Communications to the Editor

PHTHORAMYCIN, A NEW ANTIBIOTIC ACTIVE AGAINST A PLANT PATHOGEN, *PHYTOPHTHORA* SP.

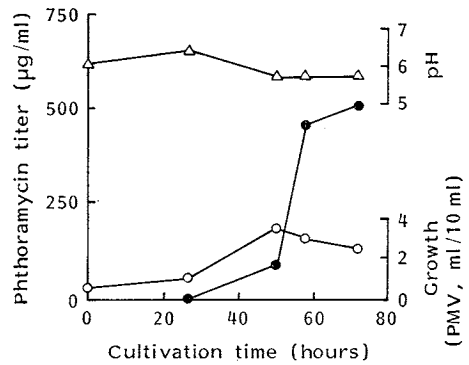
Sir:

A new antibiotic named phthoramycin was found in the cultured broth of an actinomycete, *Streptomyces* sp. WK-1875, which was isolated from a soil sample collected at the seashore of Tokyo Bay. Phthoramycin is active against filamentous fungi such as the plant pathogen *Phytophthora parasitica*. The present communication describes the fermentation, isolation, and physico-chemical and biological properties of the antibiotic.

Fermentation was carried out in a 30-liter jar fermentor containing 20 liters of a medium (glycerol 2%, soybean powder 2%, NaCl 0.3%, pH 6.8 before autoclaving) at 27°C for 3 days with agitation (250 rpm) and aeration (10 liters/minute). Antibiotic activity in the cultured broth was monitored by the paper disc method with *P. parasitica* KF-223 as a test organism (potato-glucose agar, pH 6, incubation at 27°C for 44 hours). Under the above conditions antibiotic titer reached its maximum of *ca.* 500 μg/ml at day 3 (Fig. 1).

Phthoramycin was isolated from the cultured broth, as shown in Fig. 2. The supernatant of a 3-day culture (15 liters) of strain WK-1875 was extracted with ethyl acetate (10 liters). After evaporation of the organic layer, the oilish residue (45 g) was chromatographed on a silica gel column, which was eluted stepwise with chloroform - methanol (50 : 1 ~ 5 : 1). The crude powder obtained by evaporation of the active fractions was subjected to second column chromatography on silica gel developed with benzene - acetone

Fig. 1. Phthoramycin production in a 30-liter jar fermentor.



Packed mycelial volume (PMV) measured after centrifugation (3,000 rpm, 10 minutes).

Fig. 2. Isolation procedures for phthoramycin.

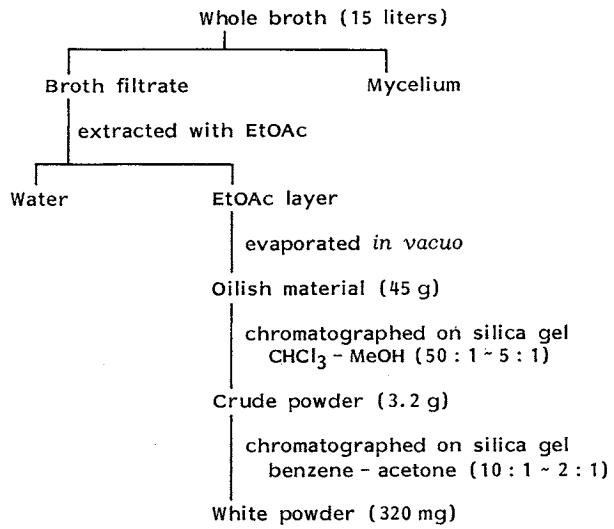
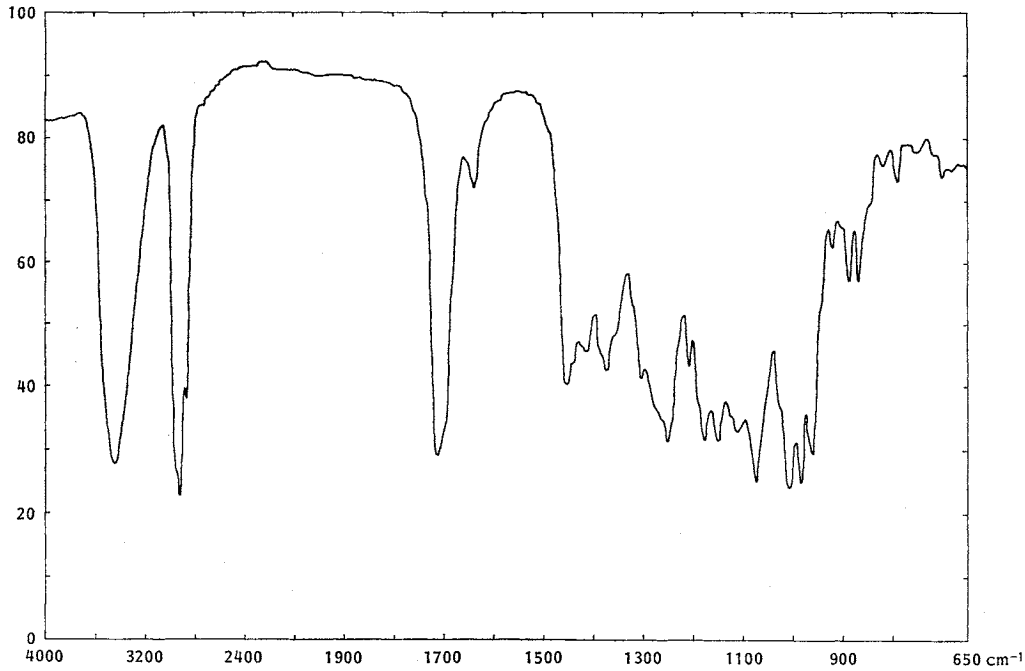


