STRUCTURE AND SYNTHESIS OF PYRROLOMYCIN A, A CHLORINATED NITRO-PYRROLE ANTIBIOTIC

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Pyrrolomycin A, a new antifungal antibiotic produced by an actinomycete, has a molecular formula $C_4H_2N_2O_2Cl_2$. Its structure was determined to be 2,3-dichloro-4-nitropyrrole by spectroscopic analysis and synthetic studies. Structure-activity relationships among the analogs of pyrrolomycin A are discussed.

In the course of our screening for new antimicrobial agents, two antibiotics named pyrrolomycins A and B (SF-2080 A and B) were discovered in the fermentation broth of an unidentified actinomycete strain. The isolation and characterization of the two agents and the structure of pyrrolomycin B have been reported in the preceding papers[1,2]. This paper describes the structure of pyrrolomycin A which was elucidated by spectroscopic and synthetic studies.

Spectroscopic Analysis

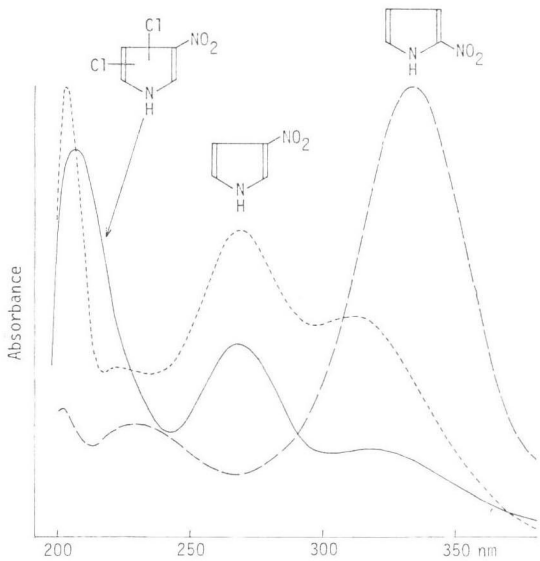
Pyrrolomycin A isolated as fine yellowish crystals showed mp 211~213°C. It is insoluble in water, but becomes soluble on the addition of sodium carbonate, forming a deep yellow solution. Elemental and high-resolution mass analyses established the molecular formula as $C_4H_2N_2O_2Cl_2$. The presence of a nitro group conjugated with an unsaturated bond was shown by fragment ions at m/z 164 (M^+-O), 150 (M^+-NO) and 134 (M^+-NO_2), IR absorption bands at 1505 and 1370 cm^{-1}, and UV absorption maxima at 268 (ε 7,400) and 323 nm (ε 11,400) in neutral and alkaline methanol, respectively.

The PMR spectrum of pyrrolomycin A in acetone-d_6 showed two singlets at 7.94 and 11.30 ppm (broad). The latter disappeared on addition of D_2O, suggesting NH or OH. When treated with excess of diazomethane in ethyl acetate, pyrrolomycin A was converted quantitatively into a crystalline derivative with formula $C_5H_4N_2O_2Cl_2$. This compound was insoluble in alkaline solution, and showed no PMR signal at low field except for a sharp singlet at 8.03 ppm. Instead a new *N*-methyl signal appeared at 3.83 ppm. These data indicated that pyrrolomycin A had an acidic NH group, which was *N*-methylated by diazomethane. Consideration of the highly unsaturated formula $C_4H_2N_2O_2Cl_2$, together with the presence of NO_2 and NH groups reasonably led to a dichloropyrrole structure. BIRCH[3] and TAKEDA[4] reported that the NH group of a 2-acylated pyrrole did not react with diazomethane but when two chlorine atoms were substituted on the pyrrole ring, the NH group reacted to afford the *N*-methylated derivative, in agreement with the reactivity of pyrrolomycin A.

