PYRROXAMYCIN, A NEW ANTIBIOTIC TAXONOMY, FERMENTATION, ISOLATION, STRUCTURE DETERMINATION AND BIOLOGICAL PROPERTIES

KENICHI YANO, JUNJI OONO, KINICHI MOGI,
TAKEMITSU ASAOKA and TOSHIAKI NAKASHIMA

Central Research Laboratories, SS Pharmaceutical Co., Ltd.,
Narita, Chiba 286, Japan

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A strain of streptomycete was found to produce a new antibiotic pyrroxamycin. This compound was isolated from the culture broth of *Streptomyces* sp. S46506. The chemical structure was determined to be 4,5-dichloro-2-(6′,8′-dichloro-4′*H*-1′,3′-benzodioxin-4′-yl)-3-nitropyrrole by its chemical character and ^{1}H and ^{13}C NMR spectral analysis. Pyrroxamycin was active against Gram-positive bacteria and dermatophytes.

In the course of our screening program for new antibiotics, a streptomycete, strain S46506, designated as *Streptomyces* sp. S46506 was found to produce a new antibiotic named pyrroxamycin (**1**). This compound was previously called SS46506A substance[1]. The chemical structure of pyrroxamycin, a new antibiotic related to pyrrolomycins, was determined to be 4,5-dichloro-2-(6′,8′-dichloro-4′*H*-1′,3′-benzodioxin-4′-yl)-3-nitropyrrole by its chemical character and ^{1}H and ^{13}C NMR spectral analysis. Pyrroxamycin was active against Gram-positive bacteria and dermatophytes.

This paper deals with taxonomy of producing strain, fermentation, isolation, physico-chemical properties, chemical structure and biological properties of pyrroxamycin.

Taxonomy

Strain S46506 was isolated from a soil sample collected at Kamagaya City, Chiba Prefecture, Japan. The medium used for actinomycete isolation was tomato juice - oatmeal agar composed of oatmeal 20 g, tomato juice (centrifuged supernatant, 10,000 rpm, 10 minutes) 100 ml, distilled water 900 ml, Bacto agar (Difco) 20 g, nystatin 40 mg, cycloheximide 40 mg and novobiocin 10 mg.

The strain has been deposited at The Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, where it has been assigned accession number FERM P-8020.

Taxonomic studies were carried out according to the methods described by SHIRLING and GOTTLIEB[2] and of WAKSMAN[3]. The color notations were from the ISCC-NBS Centroid Color Charts†. The inoculation of strain S46506 onto various media was carried out using the washed cells cultured in yeast - starch broth at 28°C for 5 days on a reciprocal shaker.

Morphological observations were made of the cultures grown at 28°C for 7~21 days on thin potato - carrot agar and water agar[4]. Mature spore chains had 10 or more spores in the form of spirals (Fig. 1). Spirals were observed in spots on the above media. The spores were oval or cylindrical and $0.5\sim0.8\times0.8\sim1.2$ μm in size with a smooth surface under an electron microscope (Fig. 2). No sporangia or flagellated spores were observed.

† U.S. Department of Commerce, National Bureau of Standards: Standard Reference Material 2107. Color Kit, consists of: SRM 2106, ISCC-NBS Centroid Color Charts, and SP 400, Color: Universal Language and Dictionary of Names.

Fig. 1. Aerial mycelium of strain S46506.
Thin potato - carrot agar, 14 days at 28°C (×600).

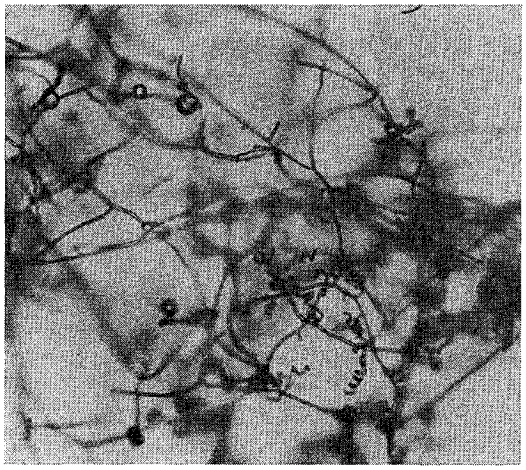


Fig. 2. Spores of strain S46506.
Thin potato - carrot agar, 14 days at 28°C.

