PYRROLOMYCINS C, D AND E, NEW MEMBERS OF PYRROLOMYCINS

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Pyrrolomycins C, D and E, new members of pyrrolomycins produced by *Actinosporangium vitaminophilum* SF-2080, have been isolated by chromatography on a basic alumina column. Three antibiotics have chlorinated pyrrole nuclei linked directly or *via* carbonyl function to the dichlorophenol moiety. Pyrrolomycins C and E are active against Gram-positive bacteria, while the spectrum of pyrrolomycin D is broad including Gram-positive, Gram-negative bacteria and fungi.

In the preceding papers[1~3], isolation, structure and biological activity of new antibiotics pyrrolomycins A (**I**) and B (**II**) from fermentation broth of *Actinosporangium vitaminophilum* SF-2080[4] was reported. We describe here the isolation, physico-chemical and biological properties of three new active agents pyrrolomycins C, D and E which were co-produced in the culture broth with pyrrolomycins A and B. The producing organism of pyrrolomycins was considered to belong to the genus Streptomyces by its chemotaxonomic features, because it did not show any morphological characteristics necessary for the genus determination. But a further investigation led to conclusion that this organism belonged to the genus Actinosporangium. Structural study of three compounds will be presented in a succeeding report[5].

Fermentation

The producing strain, *A. vitaminophilum* SF-2080[4] was inoculated into 100 ml Erlenmeyer flask which contained 20 ml of a seed medium composed of 1% glucose, 1% starch, 0.5% Polypeptone, 0.3% yeast extract, 0.2% soybean meal, 0.2% meat extract and 0.1% $CaCO_3$. pH was adjusted to 7.0 before sterilization. Fermentation was performed on a rotary shaker at 30°C for 120 hours.

The seed culture was stepwise inoculated into 800 ml and then 20 liters of the medium with the same composition as indicated before. Eight liters of the 3rd seed culture was inoculated into the 570-liter tank fermenters each containing 250 liters of the production medium. The medium consisted of 0.2% maltose syrup, 1% soybean meal, 0.5% Pharmamedia (Traders Oil Mill Co., Texas), 0.25% distillers 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). The fermentation was conducted at 28°C for 120 hours with an agitation rate of 130 rpm and air flow rate of 1.0 vol/vol/minute. The antibiotic titer was assayed by a paper-disc agar diffusion method using *Bacillus subtilis* ATCC 6633 as a test organism. After 120-hour incubation, a maximum titer (60 μg/ml as pyrrolomycin A) was obtained.

Isolation

The 120-hour cultured broth (500 liters) was adjusted to pH 3.0, extracted with 300 liters of ethyl acetate and filtered. The ethyl acetate layer was separated, concentrated in reduced pressure

and re-extracted with toluene. The toluene extract was washed with an aqueous sodium bicarbonate, and then was subjected to column chromatography over basic alumina, using the following solvent systems; (1) ethyl acetate, (2) ethyl acetate - methanol (10: 1), (3) ethyl acetate - methanol (1: 1).

At first, the column was developed with solvent system (1), and 10 g of pyrrolomycin A was obtained from the first eluate. From the following fraction, on the other hand, a new active component was obtained as yellow crystals. After the recrystallization from hot ethyl acetate, pure antibiotic pyrrolomycin E was obtained.

Although most organic substances are eluted from alumina column with solvent system (3), recovery of antibiotic titer was not quantitative. Subsequently, the alumina column was washed with an acidic solvent system (ethyl acetate - conc. hydrochloric acid, 100: 1). From this eluate, another bioactive compounds which showed strong activity against Gram-positive bacteria were obtained as a crude yellow mass. This material was separated into two compounds by further purification using silica gel column chromatography (hexane - ethyl acetate - acetic acid, 100: 20: 1). Recrystallization from hot benzene gave pure pyrrolomycins C and D as yellow needles respectively.