The location of the nitro group was easily determined by UV spectroscopy. It has been reported that 2-nitropyrrole showed a maximum at 335 nm (ε 16,900) in methanol, while 3-nitropyrrole absorbed at 268 nm (ε 7,240)[5,6]. This difference was large enough to neglect the electronic effect of chlorine substituents. The UV spectrum of pyrrolomycin A, as shown in Fig. 1, closely resembled that of 3-nitropyrrole, suggesting β-nitro substitution.

In order to determine the substitution position of the chlorine atoms, an attempt was made to correlate the coupling constant of the ring protons with their position. Though the methine signal of pyrrolomycin A appeared as a singlet in acetone-d_6, it was split into a doublet with J_{NH-CH}=3.8 Hz, by the addition of trifluoroacetic acid. This seemed to be favorable for α-coupling[5,7], but no definite conclusion could be made, since J_{1-4} and J_{1-5} coupling constants of 3-nitropyrrole have similar J values (2.6 Hz). Furthermore, even if the α-coupling were correct, there remained two possibilities for the structure of pyrrolomycin A, *i.e.* 2,3-dichloro-4-nitropyrrole and 2,4-dichloro-3-nitropyrrole. Evidently, another approach was necessary to resolve this problem, and we chose synthesis.

Fig. 1. UV spectra of pyrrolomycin A and model compounds in MeOH.



Synthetic Study

There were two routes to prepare dichloro-3-nitropyrrole, *i.e.* nitration of dichloropyrrole and chlorination of 3-nitropyrrole. The former reaction suffered from the disadvantages of the instability of dichloropyrrole and the difficulty of selective α-oriented nitration[5,8,9]. On the other hand, 3-nitropyrrole and its derivatives were stable in acidic media, and suitable for chlorination.

At first, methyl 4-nitropyrrole-2-carboxylate (**I**) was selected as starting material for the preparation of 2,4-dichloro-3-nitropyrrole (**Va**). Chlorination of **I** with sulfuryl chloride at 80°C in acetic acid afforded a mixture of **II** and **III**, which were separated by fractional crystallization. The structure of **II** was determined to be methyl 5-chloro-4-nitropyrrole-2-carboxylate by PMR spectrometry, because the

Fig. 2. 100 MHz ^{1}H NMR spectrum of pyrrolomycin A in CF_3COOH - acetone-d_6.

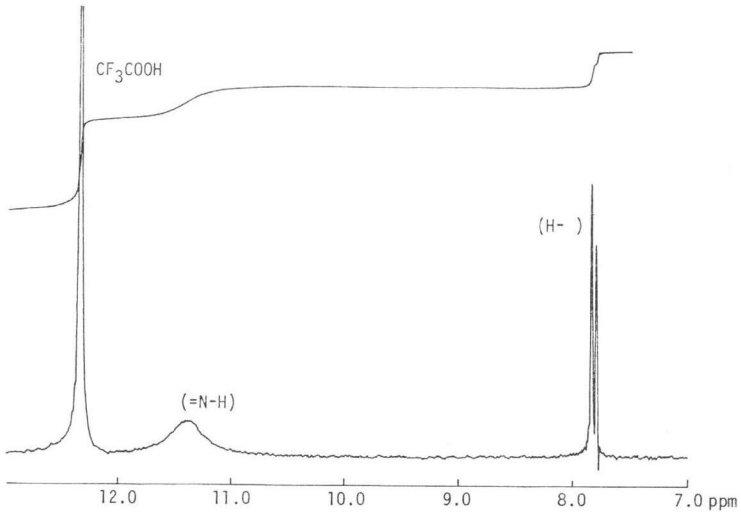


Chart 1.