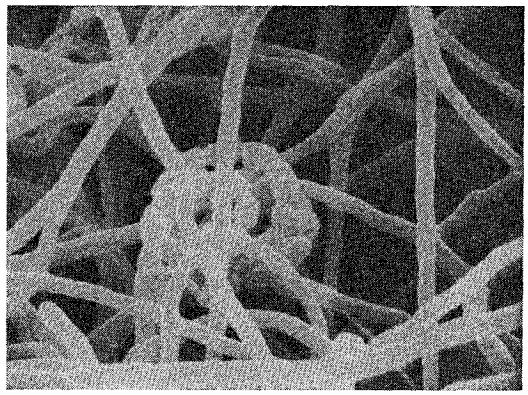


Table 1. Cultural characteristics of strain S46506.

Medium	Growth	Aerial mycelium	Reverse color	Soluble pigment
Sucrose - nitrate agar	Very poor	None	Colorless	Faint yellow
Glucose - asparagine agar	Poor	None	Pale yellow	Faint yellow
Glycerol - asparagine agar	Moderate	None	Pale to moderate yellow	Faint yellow
Inorganic salts - starch agar	Poor	None	Pale yellow	Faint yellow
Tyrosine agar	Poor	None	Light to moderate yellowish brown	Faint yellowish brown
Nutrient agar	Very poor	None	Pale yellow	Faint yellow
Yeast extract - malt extract agar	Moderate	None	Moderate yellow	Faint yellow
Oatmeal agar	Moderate	None	Pale yellow	Faint yellow
Glycerol - nitrate agar	Moderate	None	Pale yellow	Faint yellow
Calcium malate agar	Poor	None	Colorless	Faint yellow
Thin potato - carrot agar	Poor	Scant, white	Colorless	Faint yellow
Water agar	Very poor	Very scant, white	Colorless	Faint yellow

Cultural characteristics of strain S46506 grown on various media at 28°C for 14 days are shown in Table 1. Aerial mycelium was very thin on thin potato - carrot agar and water agar. No development of aerial mycelium on most media is the characteristic of this strain. The aerial mass color was in the white color series. The color of substrate mycelia was colorless to pale yellow or yellowish brown. Faint yellow or yellowish brown pigment was found in the all media used. This pigment was not pH sensitive when tested with 0.05 N NaOH or HCl.

Physiological characteristics of strain S46506 are presented in Table 2. Strain S46506 grew at 16~41°C with optimum temperature of 25~35°C on yeast - starch agar. Melanoid pigment was not formed in peptone - yeast extract - iron agar, tyrosine agar or Tryptone - yeast extract broth.

Utilization of carbon sources by strain S46506 were observed on inorganic salts - starch agar using various carbohydrates to replace starch, because strain S46506 grew scarcely on ISP medium 9. The pattern of carbon utilization by strain S46506 is shown in Table 3. D-Glucose, L-arabinose, D-mannitol, D-fructose, raffinose and D-galactose were utilized, but the utilization of the other carbon sources

Table 2. Physiological characteristics of strain S46506.

Properties observed	Characteristics
Melanin formation	Negative
Starch hydrolysis	Positive
Gelatin liquefaction	Positive
Milk coagulation	Negative
Milk peptonization	Negative
Nitrate reduction	Positive

Table 3. Carbon utilization of strain S46506.

D-Glucose	+
L-Arabinose	+
Sucrose	±
D-Xylose	±
i-Inositol	−
D-Mannitol	+
D-Fructose	+
L-Rhamnose	±
Raffinose	+
D-Galactose	+
Salicin	±
Lactose	±
D-Mannose	±

+; Utilized, ±; weakly utilized, −; not utilized.

was doubtful if at all.

The whole-cell analysis of strain S46506 according to the method of STANECK and ROBERTS[5] revealed the presence of the L-isomer of diaminopimelic acid. The result of the glycolate test[6] of whole cells suggested the presence of an acetyl group in the muramic acid of its peptidoglycans. Strain S46506 was shown to have a type PII phospholipid according to the method of LECHEVALIER and LECHEVALIER[7].

