VIRANTMYCIN, A POTENT ANTIVIRAL ANTIBIOTIC PRODUCED BY A STRAIN OF *STREPTOMYCES*

Sir:

In the course of screening for antiviral substances and interferon inducers from Actinomycetes, a new antiviral antibiotic, virantmycin, has been isolated from the culture broth of strain AM-2722, a soil isolate identified as *Streptomyces nitrosporeus*[1].

Medium composed of 2.0% of glucose, 0.5% of peptone, 0.5% of meat extract, 0.3% of dry yeast, 0.5% of NaCl, and 0.3% of $CaCO_3$ (pH 7.0) was used for seed culture and fermentation. A 7-day agar culture (modified WAKSMAN's medium) of the strain AM-2722 was inoculated into seed medium (100 ml) in a SAKAGUCHI flask and incubated for two days at 27°C. The production fermentation of virantmycin was carried out in a 50-liter jar fermentor with 30 liters of the above medium for 2 days at 27°C. Antiviral activity assays for screening, following the fermentation and for purifying virantmycin were carried out by the plaque reduction test with vesicular stomatitis virus (VSV) on a rabbit fibroblast cell line RK-13 cells. Virantmycin production in the fermentation reached a maximum at about 40 hours.

An ethyl acetate extract of the cultured broth was subjected to high performance liquid chromatography (Prep LC/System 500) on silica gel (developer: $CHCl_3$ - MeOH, 30: 1). Further purification was carried out with a silica gel preparative thin-layer chromatography (Kiesel gel 60 F_{254}, Merck, developer: benzene-acetone, 2: 1) to obtain the pure antibiotic as a white powder, mp. 42~43°C, $[\alpha]_D^{25}$ −0.5° (*c* 1, $CHCl_3$).

The molecular formula $C_{19}H_{26}NO_3Cl$ was established from the elemental analysis (C, 65.1%; H, 7.1%; N, 3.7%; Cl, 10.3%) and high resolution mass spectrum [M^+ *m/z* 351, 351.1613 calcd. for $C_{19}H_{26}NO_3Cl$, 351.1601]. The UV spectrum (in EtOH) of virantmycin showed maxima at 228 nm (ε, 3500) and 306 nm (ε, 8100). The IR spectrum (in CCl_4) exhibited absorption bands characteristic of a carboxylic acid at 3200~2400 cm^{-1} and 1690 cm^{-1}, as shown in Fig. 1. The ^{13}C-NMR spectrum (in $CDCl_3$) is shown in Fig. 2. It is soluble in organic solvents such as dimethyl sulfoxide, methanol, acetone, chloroform, benzene and ethyl acetate, and insoluble in water.

The assay of antiviral activity of antibiotic was performed by a plaque reduction test[2] which is used in the estimation of interferon activity. The following eight animal viruses were used as challenge virus: Indiana strain of vesicular stomatitis virus (VSV), Egypt Ar 339 strain of Sindbis virus (SbV), McMILLAN strain of Western equine encephalitis virus (WEE), MIYADERA strain of

Fig. 1. IR spectrum of virantmycin (CCl_4).

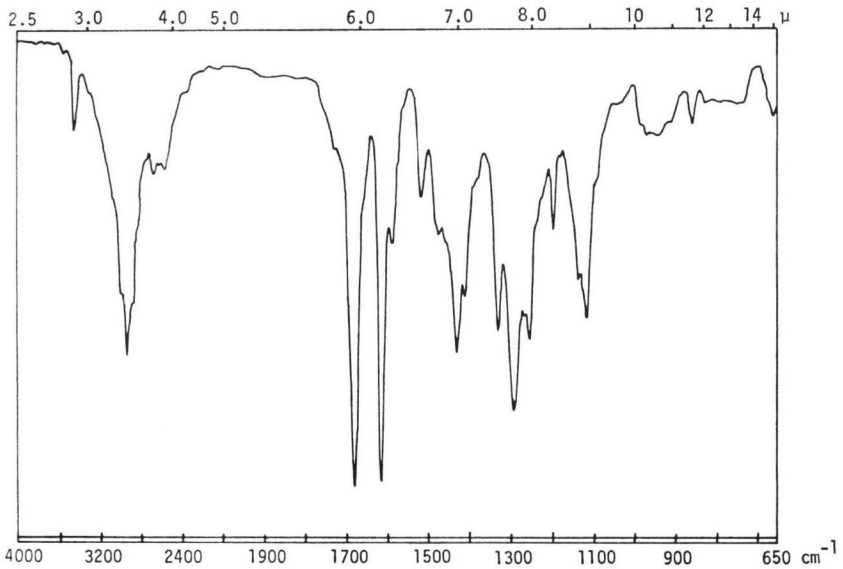

Fig. 2. ^{13}C-NMR spectrum of virantmycin ($CDCl_3$).

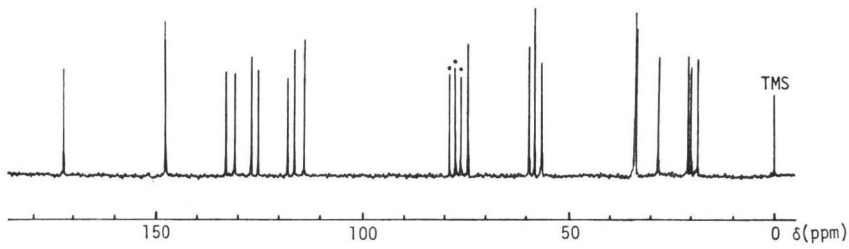

Table 1. Antiviral effect of virantmycin on several RNA and DNA viruses.

	Virus	Minimum effective dose (μg/ml)
RNA virus	VSV	0.008
	SbV	0.006
	WEE	0.003
	NDV	0.04
DNA virus	Vac-DIE	0.005
	Vac-IHD	0.004
	HSV-1	0.03
	HSV-2	0.02

Newcastle disease virus (NDV), DIE strain of vaccinia virus (Vac-DIE), IHD strain of vaccinia virus (Vac-IHD), HF strain of herpes simplex virus type 1 (HSV-1), UW strain of herpes simplex virus type 2 (HSV-2).

For testing, primary chick embryonic cells (CE cells) were prepared by trypsinization of 9-day chick embryos and cultivated with 3 ml of minimum essential medium supplemented with 10% calf serum (MEM CS 10%) in 40mm glass dishes. Monolayers of CE cells in MEM CS 2% were incubated with a solution (2 ml) of the antibiotic (10~0.001 μg/ml) in dimethyl sulfoxide-H_2O for 20 hours at 37°C. After pretreatment, the cells were washed with phosphate-buffered saline, pH 7.2. Then, plaque formation[3] was induced by infection with approximately 100 PFU (plaque forming unit) of each virus. The minimum effective dose of antibiotic (the concentration showing 50% reduction of plaque count of a challenge virus) for each virus is shown in Table 1. All viruses investigated were inhibited at very low concentrations with little difference between NDV, HSV-1 and HSV-2 and other viruses. We assume that virantmycin can exert an effect on cell membranes including specific virus receptor sites and the replication of viruses may be suppressed at a very early stage.

Virantmycin also exhibits weak antifungal activity. The minimum inhibitory concentration of the antibiotic against *Saccharomyces sake*, *Piricularia oryzae*, *Trichophyton interdigitale*, *Aspergillus niger* was 25 μg/ml.

The LD_{50} value of virantmycin in mice (CDF_1) is 5 mg/kg by intraperitoneal injection. We are now investigating the mechanism of action of virantmycin.

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