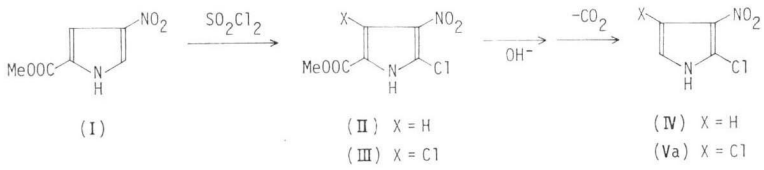
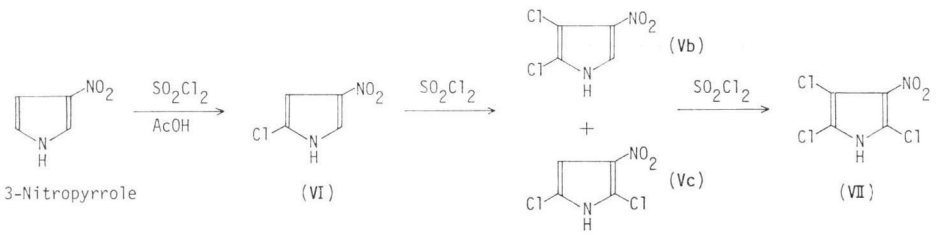


Chart 2.



signal in the starting material (**I**) at lower field disappeared in **II**. Compound **III** afforded 2,4-dichloro-3-nitropyrrole (**Va**), after alkaline hydrolysis in aqueous methanol followed by thermal decarboxylation. Compound **Va** was found to be a positional isomer of natural pyrrolomycin A, by comparing the physico-chemical properties. The monochloro derivative (**IV**) was obtained from **II** in the same manner as for **Va**.

In contrast to **I**, 3-nitropyrrole easily reacted with sulfuryl chloride at lower temperature. When equimolar sulfuryl chloride was added to the acetic acid solution of 3-nitropyrrole at 15°C, the monochloro derivative (**VI**) was the only product obtained. The structure of **VI** was presumed from the small J_{CH-CH} value (1.9 Hz) in acetone-d_6 - D_2O. With three moles of reagent and at elevated temperature, the trichloro derivative (**VII**) was obtained in good yield. However, with two moles of reagent, there were produced two compounds (**Vb** and **Vc**), accompanied by small amounts of **VI** and **VII**. These compounds (**Vb** and **Vc**) were obtained also from **VI** by treatment with one mole of reagent. The two isomers **Vb** and **Vc** were separated by preparative TLC on silica-gel developed with benzene - ethyl acetate (4: 1), and each was purified by recrystallization. Both **Vb** and **Vc** were different from **Va** previously synthesized, but one of them (**Vb**) was found to be identical with natural pyrrolomycin A by comparing physico-chemical properties.

Fig. 3. Mass spectra of three dichloro-β-nitropyrroles (EI).

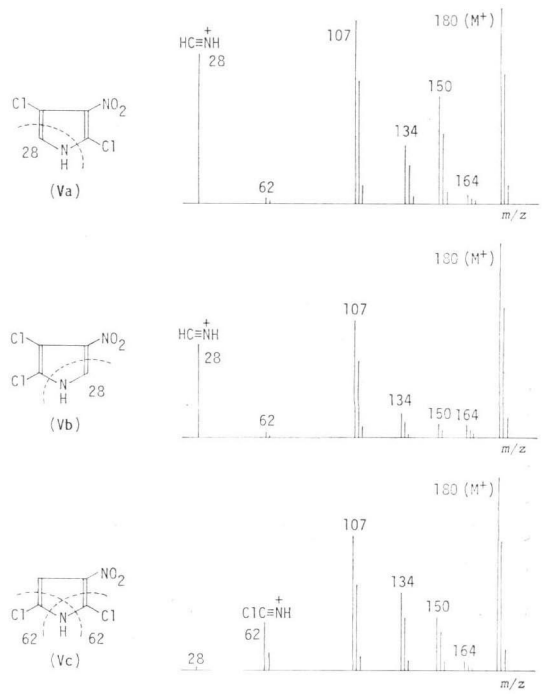
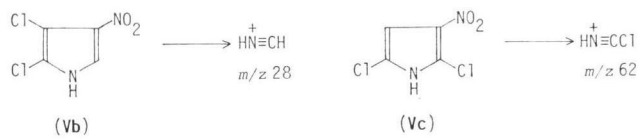


Chart 3.