The above characteristics indicated that strain S46506 belongs to the genus *Streptomyces*. Therefore, a comparison of the strain was made with the published description[8~12] of various *Streptomyces* species. However, strain S46506 was not identical with any of the previously published species of the genus *Streptomyces* although it was considered to be closely related to *Actinosporangium vitaminophilum* Shomura *et al.*[13]. Strain S46506 also coproduced pyrrolomycin C (**4**)[14] as a minor component. However, strain S46506 differed from *A. vitaminophilum* Shomura *et al.* in the morphology of sporulating aerial mycelium, soluble pigment production and carbon utilization. Therefore, strain S46506 was designated as *Streptomyces* sp. S46506.

Fermentation

The stock culture was grown on yeast - starch agar at 28°C for 14 days. A loopful of cells from the stock culture was inoculated into 500-ml flasks each containing 50 ml of a medium composed of glucose 1.0%, soluble starch 3.0%, Polypepton S (Daigo Nutritive Chemicals Ltd.) 0.5%, soybean meal 1.0%, yeast extract 0.5%, corn steep liquor 0.5%, $CaCO_3$ 0.2%. The pH of the medium was adjusted to pH 7.4 before sterilization.

The flasks were cultured at 27°C for 96 hours on reciprocal shaker. About 1 ml of the seed culture was transferred to 500-ml flasks containing 100 ml of the above medium and then cultured at 27°C for 48 hours.

A 30-liter jar fermentor was charged with 16 liters of the above medium and inoculated with 240 ml of the second seed. The fermentation was carried out at 30°C for 110 hours with aeration of 16 liters/minute and agitation of 400 rpm. The production of the active compound during fermentation was monitored by HPLC (Table 4). A typical time course of fermentation in a 30-liter jar fermentor is shown in Fig. 3. The amount of pyrroxamycin reached maximum after 96 hours fermentation.

Isolation and Purification

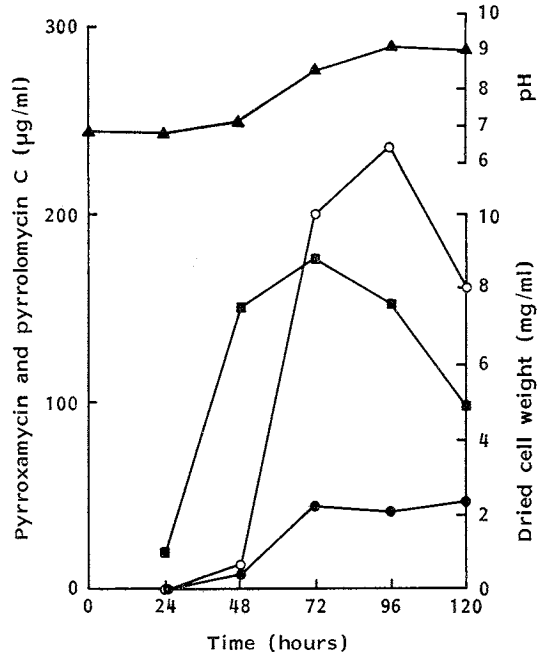
The culture broth was centrifuged to separate the mycelium from the broth. The supernatant was extracted with an equal volume of ethyl acetate. The mycelium was extracted twice with 3 liters of

acetone. The acetone extract was concentrated under reduced pressure and then the residual solution was extracted twice with ethyl acetate. After combining both of the ethyl acetate extracts, they were evaporated to dryness under reduced pressure.

The crude product was applied to a column (4×35 cm) with Silica gel 60 (Merck) and eluted with *n*-hexane - ethyl ether - acetone (9 : 3 : 0.5). The fraction containing the crude pyrroxamycin was evaporated to dryness under reduced pressure. The residue was applied to a silica gel column (4×35 cm) eluting with *n*-hexane - ethyl acetate - acetic acid (1,000 : 120 : 2) to give 2.0 g of pyrroxamycin from benzene as yellow needles.

Fig. 3. Time course of pyrroxamycin production by *Streptomyces* sp. S46506.

▲ pH, ○ pyrroxamycin, ■ dried cell weight, ● pyrrolomycin C.



Physico-chemical Properties

The physico-chemical properties of the minor component coproduced by *Streptomyces* sp. S46506 (IR, UV, ^{1}H and ^{13}C NMR spectra) were identical with those of literature data for pyrrolomycin C (**4**)[14].