Physico-chemical Properties

Pyrrolomycins C, D and E showed positive color reactions with BEILSTEIN, LEMIEUX and iodine tests but negative with ninhydrin test. Pyrrolomycins C and D are soluble in acetone, dioxane, ethyl acetate and lower alcohols, sparingly soluble in chloroform and insoluble in water. Pyrrolomycin E is less soluble than C and D in organic solvents and equally insoluble in water. Other physico-chemical properties of three antibiotics are tabulated in Table 1. The IR and UV spectra are illustrated in Figs. 1 and 2. Analysis of three pyrrolomycins indicated as high a content of chlorine atoms as pyrrolomycins A and B. Based on spectroscopic and X-ray analysis as well as synthetic correlation[5], the following structures **III**, **IV** and **V** were assigned for three antibiotics.

Biological Activity

As shown in Table 2, pyrrolomycin C was active against Gram-positive bacteria but inactive against

Table 1. Physico-chemical properties of pyrrolomycins C, D and E.

	Pyrrolomycin C	Pyrrolomycin D	Pyrrolomycin E
Appearance	Yellow needles	Yellow needles	Yellow needles
Melting point	220~221°C	195~198°C	>250°C
Elemental analysis	C 40.92, H 1.53, N 4.24, Cl 43.26	C 36.41, H 1.10, N 3.78, Cl 49.68	C 39.10, H 1.61, N 8.94, Cl 34.88
Molecular weight (MS, *m/z*)	323, 325, 327, 329	357, 359, 361, 363, 365	292, 294, 296
Molecular formula	$C_{11}H_5NO_2Cl_4$	$C_{11}H_4NO_2Cl_5$	$C_{10}H_5N_2O_3Cl_3$
UV λ_{max}^{MeOH}; nm ($E_{1cm}^{1\%}$)	220(sh, 650), 260(sh, 250), 327 (455), 340 (sh, 450)	220 (sh, 740), 336 (380), 370 (400)	221 (1340), 245 (sh, 530) 330 (190), 340 (210)
^{1}H NMR (δ, acetone-d_6)	7.09 (s), 7.69 (d), 7.76 (d), 11.12 (s), 11.75 (s, br)	7.61 (d), 7.68 (d)	6.80 (s), 7.42 (d), 7.54 (d), 10.26 (s, br)
^{13}C NMR (δ, acetone-d_6)	111.8 (s), 119.6 (d), 123.1 (s), 124.0 (s), 124.2 (s), 125.1 (s), 128.8 (s), 129.2 (d), 133.8 (d), 154.5 (s), 183.2 (s)	118.5 (s), 121.1 (s), 123.6 (s), 124.8 (s), 125.9 (s), 126.1 (s), 129.9 (d), 130.1 (s), 134.0 (d), 153.7 (s), 183.0 (s)	

Fig. 1. Time course of pyrrolomycin fermentation.