(Vb) (Vc)

Table 1. PMR spectra of chlorinated 3-nitropyrroles.

Compounds	ppm (acetone-d_6)			J (Hz)*					
	2-H	4-H	5-H	1–2	1–4	1–5	2–4	4–5	2–5
3-Nitropyrrole	7.79	6.91	6.71	3.5	2.6	2.6	1.6	3.2	2.0
I	7.96	7.28	COOMe	3.6	2.8	—	1.7	—	—
II	Cl	7.35	COOMe	—	3.0	—	—	—	—
IV	Cl	6.79	6.92	—	2.8	3.0	—	3.6	—
Va	Cl	Cl	7.10	—	—	3.1	—	—	—
Vb	7.94	Cl	Cl	3.8	—	—	—	—	—
Vc	Cl	6.78	Cl	—	2.9	—	—	—	—
VI	7.80	6.69	Cl	3.4	2.6	—	1.9	—	—

* J_{NH-CH} of **I**, **II**, **Va**, **Vb** and **Vc** were obtained in CF_3COOH - acetone-d_6

The structures of compound **Vb** and **Vc** were determined by mass spectrometry as shown in Fig. 3. When the relative intensity of the corresponding ions were compared between **Vb** and **Vc**, a fragment ion at m/z 28, attributable to $\overset{+}{H}N\equiv CH$, was more pronounced in compound **Vb**, whereas a fragment ion at m/z 62 ($\overset{+}{H}N\equiv CCl$) was more abundant in **Vc**. The difference could be ascribed to the position of chlorine atoms. Predominant formation of an ion at m/z 62, but not at m/z 28, in compound **Vc**, indicated that both α-positions were chlorinated. On the other hand, at least one hydrogen atom was suggested in α-position in compound **Vb**, based on the high abundance of an ion at m/z 28. Similarly the mass spectrum of the authentic 2,4-dichloro-3-nitropyrrole (**Va**) gave high abundance of an ion at m/z 28. Consequently, the structure of **Vb** was assigned as 2,3-dichloro-4-nitropyrrole and **Vc** was 2,5-dichloro-3-nitropyrrole.

PMR spectra of three positional isomers **Va**, **Vb** and **Vc** agreed with their respective structures. α-Protons in pyrrole derivatives resonate at lower field than β-protons, and substitution of an electronegative group causes paramagnetic shifts of the neighbouring protons[7]. In accordance with this rule, the signal of the ring proton in compound **Vb** was observed at lower field (7.94 ppm) than those of the other compounds **Va** and **Vc** (7.10 and 6.78 ppm), as shown in Table 1. Moreover, the spin-spin coupling between ring protons, which was observed in trifluoroacetic acid - acetone-d_6, gave useful structural information. Coupling constants of ring protons are summarized in Table 1, which reveal that α-coupling was larger than 3.0 with one exception and that β-coupling was smaller than 3.0, regardless of chlorine substitution. The large coupling constant (J=3.8 Hz) in **Vb** indicated α-coupling, consistent with the structure. From the above data, pyrrolomycin A was identified as 2,3-dichloro-4-nitropyrrole (**Vb**). It was found that CMR of pyrrolomycin A obtained by routine scanning gave a single peak at 120.6 ppm. After the structure was elucidated, the CMR was re-examined and finally gave 4 peaks, the strongest one at 120.6 ppm (C-5), two medium intensity at 115·9 (C-2) and 105.0 ppm (C-3), and the

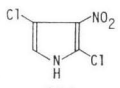
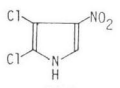
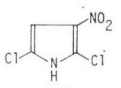
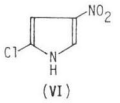
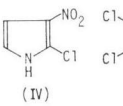
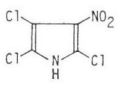
weakest broad one at 133.4 ppm (C-4). The difficulty of obtaining the last 3 peaks was apparently due to the lack of proton substituted on carbon.

