AN ANTIBIOTIC 24010 B-1

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In the previous paper[1], production, isolation and characterization of antibiotic 24010 has been reported. The antibiotic is water-insoluble, UV-absorbing, and causing morphological changes in many kinds of microorganisms, especially in phytopathogenic fungi. From these results, many resemblances between antibiotic 24010 and tunicamycin[2] were observed.

The antibiotic 24010 producing strain, tentatively named *Streptomyces* No. 24010, was revealed as a producer of three additional kinds of antibiotics, 24010 B-1,2 and 3. Among them, antibiotic 24010 B-1 was isolated and characterized.

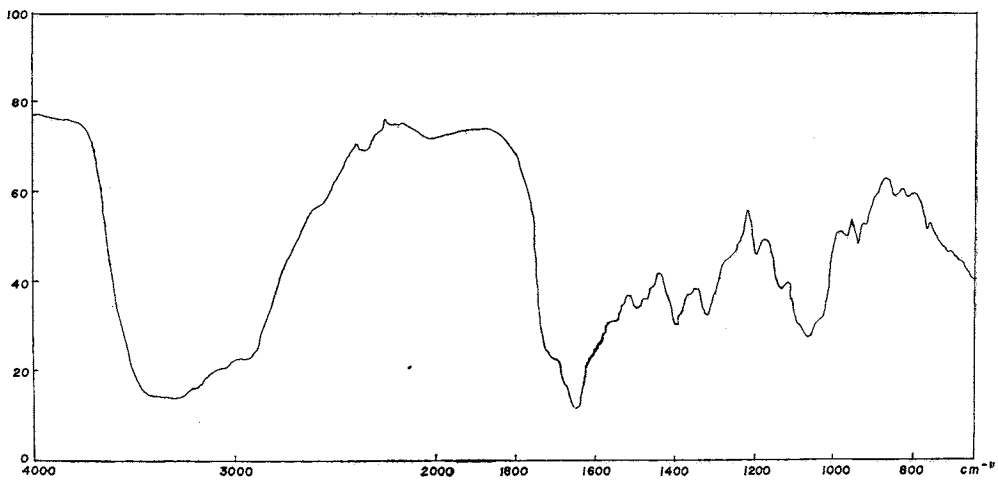
In this paper, production and isolation procedures, and chemical, physical and biological properties of antibiotic 24010 B-1 are reported.

Production and Isolation of Antibiotic 24010 B-1

Streptomyces No. 24010 was grown in conditions reported previously[1], and cultured broth was centrifuged. Clear supernatant (1,570 liters) thus obtained was passed through a 12×90 cm column (wet volume, 10 liters) of Amberlite IRC-50 (H^+ form), and the column was washed with 200 liters of water. An active fraction was eluted with 0.05 N HCl (400 liters), and the eluate was neutralized with Amberlite IR-45 (OH^- form). The neutralized solution, containing antibiotics 24010 B-1, 2 and 3, was treated with 1 kg of activated charcoal to remove inactive contaminants, mainly pigments. The colorless and clear filtrate was concentrated, and finally freeze-dried. Seventy seven grams of creamy white crude powder was obtained.

The powder (170 mg) was applied to a column (3.5×80 cm) of cellulose powder (Toyo Roshi, Type A), and chromatographed using the following solvent system: iso-propanol – acetic acid – water, 8 : 1 : 4, v/v. Flow rate was 20 ml per hour, and effluent was collected in 9 ml fractions. The antibiotics in individual fractions were monitored by ninhydrin and bioassay using *Bacillus subtilis* as a test organism. Fractions containing only antibiotic 24010 B-1 were collected, and freeze-dried to give 54 mg of white powder. This powder was further purified by chromatography on a Sephadex LH-20 column with 10% aqueous methanol solution as described by TANIYAMA *et al.*[3] The yield of pure antibiotic 24010 B-1 was 38 mg.

Fig. 1. Infrared absorption spectrum of antibiotic 24010 B-1 hydrochloride (KBr)



Properties of Antibiotic 24010 B-1

Chemical, physical and biological properties of antibiotic 24010 B-1 were determined in the form of the hydrochloride.

Antibiotic 24010 B-1 is a colorless powder, and has no characteristic absorption in the UV-region different form antibiotic 24010. The IR spectrum of this antibiotic is shown in Fig. 1. The antibiotic melts at 200~203 °C with decomposition, and consists of five elements, C 34.9 %, H 5.9 %, N 18.1 %, O 22.0 % and Cl 14.93 %. Molecular weight of the antibiotic was measured by Vapor Pressure Osmometer 301 A (Micro Labs, U.S.A.). Methanol was used as solvent, and raffinose was employed as reference. From the result, a molecular formula was tentatively determined as $C_{16}H_{29}N_6O_7Cl_2$ (M.W. 488). It gives positive test for ELSON-MORGAN, MOLISCH, anthrone, TOLLENS, ninhydrin and Na_2CO_3-$KMnO_4$ reactions. $[\alpha]_D^{18}$ shows −43.0 (*c* 1, water).

The antibiotic is soluble in water and methanol, slightly soluble in ethanol, and insoluble in ordinary organic solvents tested. The antibiotic is stable at pH 2~9 at room temperature for 48 hours, and at pH 3~9 at 60°C for 1 hour.

Antibiotic 24010 B-1 gave one spot on paper chromatogram (Toyo Roshi no. 51) using solvent systems : *n*-propanol-pyridine-acetic acid-water (15 : 10 : 3 : 12, v/v, Rf 0.39); *n*-butanol-pyridine-acetic acid-water (15 : 10 : 3 : 12, v/v, Rf 0.29); *n*-butanol saturated with water (Rf 0.05); 1.5 % aqueous NH_4Cl (Rf 0.91); 75 % aqueous phenol (Rf 0.44); a mixture of 40 ml of *n*-butanol, 10 ml of methanol, 20 ml of water and 1.5 g methyl orange (Rf 0.51); *n*-butanol-methanol-water

Table 1. Antimicrobial spectrum of antibiotic 24010 B-1.

