Communications to the editor

VERMICILLIN, A NEW METABOLITE FROM *PENICILLIUM VERMICULATUM* INHIBITING TUMOR CELLS *IN VITRO*

Sir:

In the systematic research for metabolites effective against tumor cells, the antibiotics, vermiculine[1] and vermistatin[2], effective against P 388 cells have been isolated from the filtrate of *Penicillium vermiculatum* DANG. This strain produced on a modified CZAPEK-DOX medium, in addition to the small amount of already known antibiotics, a new antibiotic which we have named vermicillin. The present paper deals with the isolation, physico-chemical and biological properties of vermicillin.

For the production of vermicillin, a medium containing 9% glucose, 0.2% $NaNO_3$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl and 0.001% $FeSO_4 \cdot 7H_2O$ adjusted to pH 6.3, was found to be suitable. The medium (100 ml in 500-ml shake flasks) was inoculated with vegetative inoculum of *P. vermiculatum* DANG. CCM F 276 (10% v/v, 42 hours old) and cultivated for 120~140 hours at 28°C on a rotary shaker at 220 r.p.m.

The clear filtrate obtained after separation of the mycelial cake was extracted three times with tetrachloromethane (ratio 2 : 1, for 30 minutes, at 24~25°C). The collected extracts were dried by filtration through anhydrous Na_2SO_4 and the solvent was removed completely under reduced pressure at 35°C. The total solids were triturated several times with petroleum ether (b.p. 30~50 °C) and the extracts so obtained were collected, concentrated under reduced pressure and allowed to stand for 18 hours at 4°C. A crude crystalline substance thus obtained was separated by silica-gel column-chromatography (Silica gel Lachema L, column diameter 25 mm, length 250 mm), using a mixture of chloroform - methanol (10: 1) for elution. The fractions containing vermicillin were collected, evaporated to dryness, and the crude vermicillin dissolved in boiling *n*-hexane. The solution was allowed to stand at 5°C overnight, whereupon pure vermicillin was obtained.

Vermicillin[3] crystallized from *n*-hexane as white cubes with neutral properties, (m.p. 96~100°C), with a slight optical rotation $[\alpha]_D^{22}$ −1.0° (*c* 0.2, chloroform). The molecule contains C, H and O. The ultraviolet absorption spectrum of vermicillin in methanol (Fig. 1) indicates maxima at 218 and 252 nm ($E_{1cm}^{1\%}$ 788 and 680 respectively). The infrared absorption spectrum in chloroform (Fig. 2) shows maximum absorption at: 860, 890, 905, 940, 960, 1050, 1060, 1100, 1240, 1290, 1340, 1405, 1445, 1460, 1560, 1580, 1600, 1640, 1685, 1720, 1770, 2850, 2950, 3000 cm^{-1}. The antibiotic is soluble in benzene, chloroform, tetrachloromethane, *n*-hexane, low aliphatic alcohols and their esters with acetic acid, petroleum ether and DMSO. The antibiotic is insoluble in water. Vermicillin gives a yellow color with concentrated sulphuric acid and methanolic solution of KOH, and dark brown with $FeCl_3$. Positive test with I_2 is obtained with vermicillin.

In *in vitro* experiments,[4] the antibiotic inhibits the utilization of ^{14}C-labelled precursors of nucleic acid synthesis, *i.e.* adenine, uridine, thymidine, and those of protein synthesis, *i.e.* L-valine in leukemia P 388 (Fig. 3) as well as in EAC, NK/Ly and L 1210 cells. From the inhibition of incorporation of uridine one can assume that the antibiotic affects preferentially synthesis of RNA. Vermicillin suppresses the *in vitro* proliferation of leukemia P 388 cells (Fig. 4). Substrates used and conditions for the culture of this strain are as described previously[5,6].

Fig. 1. Ultraviolet absorption spectrum of vermicillin (in methanol).

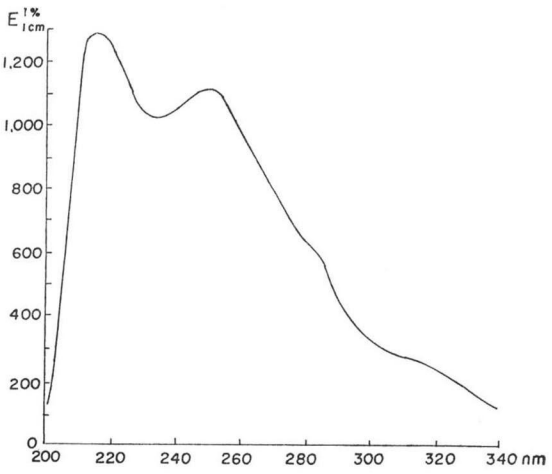


Fig. 2. Infrared absorption spectrum of vermicillin (in chloroform).

