NOTE

AN ANTIBIOTIC 24010

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In the course of our screening program of antibiotics against phytopathogenic fungi, an antibiotic was found in the cultured broth of a streptomyces, designated as *Streptomyces* No. 24010. The strain was isolated from a soil sample collected at Ikeda City, Osaka, Japan. The strain belongs to the genus *Streptomyces*, and is classified as a non-chromogenic type strain. Extensive determination of *Streptomyces* No. 24010 is in progress.

The antibiotic, named antibiotic 24010, depressed germination and mycelial growth of *Piricularia oryzae*, and caused morphological change in the mycelium in concentration less than 1 p.p.m. of the antibiotic (Fig. 1). Same phenomena were observed in another phytopathogenic fungi, which are *Glomerella lagenarium*, *Alternaria kikuchiana*, *Pellicularia sasakii*, *etc.* These morphological changes in phytopathogenic fungi caused by antibiotic 24010 are classified as "bulging effect".[1]

Streptomyces No. 24010 was inoculated into shaking flask containing 100 ml of a medium consisting of 2 % soluble starch, 1 % soybean meal, 0.3 % NaCl, 0.1 % K_2HPO_4, 0.05 % $MgSO_4 \cdot 7H_2O$ and 0.2 % $CaCO_3$, and shaken reciprocally at 30°C for 24 hours. Fifty ml of one day-old culture was seeded into 5-liter Erlenmeyer's flask containing 1 liter of the same medium. It was cultured on rotary shaker at 30°C for 72 hours.

Ten liters of the cultured broth of *Streptomyces* No. 24010 thus obtained were centrifuged, and collected mycelia were extracted with 2 liters of acetone. The extract was concentrated to 1.2 liter at 60°C, and the concentrated acetone extract was shaken with 1 liter of *n*-butanol. The organic layer was washed with water, and shaken with 1.5 liter of 2 N NaOH. The aqueous layer was adjusted to pH 3.0 with HCl, and was extracted with *n*-butanol (1 liter). After washing with water, the *n*-butanol extract was dried *in vacuo* at 60°C to give 450 mg of crude powder.

The crude powder was dissolved in 100 ml of 0.1 N ammonia water, and was applied to charcoal column (ϕ=20 mm, wet volume 100 ml). The column was washed successively with water (1 liter) and water - ethanol (1 : 1, v/v, 3 liters). An active fraction was eluted with 1 liter of *n*-butanol saturated with

Fig. 1. Morphological change in *P. oryzae* by antibiotic 24010.

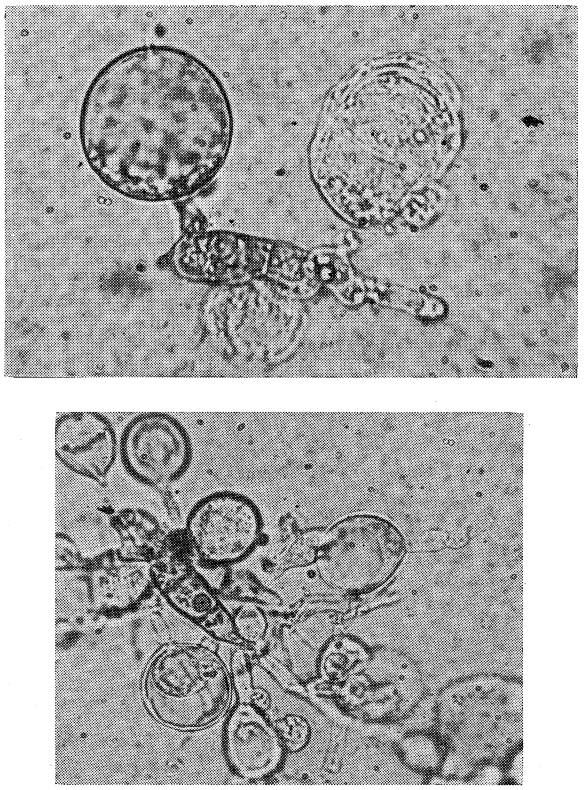

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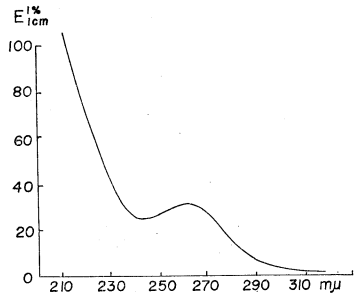
Table 1. Antimicrobial spectrum of antibiotic 24010 (I)

