

STILBELLIN, A NEW ANTIBIOTIC FROM *STILBELLA* SP.

Sir:

We wish to report the isolation of a new antibiotic, stilbellin, which was produced by a coprophilous hyphomycete, *Stilbella* sp. The fungus was obtained from deer dung and identified according to MORRIS¹⁾. Fermentations were conducted under submerged culture conditions for 7 days at 28°C in a medium containing 3% glucose, 2% peptone, 0.5% sodium chloride and 0.2% soy bean oil. The pH was adjusted to 6.8 prior to sterilization. The metabolite was recovered from the mycelium by methanol extraction and purified by direct recrystallization from methanol.

Stilbellin, a neutral polypeptide, was obtained as colorless needles, m.p. 228~230°C, $[\alpha]_D^{25} +15.9^\circ (\pm 1.5^\circ)$ (c 0.371 in methanol), ultraviolet: end absorption, and ν_{\max} (KBr-disk) 3293, 2980, 2940, 1650, 1538 (C=O and NH), 1080, and 697 cm^{-1} . It was soluble in methanol, ethanol, isopropanol, butanol, pyridine, acetic acid and dimethylformamide, and insoluble in water, chloroform, ether, ethyl acetate and acetone. The ferric chloride and ninhydrin tests were negative.

Stilbellin was hydrolyzed with 6N-hydrochloric acid and analyzed by an automatic amino acid analyzer. The following amino acids were identified: 2 mol. of proline, 2 mol. of phenylalanine, 3 mol. of hydroxy-

proline, 2 mol. of leucine, 2 mol. of glycine, 2 mol. of glutamic acid, and 1~2 mol. of an unknown amino acid, which were calculated as glycine. The molecular weight* was determined by the ARCHIBALD method of sedimentation²⁾ in methanol and by a combination of sedimentation and diffusion³⁾ in butanol. The former method afforded the molecular weight of 1470 and the latter did that of 1500. The elementary analysis was as follows: C 57.82, H 7.44, N 14.51%. ASSIGNE's tests for halogen, sulfur and phosphorus were negative.

The physico-chemical properties of stilbellin are very similar to those of an antiprotozoal anthelmintic antibiotic, anti-amoebin^{4,5,6)} which was isolated as colorless needles, m.p. 219~220°C (decomp.), $[\alpha]_D +10^\circ$. However, the amino acid composition (proline, α -aminoisobutyric acid, phenylalanine, valine, leucine, hydroxyproline, lysine and glutamic acid) differed from that of stilbellin.

Stilbellin has weak antimicrobial activity as shown in Table 1. It did not inhibit the growth of HeLa cells in culture. The acute toxicity (LD₅₀) in mice was determined intraperitoneally as 47.5 mg/kg.

Acknowledgement

The authors express their sincere thanks to Dr. M. J. THIRUMALACHAR, Research Laboratories, Hindustan Antibiotics Ltd., for a supply of anti-amoebin.

KATSUO SASAKI
HITOSHI MINATO
KEN KATAGIRI
SHOHEI HAYAKAWA
TAKASHI MATSUSHIMA

Shionogi Research Laboratory
Shionogi & Co., Ltd.
Fukushima-ku, Osaka, Japan

(Received October 26, 1970)

References

- 1) The Synnematosus Genera of the Fungi Imperfecti Western Illinois Univ. Series in the Biological Sciences No. 3 (1963) Macomb. Illinois, p. 120~121

Table 1. Antimicrobial activity of stilbellin
in vitro.

Test organisms	M.I.C. ($\mu\text{g/ml}$)
<i>Bacillus subtilis</i> PCI 219	20
<i>Bacillus anthracis</i>	5
<i>Staphylococcus aureus</i> FDA 209P	20
<i>Mycoplasma pulmonis</i>	25
<i>Escherichia coli</i>	>50
<i>Salmonella typhi</i>	>50
<i>Klebsiella pneumoniae</i>	>50
<i>Mycobacterium tuberculosis</i> H ₃₇ RV	>50
<i>Candida albicans</i>	>50
<i>Trichophyton rubrum</i>	>50
<i>Trichomonas vaginalis</i>	50
<i>Tetrahymena gelleii</i> (W)	50

* The molecular weight was determined by Dr. H. INOUE, to whom the authors express their deep gratitude.

- 2) ARCHIBALD, W. J.: A demonstration of some new methods of determining molecular weight from the data of the ultracentrifuge. *J. Phys. Colloid Chem.* 51 : 1204, 1947
- 3) SCHACHMAN, H. K.: Ultracentrifugation, diffusion, and viscometry. *Methods in Enzymology* 4 : 32, 1957
- 4) DESHMUKH, P. V. & M. G. VAIDYA: L-2-Amino-3-phenyl-1-propanol (L-phenylalanyl) as a constituent of a fungal metabolite. *Nature* 217 : 849, 1968
- 5) DESHMUKH, P. V.: Antimoebin, a new anti-protozoal-anthelmintic antibiotic. II. Chemical characterization. *Hindustan Antibiot. Bull.* 10 : 299~302, 1968
- 6) VAIDYA, M. G.; P. V. DESHMUKH & S. N. CHARI: Amino acid sequence in antimoebin. *Hindustan Antibiot. Bull.* 11 : 81~89, 1968