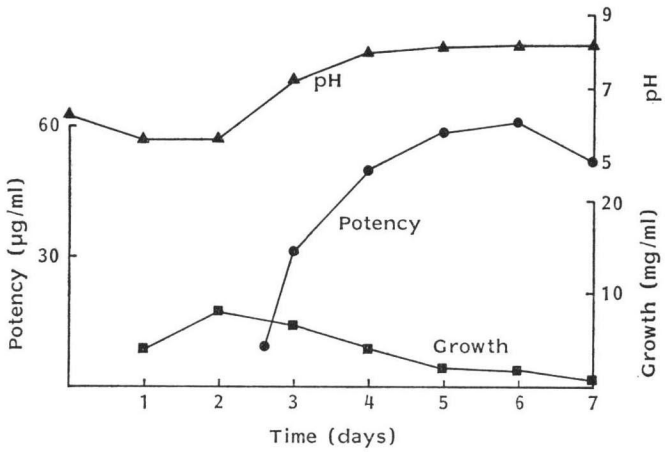
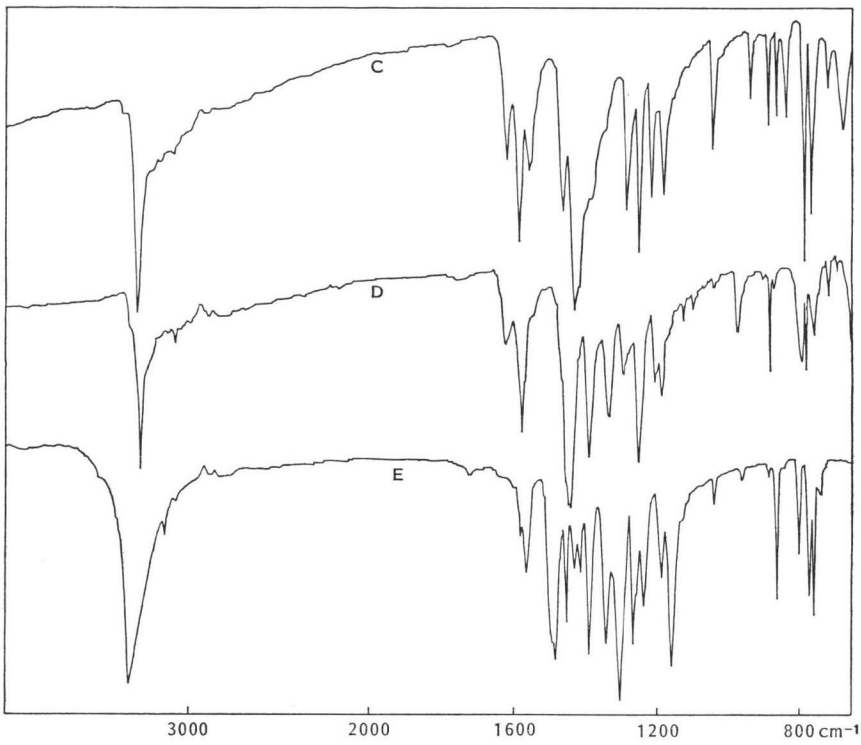


Fig. 2. IR spectra of pyrrolomycins C, D and E.



Gram-negative bacteria. Pyrrolomycin D was strongly active against Gram-positive bacteria and moderately active against Gram-negative bacteria and some fungi. Pyrrolomycins C and D are similar to pyoluteorin[8], produced by Pseudomonas, in their structure, but pyrrolomycin D is more active against Gram-positive bacteria and some of fungi than pyoluteorin. This indicated that introduction of a chlorine atom into pyrrole moiety considerably enhanced the bioactivity.

Table 2. Biological activities of pyrrolomycins C, D and E.

Test organisms	MIC (μg/ml)		
	C	D	E
Staphylococcus aureus 209P JC-1	0.20	≦0.025	1.56
S. epidermidis ATCC 14990	≦0.025	≦0.025	
Streptococcus faecalis ATCC 8043	0.39	≦0.025	1.56
Bacillus anthracis No. 119	0.10	≦0.025	≦0.05
Escherichia coli NIHJ JC-2	>100	6.25	>100
Citrobacter freundii GN-346	>100	6.25	>100
Salmonella typhi O-901-W	>100	6.25	>100
Shigella sonnei EW-33 Type I	>100	12.5	>100
Klebsiella pneumoniae PCI-602	>100	6.25	>100
Proteus vulgaris OX-19	>100	1.56	6.25
P. morganii Kono	>100	6.25	
P. mirabilis J-0013	>100	1.56	
Serratia marcescens MB-3848	>100	12.5	>100
Pseudomonas aeruginosa MB-3829	>100	25	>100
Candida albicans C-A-24	>100	100	>100
Cryptococcus neoformans Cr-1	>100	6.25	>100
Trichophyton mentagrophytes 530324	>100	6.25	100
T. interdigitale	>100	12.5	100
Aspergillus fumigatus Saito	>100	100	>100

Fig. 3. UV spectra of pyrrolomycins C, D and E.