The physico-chemical properties of **1** are summarized in Table 5. It was weakly acidic compound and obtained as pale yellow crystals. It was soluble in $CHCl_3$, acetone, MeOH and diethyl ether but insoluble in *n*-

Table 4. Chromatographic behavior of pyrroxamycin on HPLC.

Solvent system	Retention time (minutes)	
	Pyrroxamycin	Pyrrolomycin C
CH_3CN - H_2O - AcOH (80 : 20 : 0.5)	6.0	8.2

Column; Nucleosile 5C-18 4.5×250 mm. Detection; UV absorption at 320 nm. Flow rate; 1 ml/minute.

Table 5. Physico-chemical properties of **1**.

	1
Appearance	Light yellow needles
Molecular formula	$C_{12}H_6N_2O_4Cl_4$
$[\alpha]_D^{25}$ (*c* 1, acetone)	−37.2°
MW	384.002
MS (*m/z*)	382, 384, 386 (*ca.* 3 : 4 : 2)
UV λ_{max}^{MeOH} nm (ε)	220 (sh), 276 (6,800), 285 (6,950), 294 (6,500), 322 (5,300)
Rf value*	0.51
IR (KBr) cm^{-1}	3280, 1505, 1365
Anal Found:	C 36.95, H 1.43, N 7.11, Cl 37.25.
Calcd:	C 37.53, H 1.57, N 7.30, Cl 36.93.
MP (°C, dec)	216~220

* Silica gel TLC plate: Merck Art. 5715, solvent: $CHCl_3$ - acetone (20 : 1).

hexane and water. It was visualized by iodine vapor on silica gel TLC plate and showed positive reaction with Beilstein test.

In the mass spectrum of **1**, the molecular ions were observed at *m/z* 382, 384 and 386 (*ca.* 3 : 4 : 2). High resolution mass measurement for **1** showed molecular ions at *m/z* 381.9095, 383.9049 and 385.8982 (calcd for $C_{12}H_6N_2O_4{}^{35}Cl_4$, $C_{12}H_6N_2O_4{}^{35}Cl_3{}^{37}Cl$ and $C_{12}H_6N_2O_4{}^{35}Cl_2{}^{37}Cl_2$: 381.9082, 383.9052 and 385.9021, respectively), so

Fig. 4. UV spectrum of **1** (MeOH).

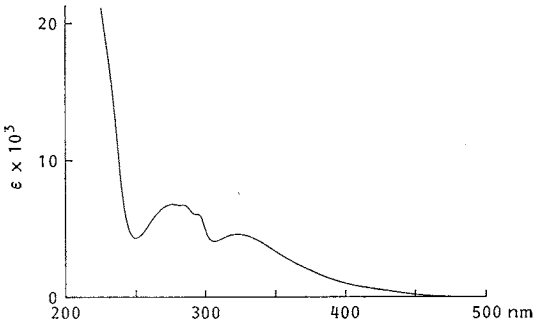


Fig. 5. IR spectrum of **1** (KBr).

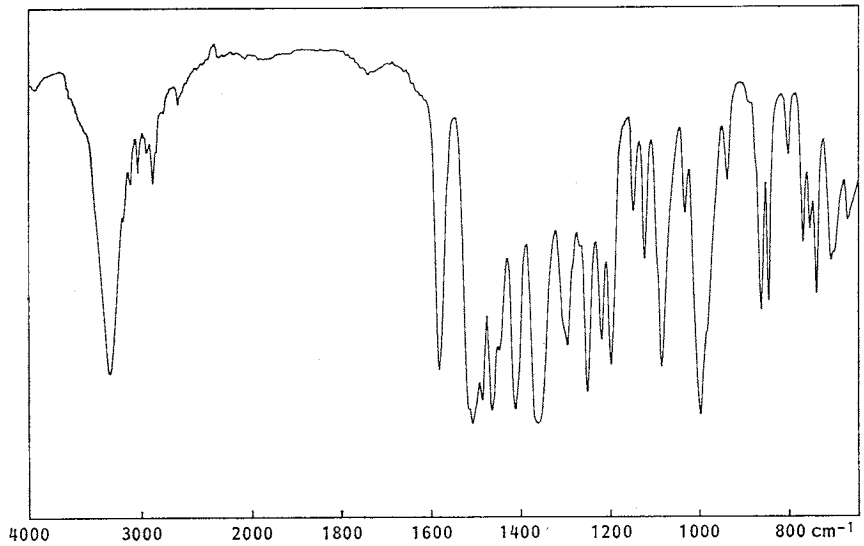
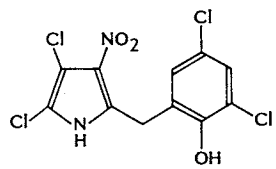
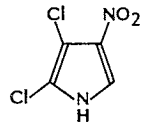


