VERTICILLIN A, A NEW ANTIBIOTIC FROM *VERTICILLIUM* SP.

Sir:

A species of *Verticillium*, an imperfect fungus isolated from a basidiocarp of *Coltricia cinnamomea* (*Polystictus cinnamomeus*), produced a new antibiotic, verticillin A. *Verticillium* sp. strain TM-759 on an agar slant was inoculated into a shaken 500-ml Sakaguchi flask containing 100 ml of a medium containing (in g/liter): glucose, 30; peptone, 20; NaCl, 5. The pH of the medium was adjusted to 6.8 prior to sterilization. The inoculated flask was incubated at 27°C for 24 hours with shaking to produce a primary seed culture. Two samples of seed culture broth (5 ml) were transferred each into a 2-liter Erlenmeyer flask containing 500 ml of the same medium and incubated on a rotary shaker (180 r.p.m.) at 27°C for 3 days. The secondary seed culture broth thus obtained was transferred into a 30-liter jar fermenter containing 20 liters of the same medium and 2 ml of Nissan Uniol D-2000 as a defoamer. Fermentation was carried out at 27~28°C for 2 days with an air rate of 20 liters/min. at a pressure of 0.5~0.7 kg/cm^2 with an agitator speed of 250 r.p.m.

The mycelium thus obtained (6 jar fermenters, wet weight 5.47 kg) was collected by centrifugal separation and extracted with acetone (10 liters) and then ethyl acetate (8 liters) with stirring at room temperature. The extracts were combined and evaporated *in vacuo* at 50~60°C. The residue was dissolved in ethyl acetate, washed with 5% sodium carbonate and water, dried over Na_2SO_4, and evaporated *in vacuo*, leaving a residue (47.55 g). This residue was added to ether (200 ml) and stirred at room temperature for 30 minutes, giving an ether-insoluble product (4.34 g), which was crystallized from pyridine-acetone to give crude verticillin A (2.52 g).

Fig. 1. Infrared spectrum of verticillin A (nujol mull).

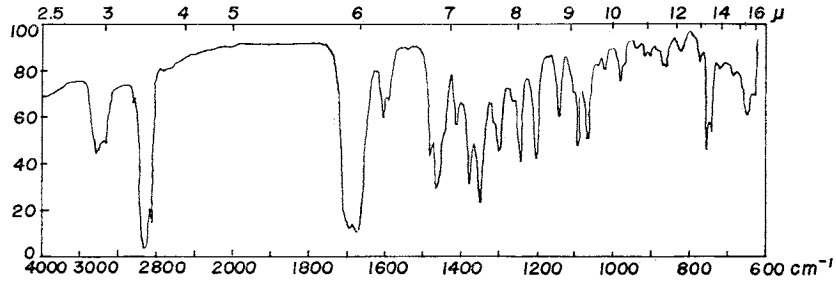
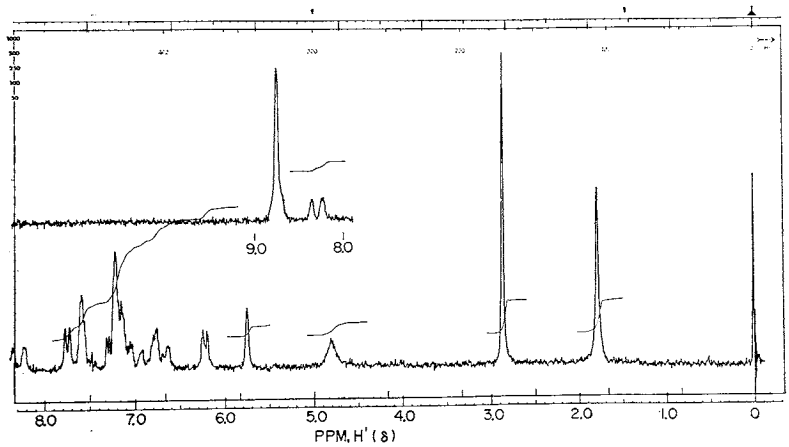


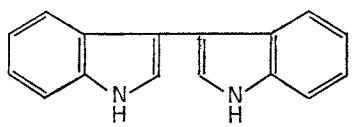
Fig. 2. NMR spectrum of verticillin A·xC$_5$H$_5$N(C$_5$D$_5$N).



