Communications to the editor

NEW CHLORINATED NITRO-PYRROLE ANTIBIOTICS, PYRROLOMYCIN A AND B (SF-2080 A AND B)

Sir:

We wish to report here the isolation and characterization of two new antimicrobial substances with broad spectra, pyrrolomycin A and B (SF-2080 A and B) from the culture broths of a strain of actinomycetes designated SF-2080.

The strain was isolated from a soil sample collected at the Chikuma River of Nagano City in Japan. A taxonomic study was undertaken by detail examination on agar and submerged culture, but the substrate mycelium of strain SF-2080 did not yield any morphologically important items so far, such as aerial mycelium, spore, nocardioform, synnemata, *etc.* However, LL-diaminopimelic acid detected from the hydrolyzate of whole cells[1] suggests that it most probably belongs to the genus *Streptomyces*.

A culture of strain SF-2080 was grown on a starch-yeast extract agar at 28°C for a week, and was used to inoculate two 5-liter Erlenmeyer flasks each containing 800 ml of a sterile seed medium. The medium consisted of 10 g soluble starch, 10 g glucose, 5 g Polypepton, 2 g meat extract, 3 g yeast extract, 2 g soybean meal and 2 g $CaCO_3$ per liter of tap water and was adjusted to pH 7.0 prior to sterilization. The flasks were incubated at 28°C for 72 hours on a rotary shaker (200 rpm). The contents were transferred into two 50-liter jar fermentors each containing 30 liters of a production medium composed of 2% maltose syrup, 1% soybean meal, 0.5% Pharma-

Fig. 1. IR spectra of pyrrolomycin A and B (KBr).

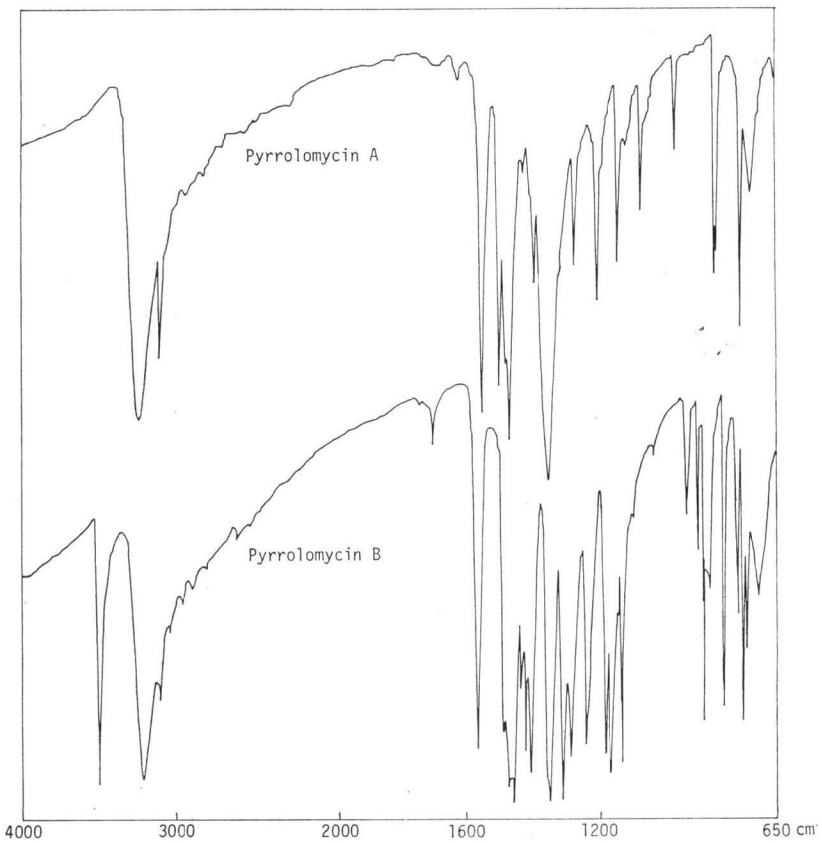


Table 1. Physico-chemical properties of pyrrolomycin A and B.

	Pyrrolomycin A	Pyrrolomycin B
Appearance	Yellow crystals	Same as A
Melting point	211~213°C	222~225°C
Specific rotation $[\alpha]_D^{25}$	0° (*c* 1, methanol)	Same as A
Elemental analysis		
Found	C 27.03, H 1.20, N 14.77, Cl 38.86	C 36.85, H 1.68, N 7.44, Cl 39.97
Calcd.	C 26.52, H 1.10, N 15.47, Cl 39.23	C 37.08, H 1.69, N 7.87, Cl 39.89
Molecular weight (MS)	180	354
Molecular formula	$C_4H_2N_2O_2Cl_2$	$C_{11}H_6N_2O_3Cl_4$
UV λ_{max}; nm ($E_{1cm}^{1\%}$)		
MeOH	268 (410), 318 (170)	277 (182), 330 (105)
0.01 N HCl-MeOH	270 (435), 318 (168)	277 (200), 332 (105)
0.01 N NaOH-MeOH	323 (630)	245 (340), 312 (343)
PMR (acetone-d_6, δ, ppm)	7.94 (s), 11.30 (s, br)	4.42 (s), 7.16 (d), 7.32 (d), 9.94 (s,br)
Rf value on silica-gel TLC		
Benzene - acetone (2: 1)	0.68	0.73
Chloroform - methanol (10: 1)	0.73	0.80
Benzene - ethyl acetate - acetic acid (100: 19: 1)	0.37	0.44