Test organism	M.I.C. μg/ml
Staphylococcus aureus FDA 209P	100
Bacillus subtilis PCI 219	1
Sarcina lutea PCI 1001	100
Erwinia carotovora IFO 3057	100
Xanthomonas oryzae IAM 1657	100
Xanthomonas citri IFO 3835	100
Escherichia coli K-12	100
Vibrio metschnikovii B-3-6	100
Mycobacterium 607	50
Pseudomonas aeruginosa IAM 1057	100
*Pseudomonas solanacearum**	1

Minimum inhibitory concentration of antibiotic 24010 by agar streak method, using nutrient agar.
* 1% glucose nutrient agar.

Fig. 2. UV spectrum of antibiotic 24010 in methanol.



water. By evaporating the active eluate, 230 mg of the antibiotic 24010 was obtained as a white powder, which gave one spot on thin-layer chromatograms of silica gel using the solvent systems : *n*-butanol – acetic acid – water (4 : 1 : 5, 4 : 1 : 2, 2 : 1 : 1, v/v), *n*-butanol saturated with water, and *n*-butanol – ethanol – water (10 : 3 : 7, v/v). The spot was visualized by one of the following methods : anisaldehyde-H_2SO_4 on heating, $KMnO_4$-Na_2CO_3 reagent, ultraviolet light irradiation, and bioassay using *Bacillus subtilis*.

Antibiotic 24010 does not show a definite melting point but decomposes at over 210°C with browning. After drying at 80°C for 3 hours, antibiotic 24010 gave analyses of C 55.07 %, H 7.82 %, N 6.33 % and O 29.92 %.

It is soluble in methanol, ethanol, aqueous *n*-butanol, pyridine, methylcellosolve, ammonia water and aqueous caustic soda solution, and insoluble in water (in neutral to acidic pH range), acetone, ethylether, *n*-hexane, ethylacetate, benzene and methylethylketone.

Table 2. Antimicrobial spectrum of antibiotic 24010 (II)

Test organism	M.I.C. μg/ml
Aspergillus oryzae L	100
Aspergillus niger ATCC 6275	100
Rhizopus nigricans EHRENBERG SN 32	50
Penicillium citrinum ATCC 9849	100
Trichophyton mentagrophytes IAM 5064	100
Glomerella lagenarium IAM 8051	50
Alternaria kikuchiana	100
Fusarium oxysporum	100
Botrysis cinerea	50
Helminthosporium sigmoideum	50
Piricularia oryzae A_1	1
Pellicularia sasakii	20
Candida albicans (Robin) Berkhout IAM 4888	50
Saccharomyces cerevisiae Hansen Kyokai-6	10

Minimum inhibitory concentration of antibiotic 24010 by agar streak method, using 1% glucose nutrient agar.

Table 3. Preventive effect of antibiotic 24010 on rice blast (greenhouse test)

Materials	Concn. (p.p.m.)	Protective value	Phytotoxicity
Antibiotic 24010	1.25	77	—
	2.5	99	—
	5	92	—
	10	97	—
	20	97	—
Kasugamycin HCl	20	98	—
Blastcidin S HCl	20	98	—
Not treated (Number of lesions per leaf)	0	0 (30.8)	—

Antibiotic 24010 gives yellow color with $KMnO_4$-Na_2CO_3, blue with anisaldehyde-sulfuric acid-heating, brown with I_2 vapor, and no color in anthrone, ninhydrin, ELSON-MORGAN, HANES-ISHERWOOD and aniline hydrogen phthalate reactions. The antibiotic exhibits dextrorotation, $[\alpha]_D^{20}$ +4.3° (*c* 0.2, aqueous *n*-butanol), and is stable at pH 2～14 at room temperature for 72 hours, and at pH 7～14 at 100°C for 10 minutes. The antibiotic is also stable for ultraviolet light irradiation (Chemical Lamp, Toshiba Electric & Co., Ltd.).

The antimicrobial activity of antibiotic 24010 determined by agar streak method is

Fig. 3. IR spectrum of antibiotic 24010 (KBr tablet).

