
 Communications to the Editor

 NEOPYRROLOMYCIN, A NEW
 CHLORINATED PHENYLPYRROLE
 ANTIBIOTIC

Sir:

In the course of screening for new antibiotics, we have isolated a new antibiotic, neopyrrolomycin (1), which inhibits the growth of Gram-positive bacteria, Gram-negative bacteria and some fungi, from cultured broth of *Streptomyces* sp. MI424-38F1. In this report, the production, isolation, physico-chemical properties, structure and biological properties of 1 are reported.

A strain which produced 1 was isolated from a soil sample collected at Bunkyo-ku, Tokyo, Japan and classified as *Streptomyces* sp. MI424-38F1.

The strain was inoculated in a 500-ml Erlenmeyer flask containing 110 ml of a medium consisting of galactose 2.0%, soy peptone 1.0%, corn steep liquor 0.5%, (NH₄)₂SO₄ 0.2%, pH 7.4 before sterilization. It was inoculated on a rotary shaker at 37°C for 3 days. Two ml of the precultured broth was transferred to each 500-ml Erlenmeyer flask containing 110 ml of production medium consisting of glycerol 1.5%, soluble starch 1.5%, soybean meal 0.5%, fish meal 1.5%, CaCO₃ 0.2% (pH 7.4). The fermentation was carried out at 27°C for 7 days on a rotary shaker. The antimicrobial activity was assayed by a cylinder-plate diffusion method using *Micrococcus luteus* as a test organism.

The antibiotic existed mainly in mycelial cake of the cultured broth. The purification procedure is shown in Fig. 1.

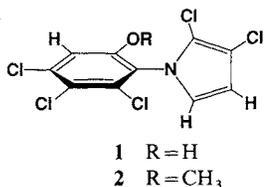
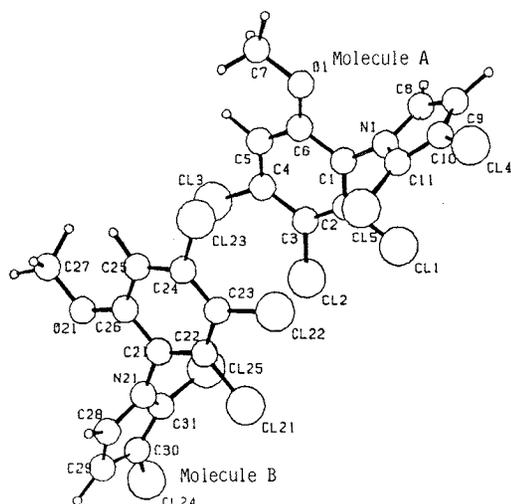
The potassium salt of 1 is colorless needles, mp 70~72°C, [α]_D²⁵ -4.8° (c 0.5, CHCl₃), soluble in MeOH, CHCl₃, EtOAc and H₂O. The free form of the antibiotic (1) was oily, soluble in MeOH, CHCl₃, (CH₃)₂CO, EtOAc but hardly soluble in H₂O. It gives positive reaction with FeCl₃ and Rydon-Smith, but negative ninhydrin reagent. The molecular formula of 1 was established to be C₁₀H₄NOCl₅ by HREI-MS (M⁺ m/z 328.8714, calcd: 328.8734). The UV spectrum of 1 in MeOH shows an absorption maximum at 299 nm (log ε 3.57). It shifted to 319 nm (log ε 3.78) in alkaline condition indicating the presence of a phenol group in the molecule. The ¹³C NMR spectrum in CDCl₃ showed ten signals at 111.68 (s), 111.88 (d), 115.05 (s), 116.63 (d), 120.63 (d), 122.98 (s), 124.10 (s), 134.57 (s), 135.94 (s) and 152.07 (s) ppm, and the ¹H NMR spectrum showed

three aromatic protons at 6.43 (d, J=4 Hz), 6.59 (d, J=4 Hz), and 7.19 ppm (s). Since the signals at 6.43 and 6.59 ppm were observed to have large J_{CH} values of 181 Hz and 196 Hz in ¹H-detected heteronuclear multiple-quantum coherence (HMQC) spectrum, they were assignable to the protons on the pyrrole ring. The singlet at 7.19 ppm and the unsaturation number of 7, which was calculated from the molecular formula, suggested the presence of a pentasubstituted benzene and a trisubstituted pyrrole. The phenol signal was observed at 5.49 ppm as a broad signal. Treatment of 1 with dimethyl-sulfate afforded an O-methyl derivative (2), which showed one methyl signal at 3.82 ppm, and ¹³C signal at 56.72 ppm. These findings indicate that the pentasubstituted benzene moiety links to a nitrogen atom of the pyrrole ring (Fig. 2). The complete structure of 1 was established by X-ray crystallographic analysis of the O-methyl derivative (2).