media (Traders Oil Mill Co., Texas), 0.25% distiller's solubles, 0.0005% $FeSO_4 \cdot 7H_2O$, 0.00005% $NiCl_2 \cdot 6H_2O$, 0.00005% $CoCl_2 \cdot 6H_2O$ and 0.1% $CaCO_3$ (pH 7.0, prior to sterilization). Fermentation was carried out at 28°C for 120 hours using an agitation rate of 350 rpm and an air flow rate of 1.0 vol/vol/minute. The antimicrobial activities were assayed by a paper-disc agar-diffusion method using *Bacillus subtilis* ATCC 6633.

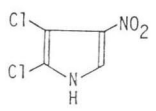
The filtrate (pH 8.5) of fermented broths and the mycelia were extracted separately with ethyl acetate (40 liters) and 50% aqueous acetone (20 liters), respectively. The latter extract was concentrated to evaporate acetone, the aqueous resultant was extracted with ethyl acetate (25 liters) and combined with the former extract. The combined extract was evaporated to dryness to furnish a tarry substance. The residue was dissolved in *n*-hexane (1.5 liters), filtered to remove the insoluble materials, and the solution was passed through an alumina column (400 g). After washing with *n*-hexane (800 ml), the column was developed with ethyl acetate (900 ml) and ethyl acetate - methanol (9: 1, 500 ml) successively. The resulting former and latter active eluates were pooled as pyrrolomycin A and B fractions, respectively. After evaporating to

Table 2. MIC values of pyrrolomycin A (PM-A) and B (PM-B).

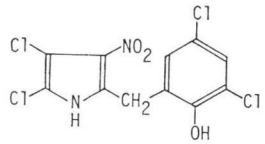
Test organisms	MIC (mcg/ml)	
	PM-A	PM-B
Staphylococcus aureus 209P JC-1	3.13	0.20
Staphylococcus epidermidis ATCC 14990	6.25	0.10
Streptococcus faecalis ATCC 8043	6.25	0.39
Bacillus anthracis No. 119	1.56	0.10
Escherichia coli NIHJ JC-2	6.25	12.5
Citrobacter freundii GN-346	6.25	12.5
Salmonella typhi O-901-W	3.13	12.5
Shigella sonnei EW-33 Type I	6.25	12.5
Klebsiella pneumoniae PCI-602	12.5	12.5
Proteus vulgaris OX-19	6.25	12.5
Proteus morganii Kono	6.25	12.5
Proteus mirabilis J-0013	6.25	12.5
Serratia marcescens MB-3848	6.25	25
Pseudomonas aeruginosa MB-3829	12.5	12.5
Candida albicans C-A-24	100	100
Cryptococcus neoformans Cr-1	25	100
Trichophyton mentagrophytes 530324	6.25	100
Trichophyton interdigitale	3.13	100
Aspergillus fumigatus Saito	25	100

dryness, pyrrolomycin A (0.5 g) and B (0.13 g) were obtained in crystalline state from hot benzene solutions.

Both pyrrolomycin A and B showed positive color reactions with BEILSTEIN, LEMIEUX and iodine tests, but negative ninhydrin and ferric chloride reactions. They are soluble in acetone, dioxane, ethyl acetate and lower alcohols, sparingly soluble in chloroform, and insoluble in water. Other physico-chemical properties of two crystalline antibiotics are tabulated in Table 1. The IR spectra are illustrated in Fig. 1. Analysis of pyrrolomycin A, and in particular pyrrolomycin B indicated a high content of chlorine atoms, and the structural studies described in subsequent papers[2,3] revealed that they are chlorinated 3-nitropyrroles having the following structures **I** and **II**.



Pyrrolomycin A (I)



Pyrrolomycin B (II)

Pyrrolomycin A and B are active against Gram-positive and Gram-negative bacteria and some genera of fungi, as shown in Table 2. LD_{50} values of pyrrolomycin A and B determined in JCL-ICR mice by intraperitoneal route were 21.2 mg/kg and *ca.* 100 mg/kg, respectively.

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References

1) BECKER, B.; M. P. LECHEVALIER, R. E. GORDON & H. A. LECHEVALIER: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. 12: 421~423, 1964
2) KOYAMA, M.; Y. KODAMA, T. TSURUOKA, N. EZAKI, T. NIWA & S. INOUYE: Structure and synthesis of pyrrolomycin A, a chlorinated nitropyrrole antibiotic. J. Antibiotics 34(12), 1981 (in press)
3) KANEDA, M.; S. NAKAMURA, N. EZAKI & Y. IITAKA: Structure of pyrrolomycin B, a chlorinated nitro-pyrrole antibiotic. J. Antibiotics 34: 1366~1368, 1981