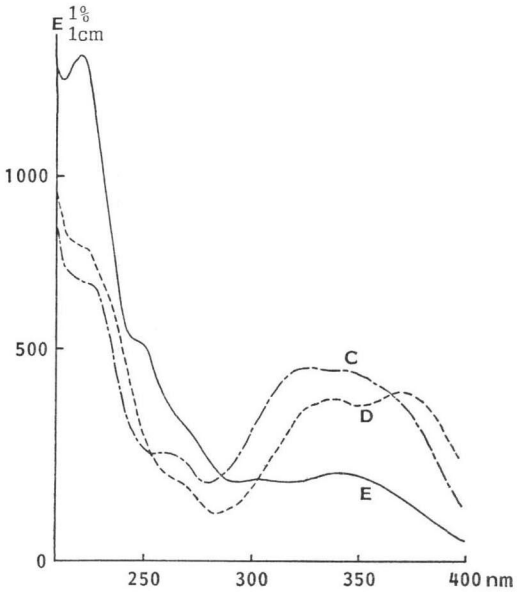
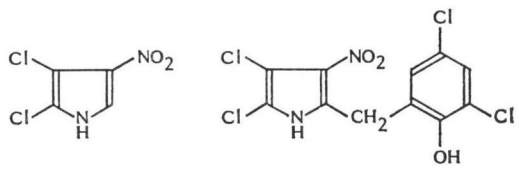


Chart 1.



Pyrrolomycin A (I) Pyrrolomycin B (II)

The acute toxicity of pyrrolomycins C and D by intraperitoneal dosing to JCL-ICR mice were 50 mg/kg and 20 mg/kg, respectively.

Pyrrolomycin E was weaker than C in anti-Gram-positive activity, but active against some Gram-negative bacteria.

Chart 2. Isolation of pyrrolomycins.

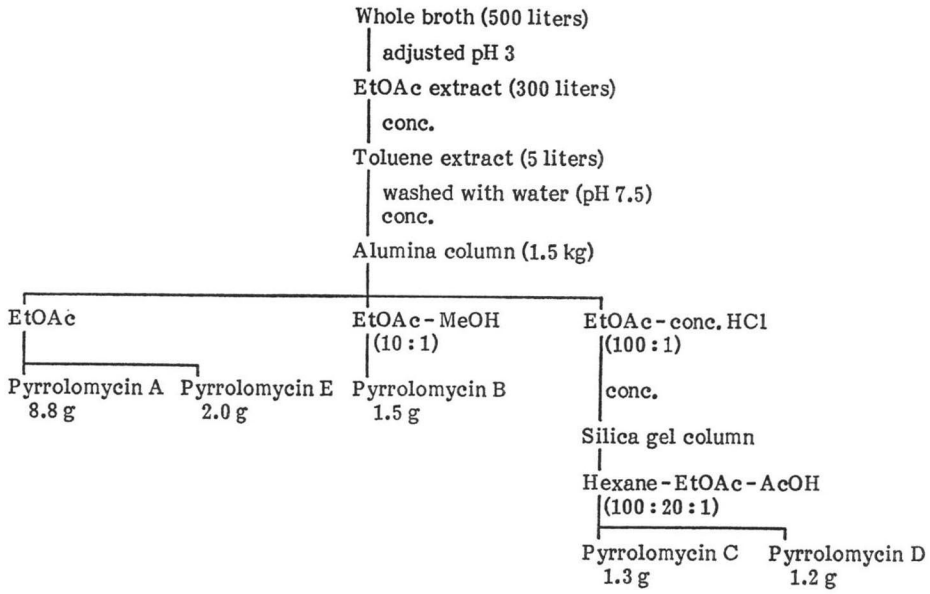
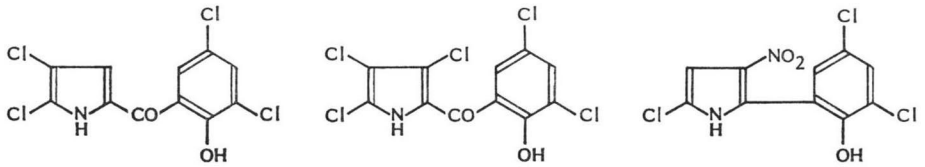


Chart 3.



Pyrrolomycin C (III) Pyrrolomycin D (IV) Pyrrolomycin E (V)

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