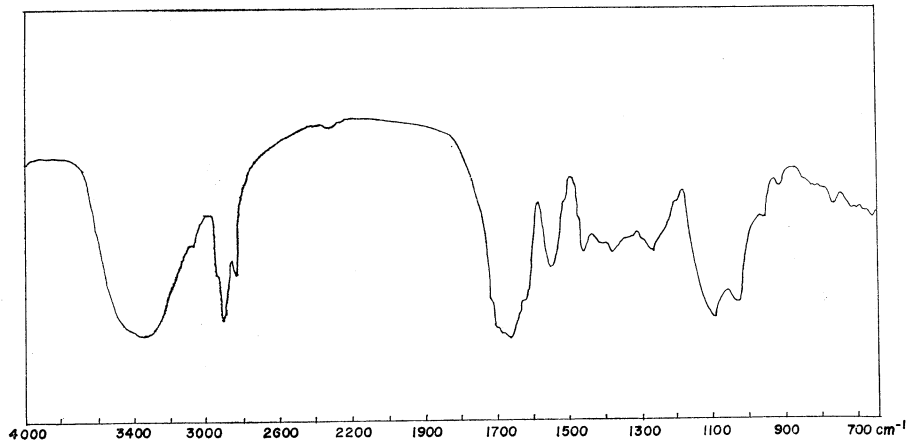
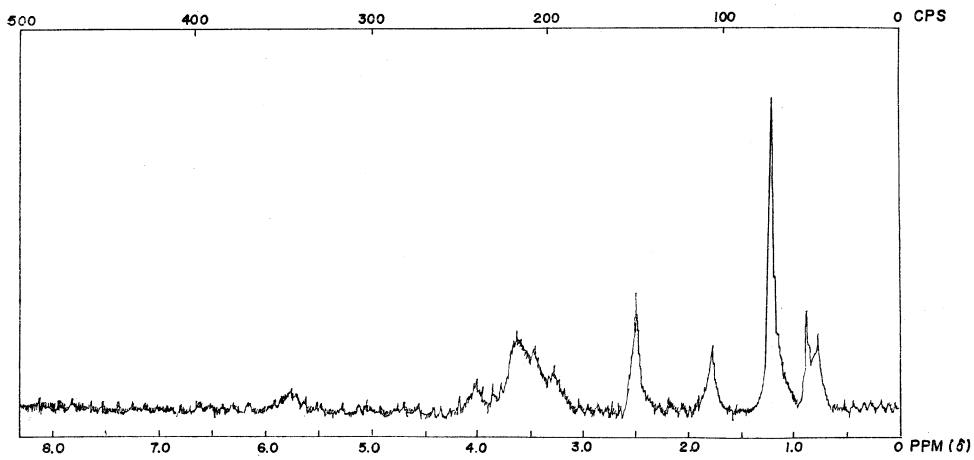


Fig. 4. NMR spectrum of antibiotic 24010 in $(CD_3)_2SO$.



shown in Tables 1 and 2. The antibiotic is active against some bacteria, yeast and fungi.

Preventive activity of the antibiotic against rice blast was measured by the method reported by UMEZAWA *et al.*[2] in green house. At three leaves' stage, infant rice plants were used for test. After 2 hours of drug supplement, a spore suspension of *Piricularia oryzae* A_1, grown on Japanese barley medium, was sprayed to rice plants. Antibiotic shows a potent preventive effect against rice blast at the concentration of 2.5 p.p.m. as shown in Table 3.

Acid hydrolysate of antibiotic 24010 (6 N HCl, 105°C, 20 hours) gave uracil, and a ninhydrin-positive spot was detected by paperchromatography.

From these results, antibiotic 24010 has some similarities and differences with polyoxins[3,4] and bulgerin[5], which are water soluble, UV-absorbing, active against only phytopathogenic fungi and cause bulging of mycelia of phytopathogens, but antibiotic 24010 is clearly different from polyoxins and bulgerin in solubility and antimicrobial spectrum.

To clarify the relationship between chemical structure and biological properties of polyoxins, bulgerin and antibiotic 24010, chemical study is in progress.

When we had finished the studies described above, the paper on tunicamycin by TAKATSUKI *et al.*[6] was published. Antibiotic 24010 is very similar to tunicamycin in chemical, physical and biological properties.

The identity or difference between antibiotic 24010 and tunicamycin is not certain and will have to determine by direct comparison.

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