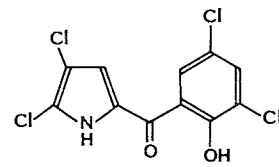
Fig. 6. Structures of the pyrrolomycins.



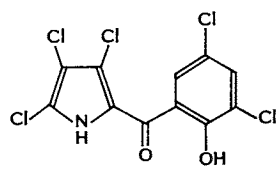
Pyrrolomycin B (**2**)



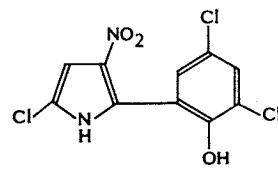
Pyrrolomycin A (**3**)



Pyrrolomycin C (**4**)

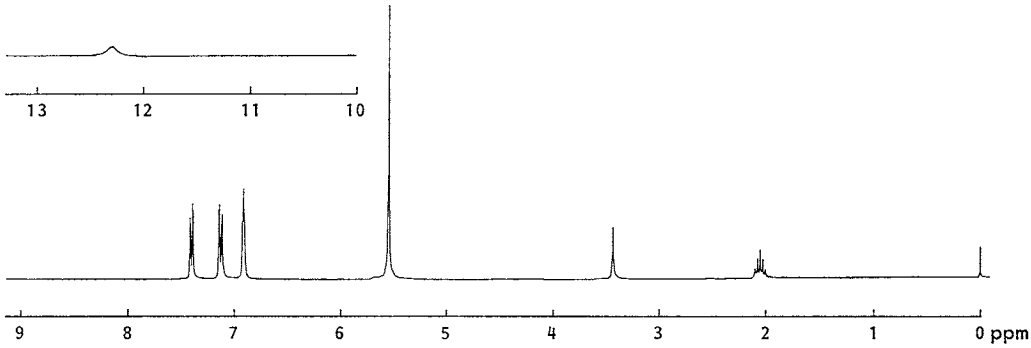


Pyrrolomycin D (**5**)

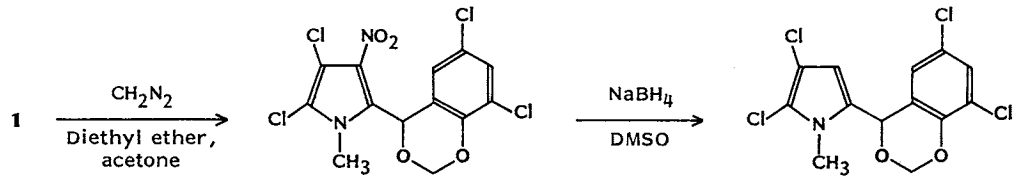


Pyrrolomycin E (**6**)

Fig. 7. ^{1}H NMR spectrum of **1** (90 MHz, acetone-d_6).



Scheme 1. Syntheses of *N*-methyl derivative (**7**) and denitro derivative (**8**).



molecular formula of **1** was determined to be $C_{12}H_6N_2O_4Cl_4$. The major mass fragment ions of **1** were observed at *m/z* 306, 308 and 310 (M$-$$CH_2O-NO_2$, *ca.* 3 : 4 : 2). Based on the elemental analysis, the molecular formula of **1** was also determined to be $C_{12}H_6N_2O_4Cl_4$.

The UV and IR spectra of **1** are shown in Figs. 4 and 5, respectively. These spectra were similar to those of the known antibiotic, pyrrolomycin B (**2**)[15,16].

Table 6. ^{13}C NMR spectra of **1***, **7*** and **3**[18].