The structure of **Vb** was further confirmed by X-ray crystallographic analysis. Compound **Vb** crystallized as a monoclinic system, with space group P $2_{1/n}$, and the following lattice constants, a= 7.356 Å, b=13.664 Å, c=7.290 Å, cos β=−0.4638 and z=4. The details of analysis will be reported separately.

Biological Activities of Chlorinated 3-Nitropyrroles

Except for carboxylic acid derivatives **II** and **III**, all the chlorinated 3-nitropyrroles synthesized showed antimicrobial activity, and MIC's against various microorganisms are listed in Table 2. It was

Table 2. Biological activities of halogenated nitropyrroles.

Organism	 (Va)	 (Vb)	 (Vc)	 (VI)	 (IV)	 (VII)
Staphylococcus aureus 209P JC-1	>100	1.56	12.5	25	6.25	100
Staphylococcus aureus Smith (1)	>100	6.25	25	50	25	100
Staphylococcus aureus No. 26	>100	6.25	12.5	25	6.25	100
Staphylococcus epidermidis ATCC 14990	>100	6.25	25	50	25	100
Staphylococcus epidermidis 109	>100	6.25	25	50	12.5	100
Streptococcus faecalis ATCC 8043	>100	6.25	50	50	25	100
Bacillus anthracis No. 109	>100	1.56	25	50	6.25	50
Escherichia coli NIHJ JC-2	>100	6.25	50	50	12.5	50
Escherichia coli No. 29	>100	6.25	25	50	12.5	50
Escherichia coli W3630 RGN823	>100	6.25	25	50	6.25	50
Escherichia coli JR66/W677	>100	6.25	25	50	6.25	50
Citrobacter freundii GN-346	>100	6.25	25	50	12.5	50
Salmonella typhi O-901-W	>100	3.13	12.5	25	6.25	50
Salmonella enteritidis No. 11	>100	6.25	25	50	6.25	50
Salmonella typhimurium LT-2	>100	6.25	25	50	12.5	50
Salmonella sp. D-0001	>100	6.25	25	100	12.5	50
Shigella sonnei EW-33 Type I	>100	6.25	25	50	6.25	50
Klebsiella pneumoniae PCI-602	>100	12.5	25	50	12.5	50
Klebsiella pneumoniae 22#3038	>100	6.25	50	100	12.5	50
Proteus vulgaris OX-19	>100	6.25	25	50	6.25	50
Proteus rettgeri J-0026	>100	6.25	25	50	6.25	50
Proteus morganii Kono	>100	6.25	25	50	6.25	50
Proteus mirabilis J-0013	>100	6.25	50	100	12.5	50
Serratia marcescens MB-3848	>100	6.25	50	100	12.5	50
Pseudomonas aeruginosa MB-3829	—	12.5	50	100	—	—
Candida albicans C-A-24	>100	100	100	100	>100	>100
Cryptococcus neoformans Cr-1	50	25	25	100	>100	>100
Trichophyton mentagrophytes 530324	12.5	6.25	12.5	25	100	50
Trichophyton interdigitale	25	3.13	3.13	25	100	50
Aspergillus fumigatus Saito	50	25	50	100	>100	100

found that synthetic **Vb** was indistinguishable from natural pyrrolomycin A in biological activity. Also noteworthy was that dichloro compounds, in particular compound **Vb**, showed relatively high activity as compared to mono- and trichloro derivatives. *N*-Methylation of these compounds resulted in considerable reduction of bioactivity, suggesting an essential role of the NH group for activity.

Experimental

Melting points were determined on a Yamato MP-21 apparatus in glass capillary tubes. PMR spectra were recorded in *ca* 3% solution containing TMS as internal reference, on a Varian XL-100 system, CMR spectra on a JEOL FX-200 spectrometer, IR spectra in KBr disks on a Hitachi 269-10 infrared spectrophotometer, UV spectra in methanol on a Hitachi 200-20 spectrophotometer and mass spectra on a Hitachi M-80 mass spectrometer. TLC was carried out by using silica gel plates F_{254} (E. Merck) and a mixed solvent of benzene - ethyl acetate (5: 1).

