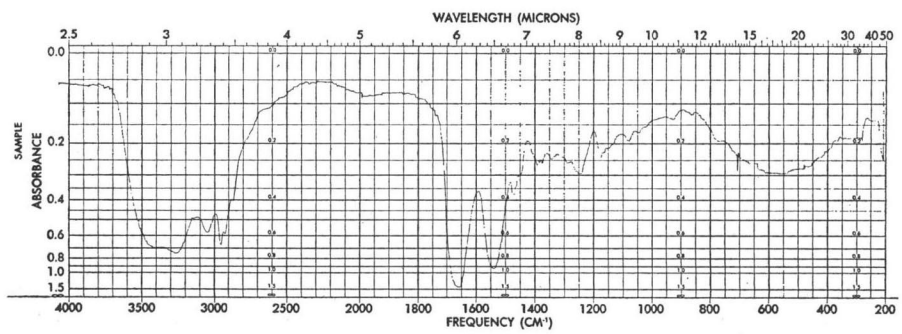

Table 1. Chromatography of polymyxin F and polymyxin B

System	Rf×100	
	Polymyxin B	Polymyxin F
Whatman No. 1 paper; BuOH - pyridine - HOAc - water, 15: 10: 3: 12	30	57
Whatman No. 1 paper; BuOH - HOAc - water, 4: 1: 5 (upper phase)	45	69
Gelman ITLC, type SAF*; PrOH - conc. NH_4OH, 7: 3	62	69
Gelman ITLC, type SAF; PrOH - pyridine - HOAc - water, 15: 10: 3: 12	85	85
Gelman ITLC, type SAF; BuOH - HOAc - water, 3: 1: 1	27	56

* Glass microfiber sheets impregnated with silicic acid.

Table 2. Antimicrobial activity of polymyxin F and polymyxin B

Organism	Minimum inhibitory concentration (μg/ml)*	
	Polymyxin F hydrochloride	Polymyxin B sulfate
Staphylococcus aureus FDA209P	50	50
Streptococcus pyogenes C203	6.3	4.7
Escherichia coli ATCC10536	2.4	0.1
Escherichia coli SC8294	2.4	0.2
Pseudomonas aeruginosa SC8329	1.6	0.8
Candida albicans SC5314	>100	75

* Minimum inhibitory concentrations were determined by two-fold broth dilution assay.

The nature of the antibiotic was further explored by 2,4-dinitrophenylation of the amino groups. Amino acid analysis of the acid hydrolysate of the resulting derivative shows that Dab and γ-dinitrophenyl-Dab are produced in a ratio of 1: 4. The presence of Dab in this hydrolysate supports the presence of a cyclic peptide. The presence of four amino groups was confirmed by the method of partial substitution[1], using dinitrophenylation and separating the mixture of derivatives by electrophoresis[2].

Polymyxin F possesses a pattern of antimicrobial activity that is similar to that of polymyxin B as seen from the data in Table 2. The antibiotic, when administered subcutaneously to mice, has an LD_{50} of 50 mg/kg but gives protection with an ED_{50} of 4.2 mg/kg against an *Escherichia coli* SC8294 infection. Polymyxin B has an LD_{50} of 50 mg/kg and an ED_{50} of 1.4 mg/kg in control experiments.

The chemical, physical and biological data that have been obtained for the antibiotic produced by *Bacillus circulans* ATCC 31228 indicate that it is a new member of the polymyxin group[3]. The most closely related polymyxins are polymyxin D[4] and polymyxin S_1[5], the only other antibiotics of this group that contain serine and that have five instead of six Dab residues.

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