No.	1	7	3
2	130.7 s	128.7 s	120.6 d
3	131.7 s	133.4 s	133.4 s
4	106.2 s	105.8 s	105.0 s
5	116.6 s	120.0 s	115.9 s
2′	91.5 t	93.1 t	
4′	69.4 d	69.6 d	
4′a	124.7 s	124.9 s	
5′	126.3 d	125.3 d	
6′	126.7 s	127.2 s	
7′	130.1 d	130.2 d	
8′	123.2 s	123.2 s	
8′a	148.9 s	148.9 s	
NCH_3		34.2 q	

* Measured in acetone-d_6 at 22.5 MHz.

Structure Determination

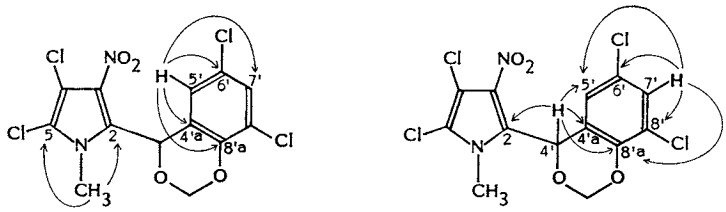
The spectral similarity to **2**, together with the highly unsaturated nature with four chlorine atoms and a nitro group in the molecule, suggested that **1** was a new member of chlorinated nitropyrrole antibiotics such as **2**[15,16], pyrrolomycins A (**3**)[15,17], C (**4**)[14,18], D (**5**)[14,18] and E (**6**)[14,18] (Fig. 6). The IR spectrum for **1** showed the absorption bands due to a nitro group (1505 and 1365 cm^{-1}) and a NH group (3280 cm^{-1}). In the ^{1}H NMR spectrum (Fig. 7) of **1**, the signal at δ 12.26 (1H, br, D_2O exchangeable) was assigned to a NH proton. Methylation of **1** (Scheme 1) with diazomethane afforded the NCH_3 derivative (**7**), which showed the ^{1}H signal at δ 3.54 and ^{13}C signal (Table 6) at δ 34.2 (q), corresponding to the newly formed NCH_3 group. Treatment of **7** in DMSO with sodium borohydride afforded denitro derivative (**8**), which showed 3-H signal at δ 5.90 (1H, s). As well as the results described above, the ^{13}C chemical shifts of C-3, C-4 and C-5 in **1** in comparison with those in **3** showed

Table 7. Antimicrobial spectra of pyrroxamycin.

Microorganisms	MIC (μg/ml)	Microorganisms	MIC (μg/ml)
Staphylococcus aureus FDA 209P	0.78	*Aspergillus niger* ATCC 9642	>100
S. aureus Terajima	0.78	*Trichophyton rubrum* NHL J	1.56
S. aureus Smith	0.20	*T. mentagrophytes* QM 248	1.56
S. epidermidis ATCC 12228	0.20	*T. mentagrophytes* IFO 5812	1.56
Micrococcus luteus ATCC 9341	1.56	*T. tonsurans* IFO 5928	1.56
M. lysodeikticus IFO 3333	0.78	*Microsporum gypseum* IFO 8231	3.12
Bacillus subtilis ATCC 6633	0.78	*M. audouinii* IFO 6074	3.12
Escherichia coli O-1	>100	*M. cookei* IFO 8303	1.56
Pseudomonas aeruginosa IFO 13736	>100	*Epidermophyton floccosum* IFO 9045	6.25
Proteus vulgaris OXK	1.25	*Pyricularia oryzae* IAM 5016	0.025
Candida albicans Yu-1200	>100		

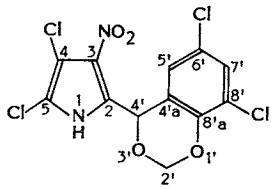
Agar dilution method (Mueller-Hinton agar for bacteria, SABOURAUD's agar for fungi and yeasts).

Fig. 8. ^{13}C-^{1}H Long range selective proton decoupling experiments for **7**.



the presence of a 2-substituted-4,5-dichloro-3-nitropyrrole moiety in the structure of **1**. The chemical shifts of C-2 in **1** and **7** were suggested to be 130.7 and 128.7, respectively, by comparison of ^{13}C NMR data for **1**, **3** and **7**.

Fig. 9. The structure of **1**.



The remaining part of **1**, attached to C-2 of the pyrrole moiety, consisted of $C_8H_5O_2Cl_2$. The structure of this part was determined mainly based on ^{1}H and ^{13}C NMR spectral experiments.