1-Methyl-2,3-dichloro-4-nitropyrrole (*N*-methyl-pyrrolomycin A)

Pyrrolomycin A (181 mg) was treated with an excess of diazomethane in ethyl acetate. After 1 hour excess reagent was decomposed by the addition of 5 N hydrochloric acid, and reaction mixture was concentrated to dryness. The residue was crystallized from ethanol, and 1-methyl-2,3-dichloro-4-nitropyrrole was obtained as colorless crystals, mp 122~123°C. *Anal.* Calcd. for $C_5H_4N_2O_2Cl_2$: C, 30.80; H, 2.07; N, 14.36; Cl, 36.36%. Found: C, 30.75; H, 2.04; N, 14.49; Cl, 36.52%. PMR $\delta_{TMS}^{acetone\text{-}d_6}$ ppm: 8.03 (H-2), 3.82 (N-Me).

Methyl 3,5-Dichloro-4-nitropyrrole-2-carboxylate (**III**) and Methyl 5-Chloro-4-nitropyrrole-2-carboxylate (**II**)

Methyl 4-nitropyrrole-2-carboxylate (**I**, 3.40 g)[5] was suspended in 100 ml of acetic acid, and to this suspension was added 2.43 ml of sulfuryl chloride. The mixture was stirred at room temperature for 18 hours and heated at 80°C for 1 hour. The reaction mixture was cooled, concentrated to dryness, and extracted with 100 ml of ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate, dried over sodium sulfate, and then concentrated to dryness. The residue was recrystallized twice from benzene, and 0.45 g (9%) of methyl 3,5-dichloro-4-nitropyrrole-2-carboxylate (**III**) was obtained as colorless crystals. This sample was contaminated with a small amount of the monochloro derivative (**II**), but further purification was not made.

From the mother liquor was obtained pure methyl 5-chloro-4-nitropyrrole-2-carboxylate (**II**) as colorless crystals. Yield 1.80 g (42%), mp 202~204°C, PMR $\delta_{TMS}^{acetone\text{-}d_6}$ ppm: 7.35 (H-3), 3.89 (O-Me).

2,4-Dichloro-3-nitropyrrole (**Va**)

Four hundred and fifty mg of methyl 3,5-dichloro-4-nitropyrrole-2-carboxylate (**III**) was dissolved in 20 ml of methanol, and 10 ml of 10% aqueous sodium hydroxide was added. The reaction mixture was kept at 75~80°C for 1 hour, then cooled, and methanol was removed by distillation under reduced pressure. Residual solution was acidified with 5 N hydrochloric acid, and extracted with 100 ml of ethyl acetate. The extract was washed with 5% sodium hydrogen carbonate, then with saturated aqueous sodium chloride, and dried over sodium sulfate. The ethyl acetate solution was concentrated to dryness, and 386 mg of the free acid was obtained as colorless powder. Three hundred mg of this sample were dissolved in 10 ml of ethylene glycol and heated at 190°C 1 hour for thermal decarboxylation. The reaction mixture was cooled and extracted with 50 ml of ethyl acetate, followed by washing with sodium hydrogen carbonate solution and water. The ethyl acetate layer was dried over sodium sulfate and concentrated to dryness. Crude product was purified by preparative TLC on silica gel developed with benzene - ethyl acetate (5: 1). Pure 2,4-dichloro-3-nitropyrrole (**Va**) was obtained as yellow needles. Yield 92 mg (27% from **III**), mp 184~186°C. *Anal.* Calcd. for $C_4H_2N_2O_2Cl_2$: C, 26.52; H, 1.10; N, 15.47; Cl, 39.32%. Found: C, 27.13; H, 1.14; N, 15.67; Cl, 39.32%. UV λ_{max}^{MeOH} 268 nm (ε 7,900).