Fig. 1. Isolation and purification of neopyrrolomycin.

```

  Whole broth (5 liters)
  |
  | filtered
  |
  Mycelial cake
  |
  | extracted with acetone (2 liters)
  |
  Filtrate
  |
  | concd in vacuo
  |
  | extracted with EtOAc (1 liter)
  |
  Organic layer
  |
  | evaporated in vacuo
  |
  Dark green oil (2.7 g)
  |
  Centrifugal partition chromatography
  |
  | acetonitrile - n-hexane
  |
  Active fraction
  |
  | evaporated in vacuo
  |
  Yellow oil (144 mg)
  |
  | crystallized from 33% KOH
  |
  Crystals (filtered)
  |
  | dissolved in H2O
  |
  | extracted with EtOAc
  |
  Organic layer
  |
  | dried over Na2SO4
  |
  | evaporated in vacuo
  |
  | crystallized from CHCl3 - n-heptane
  |
  Neopyrrolomycin K-salt (48.8 mg)
  
```

Fig. 2. Structures of neopyrrolomycin (1) and *O*-methyl derivative (2).Fig. 3. Molecular structure of *O*-methylneopyrrolomycin (2).

Crystal of **2** were grown in a *n*-heptane solution as colorless thick plates. A small specimen of dimensions approximately $0.35 \times 0.35 \times 0.15$ mm was cut from the crystal.

The lattice constants and intensity data were obtained from the data collected by a Philips PW1100 diffractometer using $\text{CuK}\alpha$ radiation monochromated by a graphite plate. Crystal data are given below: *O*-Methyl derivative (**2**), $\text{C}_{11}\text{H}_6\text{NOCl}_5$, MW 345.4, Monoclinic, space group $P2_1$, $Z=4$, Lattice constants: $a=8.564(5)$, $b=23.838(10)$, $c=7.246(5)$ Å, $\beta=115.09(5)^\circ$, $U=1,340$ Å³, $D_x=1.713$ g cm⁻³, μ for $\text{CuK}\alpha=100$ cm⁻¹.

The crystal structure was determined by direct methods and refined by the method of least-squares with block-diagonal-matrix approximations. The final R value for 2,527 independent reflections was 0.06 allowing for the dispersion corrections of atomic scattering factors of C, N, O and Cl atoms for $\text{CuK}\alpha$ radiation^a. The absolute configuration was

^a The atomic parameters, bond lengths and angles have been sent to the Cambridge Crystallographic Centre.

Table 1. Antimicrobial activities of neopyrrolomycin.

Test organisms	Medium*	MIC (μg/ml)
<i>Staphylococcus aureus</i> Smith	a	0.39
<i>S. aureus</i> MS9610	a	0.39
<i>S. aureus</i> No. 5	a	0.39
<i>S. aureus</i> No. 17	a	0.39
<i>Micrococcus luteus</i> FDA 16	a	0.78
<i>Bacillus subtilis</i> NRRL B-558	a	0.78
<i>Corynebacterium bovis</i> 1810	a	0.78
<i>Escherichia coli</i> NIHJ	a	1.56
<i>E. coli</i> K-12	a	3.12
<i>E. coli</i> K-12 ML1629	a	25
<i>Shigella dysenteriae</i> JS11910	a	6.25
<i>Salmonella typhi</i> T-63	a	12.5
<i>Proteus mirabilis</i> IFM OM-9	a	6.25
<i>Pseudomonas aeruginosa</i> A3	a	25
<i>Klebsiella pneumoniae</i> PCI 602	a	12.5
<i>Mycobacterium smegmatis</i> ATCC 607	a	0.78
<i>Candida pseudotropicalis</i> F-2	b	3.12
<i>C. albicans</i> 3147	b	12.5
<i>Xanthomonas oryzae</i>	b	3.12
<i>Aspergillus niger</i> F-16	b	6.25
<i>Trichophyton asteroides</i> 429	b	3.12
<i>Saccharomyces cerevisiae</i> F-7	b	6.25
<i>Cryptococcus neoformans</i> F-10	b	1.56
<i>Cochliobolus miyabeanus</i>	b	12.5
<i>Pyricularia oryzae</i>	b	3.12
<i>Pellicularia sasakii</i>	b	6.25

* a: Mueller-Hinton agar 37°C, b: nutrient agar + glucose 1%, 27°C.

determined by the anomalous dispersion method. 383 Friedel pairs were chosen for which the difference of observed structure factor between the pair of reflections was found to be more than 5% of the mean value of their structure factors and the difference exceeds twice of their mean standard deviation. Of these, 353 pairs showed clearly the absolute configuration shown in Fig. 3 by comparison with the calculated structure factor differences.