The five protons in this part consisted of dioxygenated methylene protons (δ 5.54), oxygenated methine proton (δ 6.89) and two aromatic protons (δ 7.14 and 7.42), respectively. Two aromatic protons cited above, bearing meta position to each other (J=2.4 Hz), showed long range couplings (J= 0.9 Hz, each) with the oxygenated methine proton at δ 6.89. The ^{13}C NMR signals of this part revealed the following functional groups, C= ×4, CH= ×2, CHO ×1, OCH_2O ×1, which accounted for five protons (Table 6). The ^{1}H-^{13}C selected proton decoupling experiments showed that each of the protons at δ 7.14, 7.42, 6.89 and 5.54 was connected to each of the carbons at δ 126.3, 130.1, 69.4 and 91.5, respectively. The six carbons at δ 148.9, 130.1, 126.7, 126.3, 124.7 and 123.2 can be assigned to those of tetra-substituted benzene ring, in which the carbon at δ 148.9 was assigned to oxygenated carbon. In consideration of the data of ^{1}H and ^{13}C NMR data cited above, it was shown that tri-substituted **1**, 3-benzodioxin ring was present, in which two chlorines and the substituted pyrrole ring cited earlier

were the substituents. In order to determine the positions of the substituents, ^{13}C-^{1}H long range selective proton decoupling experiments were applied on **7** (Fig. 8). Irradiation of NCH_3 confirmed the assignments of C-2 and C-5. Irradiation of 4'-H proved the connection between C-2 and C-4'. Further, irradiations of 4'-H, 5'-H and 7'-H confirmed the assignments of the carbons (Table 6) and the structure of the substituted benzene ring as shown in Fig. 8 considering that the irradiated protons and affected carbons were separated by two or three bonds.

Consequently, the planer structure of **1** was determined to be 4,5-dichloro-2-(6',8'-dichloro-4'*H*-1',3'-benzodioxin-4'-yl)-3-nitropyrrole as shown in Fig. 9.

Biological Properties

Minimal inhibitory concentrations of pyrroxamycin against various microorganisms are shown in Table 7.

Pyrroxamycin was active against Gram-positive bacteria and dermatophytes but not acive against Gram-negative bacteria and yeasts.

Acute toxicity of pyrroxamycin was examined with mice. An oral dosage of 12.5 mg/kg or more killed all the mice but at 6.25 mg/kg, all survived.

Experimental

General

Melting points were determined with Yanagimoto micro hot plate apparatus and were uncorrected. Electron impact mass spectrum (EI-MS) were obtained on a Jeol JMS DX303 mass spectrometer. The UV spectrum was recorded on a Hitachi 200-20 spectrometer. IR spectra were recorded on a Jasco A102 IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol FX 90Q spectrometer. The optical rotation was determined with Jasco DIP-4 digital polarimeter.

N-Methylation of **1** with Diazomethane

To a solution of **1** (105 mg, 0.27 mmol) in acetone (5 ml) was added a diethyl ether solution of diazomethane (3 ml, *ca.* 1 mmol) at room temp. After the reaction mixture was stirred for 0.5 hour at room temp, solvent was removed and the resulting reddish residue was chromatographed on a silica gel column using $CHCl_3$ to give **7** as pale yellow needles (50 mg, 45.9%): MP 141~143°C; IR (KBr) cm^{-1} 1510, 1460, 1410, 1340, 1320; MS *m/z* 396, 398, 400 (*ca.* 3 : 4 : 2); ^{1}H NMR (acetone-d_6) δ 7.43 (1H, dd, 7'-H), 7.15 (1H, m, 4'-H), 7.00 (1H, dd, 5'-H), 5.67 (2H, ABq, 2'-H), 3.45 (3H, s, NCH_3); ^{13}C NMR (Table 6).