Verticillin A* was obtained as pale yellow plates, $C_{30}H_{28}O_6N_6S_4 \cdot CHCl_3$, m.p. 199~213°C (decomp.) (from chloroform), pale yellow needles, $C_{30}H_{28}O_6N_6S_4 \cdot \frac{2}{3}$pyridine, m.p. 202~217°C (decomp.) (from pyridine), or a pale yellow amorphous powder, $C_{30}H_{28}O_6N_6S_4$, m.p. 203~214°C (decomp.) (from tetrahydrofuran), and showed $[\alpha]_D$+727.5°, λ_{max}^{dioxan} 306 mμ (ε 6300), and infrared absorptions at 3420, 3335, 1703, 1694, 1675, 1608, 1594, 1350, 1300, 1246, 1202, 1141, 1092, 1064, 982, 753, and 745 cm^{-1} (Fig. 1). The NMR spectrum is shown in Fig. 2. On acetylation with acetic anhydride in pyridine, verticillin A gave an acetate as a yellow powder, $C_{32}H_{30}O_7N_6S_4$, m.p. 220~243°C (decomp.) (from chloroform-methanol).

Moreover, verticillin A showed an ion at m/e 64 due to loss of S_2[2] in the mass spectrum** and four COTTON effects, a positive at 236, a negative at 272, a positive at 307, and a negative at 375 mμ in circular dichroism.

The presence of acid amide bands in the infrared spectrum in conjunction with the positive COTTON effect at 236 mμ indicates the presence of diketopiperazine[3~5] moiety in verticillin A. The COTTON effects at 272, 307, and 375 mμ are assigned to the disulfide chromophore[6~8], the presence of which is supported by the mass spectrum. These data suggest that verticillin A has a disulfide-bridged diketopiperazine system. Furthermore, as verticillin A gave diindolyl-(3,3') (I) on treatment with alkali, it may be a compound belonging to the gliotoxin group[5~9].



(I)

For biological evaluation, verticillin A acetate was used, as it is more soluble in water.

Antimicrobial activity was found against Gram-positive bacteria and mycobacteria but not against Gram-negative bacteria and fungi (Table 1).

Table 1. *In vitro* antimicrobial activity of verticillin A acetate

Test organisms	MIC (mcg/ml)
Bacillus subtilis PCI 219	2
Bacillus anthracis	10
Staphylococcus aureus FDA 209P	2
Mycobacterium tuberculosis H37Rv	1
Escherichia coli	>50
Salmonella typhosa	>50
Candida albicans M-9	>50
Aspergillus niger	>50
Trichophyton rubrum	>50
Epidermophyton floccosum	>50
Trichomonas vaginalis	12.5

Table 2. Antitumor effect of verticillin A acetate on EHRLICH ascites carcinoma (TPCV method)[10]

Sample	Dose (mg/kg/day)	No. of deaths	Tumor index	Effect
Verticillin A	5	5/5	—	Toxic
	2.5	3/5	—	Toxic
	1.0	0/5	0.17	++
	0.5	0/5	0.37	+
	0.25	0/5	0.83	−

The cytotoxic effect (ED_{50}) against HeLa cells was 0.2 μg/ml. Antitumor effect was observed against EHRLICH ascites tumor as shown in Table 2. The antiviral activity of this antibiotic against poliovirus (Type 1) using GM cells seems to be associated with its cytotoxicity even though the multiplicity of infection was low (0.05). Although this antibiotic has the disulfide-bridged diketopiperazine moiety[11], its ability to inhibit the multiplication of poliovirus seems to be very weak.

The acute toxicity (LD_{50}) in mice was determined intraperitoneally as 7.6 mg/kg, observed 10 days after injection as the antibiotic showed delayed toxicity.

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* Elemental analyses were carried out for crystal-solvent-free samples, after quantitative analyses of crystal solvents by use of an apparatus[1] of the carbon and the hydrogen determination combining with differential thermal analysis.

** The parent peak (M^+) could not be observed under any conditions, but the molecular weight was shown to be 674 by vapor pressure osmometry.

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