The crystal contains two crystallographically independent molecules A and B. Fig. 3 shows their molecular structure along with the absolute configuration. The molecule consists of two aromatic moieties: a benzene moiety and a pyrrole moiety. Both moieties have no asymmetric group and the enantiometry arises mainly from the twisting of the two moieties: the torsion of the C-N bond between them. It is an example of atropisomerism.

The average aromatic C-C distances of the benzene ring are 1.393 in molecule A and 1.392 Å in molecule B which are quite close to that found in usual aromatic compounds, 1.395 ± 0.003 Å. Four

C-N distances in the pyrrole rings, 1.373 to 1.396 Å are common for the partial double bond in heterocyclic systems while those found in the linkage between benzene and pyrrole rings, 1.419(12) and 1.426(12) Å are characteristic of the conjugated systems such as found in ϕ -N bond in acetanilide.

The torsional angles along these bonds, C2-C1-N1-C11 and C22-C21-N21-C31 are similar to each other, -78.1 (1.2) $^\circ$ and -82.4 (1.1) $^\circ$, respectively. These are responsible for the optical activity of this molecule. Steric interactions between the substituents of benzene and pyrrole rings may hold the molecule in such a twisted state as found in binaphthyl and biphenyl derivatives ((+)-2,2'-dihydroxy-1,1'-binaphthalene-3,3'-dicarboxylic acid dimethyl ester¹) and 1,3,5-triphenylbenzene²).

The antibiotic (**1**) is related to pyrrolnitrin³) and pyrrolomycins⁴⁻⁶), which consist of chlorinated pyrrole and substituted benzene. The antibiotic (**1**) is different from other antibiotics in the mode of linkage between pyrrole and benzene ring.

The antimicrobial activities of **1** are shown in Table 1. The acute toxicity (LD₅₀) of **1** in ICR mice was 75 mg/kg (ip).

TAKASHI NOGAMI
YASUHIRO SHIGIHARA
NAOKO MATSUDA
YOSHIKAZU TAKAHASHI
HIROSHI NAGANAWA
HIKARU NAKAMURA
MASA HAMADA
YASUHIKO MURAOKA
TOMOHISA TAKITA
YOICHI IITAKA[†]
TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

[†]Faculty of Pharmaceutical Sciences,
Teikyo University,
Sagamiko-cho, Tsukui-gun,
Kanagawa-ken 199-01, Japan

(Received March 30, 1990)

References

- 1) AKIMOTO, H. & Y. IITAKA: Determination of the crystal structure and the absolute configuration of (+)-2,2'-dihydroxy-1,1'-binaphthalene-3,3'-dicarboxylic acid dimethyl ester bromobenzene solvate. *Acta Cryst. B*25: 1491~1500, 1969
- 2) FARAG, M. S.: The crystal structure of 1,3,5-triphenylbenzene. *Acta Cryst. B*7: 117~121, 1954
- 3) IMANAKA, H.; M. KOUSAKA, G. TAMURA & K. ARIMA: Studies on pyrrolnitrin, a new antibiotic. III. Structure of pyrrolnitrin. *J. Antibiotics, Ser. A* 18: 207~210, 1965
- 4) KANEDA, M.; S. NAKAMURA, N. EZAKI & Y. IITAKA: Structure of pyrrolomycin B, a chlorinated nitro-pyrrole antibiotic. *J. Antibiotics* 34: 1366~1368, 1981
- 5) KOYAMA, M.; N. EZAKI, T. TSURUOKA & S. INOUE: Structural studies on pyrrolomycins C, D and E. *J. Antibiotics* 36: 1483~1489, 1983
- 6) NAKAMURA, H.; K. SHIOMI, H. IINUMA, H. NAGANAWA, T. OBATA, T. TAKEUCHI, H. UMEZAWA, Y. TAKEUCHI & Y. IITAKA: Isolation and characterization of a new antibiotic, dioxapyrrolomycin, related to pyrrolomycins. *J. Antibiotics* 40: 899~903, 1987