2-Chloro-3-nitropyrrole (IV)

Alkaline hydrolysis of compound **II** (60 mg) afforded 502 mg of free acid, mp 246~248°C (dec.). Thermal decarboxylation of the free acid (410 mg), and working up as described above gave 270 mg of crude product. This was purified by a similar procedure as above to give 124 mg (37% from **II**) of pure 2-chloro-3-nitropyrrole (**IV**) as brown crystals, mp 173~175°C.

2-Chloro-4-nitropyrrole (VI)

One hundred and thirteen mg of 3-nitropyrrole was dissolved in 10 ml of acetic acid, and under cooling at 15°C were added in one portion 1.36 ml of 10% sulfuryl chloride in dichloromethane. The reaction mixture was maintained at 10~15°C for 4 hours, and then acetic acid was removed by evaporation. Recrystallization of the residual solid from benzene gave 2-chloro-4-nitropyrrole (**VI**) as colorless needles. Yield 79 mg (54%), mp 159~164°C. *Anal.* Calcd. for $C_4H_3N_2O_2Cl$: C, 32.78; H, 2.06; N, 19.12%. Found: C, 33.02; H, 2.01; N, 18.77%. UV λ_{max}^{MeOH} 259 nm (ε 6,500), 320 (4,700).

2,4,5-Trichloro-3-nitropyrrole (VII)

To a solution of 3-nitropyrrole (112 mg) in 10 ml of acetic acid, was added 405 mg of sulfuryl chloride. The mixture was stirred for 4 hours at room temperature and evaporated to dryness. Residual solid (230 mg) was extracted with 50 ml of ethyl acetate and the organic layer was washed with aqueous sodium hydrogen carbonate, and dried over sodium sulfate. Evaporation of solvent and recrystallization from benzene afforded 2,4,5-trichloro-3-nitropyrrole (**VII**) as brown prisms. Yield 123 mg (57%), mp 182°C (dec.). *Anal.* Calcd. for $C_4HN_2O_2Cl_3$: C, 22.30; H, 0.47; N, 13.00%. Found: C, 23.77; H, 0.66; N, 12.45%. UV λ_{max}^{MeOH} 263 nm (ε 5,100), 316 (4,400).

2,5-Dichloro-3-nitropyrrole (Vc) and 2,3-Dichloro-4-nitropyrrole (Vb)

To a solution of 3-nitropyrrole (112 mg, 1 mmole) in 10 ml of acetic acid was added 2.7 ml (2 mmole) of 10% sulfuryl chloride in dichloromethane. The mixture was stirred for 2.5 hours at room temperature, and was concentrated to dryness. The residue was dissolved in ethyl acetate (5 ml), and evaporated again to give a mixture of chlorinated products (173 mg) as a yellow solid. These were separated by preparative TLC on silica gel developed with benzene - ethyl acetate (4: 1). The following fractions were obtained; (a) 85 mg (47%) of 2,5-dichloro-3-nitropyrrole (**Vc**), which was recrystallized from benzene, mp 147~150°C, *Anal.* Calcd. for $C_4H_2N_2O_2Cl_2$: C, 26.52; H, 1.10; N, 15.47%. Found: C, 27.01; H, 1.09; N, 14.66%. UV λ_{max}^{MeOH} 255 nm (ε 6,100), 329 (4,700). (b) 46 mg of a mixture of compounds **VI** and **Vb**. (c) 24 mg (13%) of 2,3-dichloro-4-nitropyrrole (**Vb**), recrystallized from benzene - ethyl acetate (10: 1), mp 211~213°C. *Anal.* Calcd. for $C_4H_2N_2O_2Cl_2$: C, 26.52; H, 1.10; N, 15.47%. Found: C, 26.90; H, 1.16; N, 15.30%. Compound **Vb** was identical with natural pyrrolomycin A in physico-chemical properties. (d) 17 mg (8%) of compound **VII**.

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