Fig. 3. IR spectrum of phthoramycin (KBr).



solutions (10: 1~2: 1) as eluants. The active fractions were collected and concentrated to give white powder of pure phthoramycin (320 mg). It gave a single spot on silica gel TLC with several solvent systems using 40% H_2SO_4 for visualization. The white powder gave a single peak by HPLC monitored at 220 nm.

Phthoramycin (mp 116~119°C, $[\alpha]_D^{18}$ −12.1° (*c* 0.5, methanol)) is soluble in methanol, ethyl acetate, and chloroform, but is insoluble in water and *n*-hexane. The high resolution electron impact (HREI)-MS of the antibiotic gave a peak at *m/z* 722 (*m/z* 722.460, $M^+ - H_2O$, calcd for $C_{40}H_{66}O_{11}$, 722.461). The fast atom bombardment (FAB)-MS of the pentaacetyl derivative of phthoramycin gave the molecular ion peak at *m/z* 950. These results together with ^{13}C NMR spectroscopic analyses proposed the molecular formula $C_{40}H_{68}O_{12}$ (MW 740.97) for phthoramycin. The UV spectrum of the antibiotic exhibits only end absorption. The IR spectrum (KBr, cm^{-1}) shows absorption bands at 2910, 1717, 1452, 1372, 1250, 1180, 1150, 1072, 1010, 980, 960 (Fig. 3). None of the known antibiotics share the physico-chemical properties of phthoramycin. X-14931A[1] bears some resembrance to phthoramycin, but is differentiated by molecular formula ($C_{40}H_{66}O_{11}$ vs. $C_{40}H_{68}O_{12}$).

Table 1. Antifungal spectrum of phthoramycin.

Test organism	MIC (μg/ml)
Candida albicans KF-1	>100
Saccharomyces sake KF-26	>100
Aspergillus niger KF-103	>100
Piricularia oryzae KF-180	6.25
Mucor racemosus IFO 4581	3.12
Phythophthora parasitica IFO 4783	1.56

Potato-glucose agar (pH 6), 27°C, 3 days.

Therefore it is concluded that phthoramycin is a new antibiotic.

Phthoramycin inhibits the growth of filamentous fungi: *Mucor racemosus*, and plant pathogens such as *Piricularia oryzae* and *Phythophthora parasitica* (Table 1). It is marginally active against the yeasts and bacteria tested. The antibiotic also inhibits the growth of radish seedling. Preliminary results showed that phthoramycin inhibits cellulose synthesis by resting cells of *Acetobacter xylinum*, an acetic acid bacterium known to produce extracellular cellulose[2].

The acute toxicity of phthoramycin in mice (LD_{50} value) was 30 mg/kg (ip) and 100 mg/kg (po).

Detailed description of the producing micro-

organism, chemical structure, and mode of action of the antibiotic will be reported elsewhere.

SATOSHI ŌMURA
YOSHITAKE TANAKA
KAZUYOSHI HISATOME
SATOMI MIURA
YŌKO TAKAHASHI
AKIRA NAKAGAWA

The Kitasato Institute, and
School of Pharmaceutical Sciences
Kitasato University,
Minato-ku, Tokyo 108, Japan

HARUMITSU IMAI

Central Research Laboratories,
Yamanouchi Pharmaceutical Co., Ltd.,
Itabashi-ku, Tokyo 174, Japan

H. BOYD WOODRUFF

Soil Microbiology Associates, Inc.,
Valley Road, Watchung, N.J. 07060,
U.S.A.

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