Denitration of **7** with Sodium Borohydride

To a solution of **7** (480 mg, 1.2 mmol) in DMSO (5 ml) was added a sodium borohydride (1.0 g) in one portion. The reaction mixture was stirred over night at room temp. The solution was poured on 100 ml of ice water, and was extracted twice with 50 ml of EtOAc. Extracts were collected, washed with water and dried. Solvent was removed and the resulting residue was chromatographed on a silica gel column using $CHCl_3$ to give **8** as colorless crystals (250 mg, 58.7%): MP 91~96°C; IR (KBr) cm^{-1} 1460, 1400; MS *m/z* 351, 353, 355 (*ca.* 3 : 4 : 2); ^{1}H NMR ($CDCl_3$) δ 7.33 (1H, dd, 7'-H), 6.87 (1H, dd, 5'-H), 5.90 (1H, s, 3-H), 5.89 (1H, m, 4'-H), 5.26 (2H, s, 2'-H), 3.57 (3H, s, NCH_3).

Addendum in Proof

During the publication procedure for this article, it was reported that culture LL-F42248, preliminarily identified as an unusual *Streptomyces* species, produced an antibiotic designated as LL-F42248α (G.T. CARTER *et al.*, J. Antibiotics 40: 233~236, 1987). The physico-chemical properties and structure of pyrroxamycin are identical to those of compound LL-F42248α.

Our patent has been published[1].

References

1) OONO, J.; K. YANO, T. ASAOKA, K. MOGI & T. NAKASHIMA (SS Pharm.): A new antibiotic SS46506A and its preparation. Jpn. Kokai 152295 ('86), July 10, 1986
2) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
3) WAKSMAN, S. A. (*Ed.*): The Actinomycetes. Classification, Identification and Descriptions of Genera and Species. Vol. 2. Williams & Wilkins Co., Baltimore, 1961
4) HIGGINS, M. L.; M. P. LECHEVALIER & H. A. LECHEVALIER: Flagellated actinomycetes. J. Bacteriol. 93: 1446~1451, 1967
5) STANECK, J. L. & G. D. ROBERTS: Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl. Microbiol. 28: 226~231, 1974
6) UCHIDA, K. & K. AIDA: Acyl type of bacterial cell wall: its simple identification by colorimetric method. J. Gen. Appl. Microbiol. 23: 249~260, 1977
7) LECHEVALIER, M. P. & H. A. LECHEVALIER: The chemotaxonomy of actinomycetes. *In* A Actinomycete Taxonomy. SIM Special Publication No. 6. *Eds.*, A. DIETZ & D. W. THAYER, pp. 227~291, Society for Industrial Microbiology, Arlington, 1980
8) BUCHANAN, R. E. & N. E. GIBBONS (*Ed.*): BERGEY's Manual of Determinative Bacteriology. 8th Ed., Williams & Wilkins Co., Baltimore, 1974
9) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. Int. J. Syst. Bacteriol. 18: 69~189, 1968
10) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. Int. J. Syst. Bacteriol. 18: 279~392, 1968
11) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. Int. J. Syst. Bacteriol. 19: 391~512, 1969
12) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. 22: 265~394, 1972
13) SHOMURA, T.; S. AMANO, J. YOSHIDA, N. EZAKI, T. ITO & T. NIIDA: *Actinosporangium vitaminophilum* sp. nov. Int. J. Syst. Bacteriol. 33: 557~564, 1983
14) EZAKI, N.; M. KOYAMA, T. SHOMURA, T. TSURUOKA & S. INOUYE: Pyrrolomycins C, D and E, new members of pyrrolomycins. J. Antibiotics 36: 1263~1267, 1983
15) EZAKI, N.; T. SHOMURA, M. KOYAMA, T. NIWA, M. KOJIMA, S. INOUYE, T. ITŌ & T. NIIDA: New chlorinated nitro-pyrrole antibiotics, pyrrolomycin A and B (SF-2080 A and B). J. Antibiotics 34: 1363~1365, 1981
16) KANEDA, M.; S. NAKAMURA, N. EZAKI & Y. IITAKA: Structure of pyrrolomycin B, a chlorinated nitro-pyrrole antibiotic. J. Antibiotics 34: 1366~1368, 1981
17) KOYAMA, M.; Y. KODAMA, T. TSURUOKA, N. EZAKI, T. NIWA & S. INOUYE: Structure and synthesis of pyrrolomycin A, a chlorinated nitro-pyrrole antibiotic. J. Antibiotics 34: 1569~1576, 1981
18) KOYAMA, M.; N. EZAKI, T. TSURUOKA & S. INOUYE: Structural studies on pyrrolomycins C, D and E. J. Antibiotics 36: 1483~1489, 1983