Test organism	M.I.C. μg/ml
Micrococcus flavus IFO 3242	2.5
Serratia marcescens IFO 3046	>100
Corynebacterium spedonicum	100
Proteus vulgaris	25
Bacillus subtilis	≦ 1
Pseudomonas aeruginosa	25
Escherichia coli K-12	2.5
Escherichia coli ML 3748	2.5
Staphylococcus aureus FDA 209 P	2.5
Staphylococcus aureus STF	25
Staphylococcus aureus SS 317	5
Xanthomonas citri IFO 3835	>100
Xanthomonas oryzae IAM 1657	>100
Mycobacterium 607	1
Vibrio metchnikovii IAM 1039	2.5
Candida albicans IFO 0583	50
Saccharomyces cerevisiae Hansen Kyokai-6	25
Glomerella lagenarium IAM 8053	>100
Trichophyton mentagrophytes IAM 5064	≧100
Alternaria kikuchiana Tanaka IAM 5005	50
Fusarium oxysporum	100
Aspergillus oryzae L	>100
Aspergillus niger ATCC 6275	>100
Rhizopus nigricans	≧100
Penicillium citrinum	≧100

Minimum inhibitory concentration of antibiotic 24010 B-1 by agar streak method, using nutrient agar (bacteria and yeast), malt-yeast extract agar (fungi) and 5% glycerin-nutrient agar (*Mycobacterium* 607)

Table 2. Comparison of M.I.C. values between antibiotic 24010 B-1 and neothricin

Test organisms	M.I.C. (μg/ml)	
	24010 B-1	Neothricin
Bacillus subtilis	1	10
Staphylococcus aureus FDA 209 P	2.5	10
Pseudomonas aeruginosa	25	100
Xanthomonas oryzae	>100	5
Trichophyton mentagrophytes	≧100	20

Fig. 2. Comparison of thin-layer chromatogram of antibiotic 24010 B-1, racemomycins and BY-81.

Adsorbent : Avicel (Funakoshi)
Developer : *n*-propanol-pyridin-acetic acid-water (15 : 10 : 3 : 12, v/v)
Detection : ninhydrin
I : BY-81 (hydrochloride)
II : antibiotic 24010 B-1 (hydrochloride)
III : racemomycin A,B and C (hydrochloride)

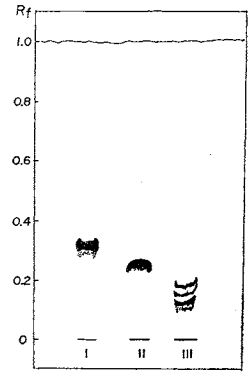


Table 3. Comparison of antibiotic 24010 B-1 with known antibiotics.

Antibiotics	24010 B-1	Neothricin	Racemomycin A	Streptothricin	BY-81
Salt form	HCl	HCl	H_2SO_4	H_2SO_4	HCl
m.p. (°C)	200~203 (dec.)	207~213 (dec.)	213~214 (dec.)	213~217 (dec.)	142~143 (dec.)
$[\alpha]_D$	−43.0 (18°C)	−43.27 (20°C)	−34.8 (14°C)	−49 (25°C) (HCl)	−60 (20°C)
FEHLING	−	+		+	doubtful
BENEDICT	−	+		+	doubtful
TLC			*		*

* See Fig. 2.

(40 : 10 : 20, v/v, Rf 0.03); benzene - methanol (4 : 1, v/v, Rf 0.0); water (Rf 0.05); water - saturated *n*-butanol containing 0.2% (w/v) *p*-toluenesulfonic acid (Rf 0.05).

The antimicrobial spectrum of antibiotic 24010 B-1 is shown in Table 1.

When antibiotic 24010 B-1 is injected intravenously to mice, the LD_{50} was 150 mg/kg. Mice survived more than 7 days after intravenous administration of doses 100 mg/kg or less.

Comparison with Known Antibiotics

Chemical, physical and biological properties of antibiotic 24010 B-1 show some resemblance to the known antibiotics such as neothricin[4], racemomycin A[5], BY-81[6] and streptothricin[7].

Melting point, optical rotation, heat stability and Rf values by PPC and TLC of antibiotic 24010 B-1 are very similar to those of neothricin. The former gives negative test for FEHLING and BENEDICT reactions, but the latter is positive for these reactions. Both antibiotics show different antimicrobial properties as shown in Table 2.

In comparison with racemomycins and BY-81, antibiotic 24010 B-1 is easily separable on a thin-layer chromatogram of Avicel plate (Funakoshi) as shown in Fig. 2.

Streptothricin sulfate shows a positive test for FEHLING and BENEDICT reactions. Summarized comparison data of antibiotic 24010 B-1, neothricin, racemomycin A, BY-81 and streptothricin are shown in Table 3.

From these results, antibiotic 24010 B-1 seems to be a new, water soluble and basic antibiotic.

Discussion

As mentioned above, antibiotic 24010 B-1 is active against gram-positive and negative bacteria, and is a water-soluble and basic antibiotic. Cross resistance with streptothricin and delayed toxicity were observed with antibiotic 24010 B-1. Consequently, antibiotic 24010 B-1 is classified in the streptothricin group of antibiotics.

In addition to biological activity of antibiotic 24010 B-1, chemical studies are in progress. Isolation and characterizations of antibiotics 24010 B-2 and 3 are also in progress.

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