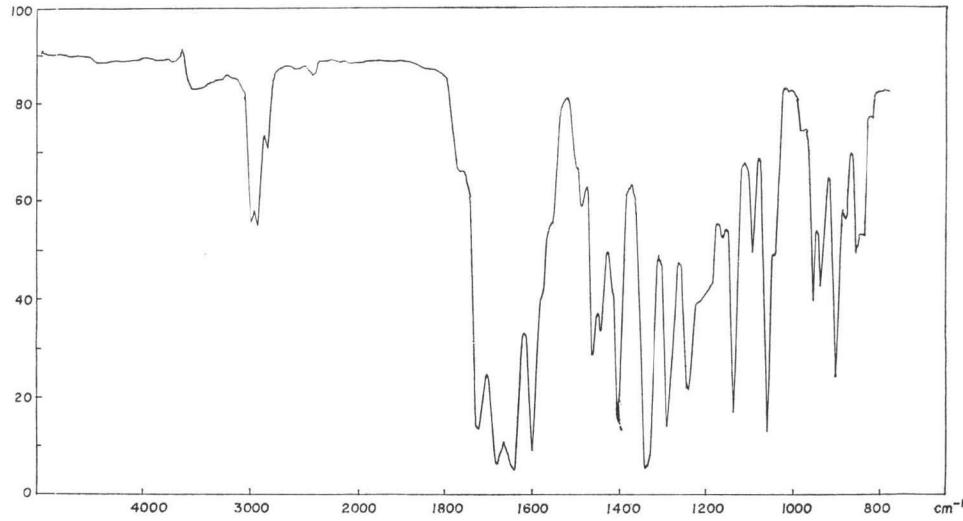


Fig. 3. Inhibition of incorporation of ^{14}C-labelled precursors into P388 cells by vermicillin.

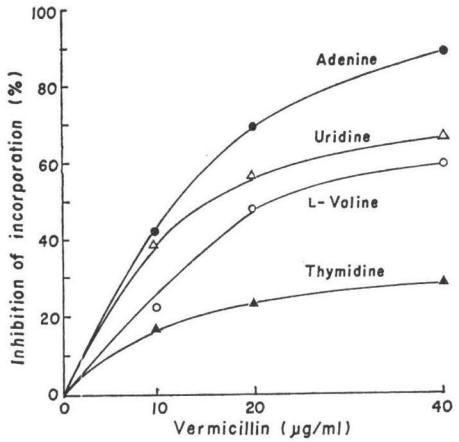
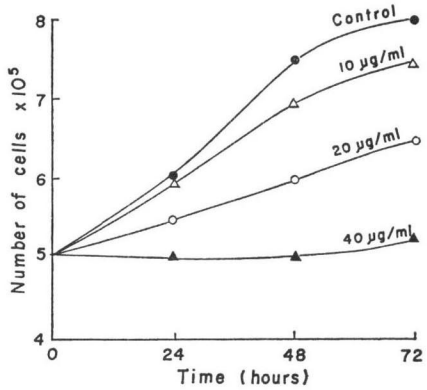


Fig. 4. *In vitro* inhibition of proliferation of P388 cells by vermicillin.



The acute LD_{50} in mice was found to be 270 mg/kg determinated by intraperitoneal injection with arabic gum. *In vivo* vermicillin administered in the form of oil-suspension (up to 50 mg/kg), was not active against P 388 and L 1210 tumors. Vermicillin showed inhibitory activities against Gram-negative bacteria at 100 μg/ml, but was ineffective against Gram-positive bacteria, *Candida pseudotropicalis* and *Tritrichomonas foetus*.

Up to the present, the following polysaccharide metabolites of *P. vermiculatum* have been isolated: luteic acid[7,8], mucilate[9] and talaron[10]. From the culture filtrate of *P. vermiculatum* DANG. strain CCM F 276 were isolated vermiculine[1], the aglycoside macrolide lactone[11~14], and vermistatin[2]. Vermicillin differs from all the foregoing metabolites in its physico-chemical and biological properties. Luteic acid and mucilate are biologically inactive. Although the three antibiotics, talaron, vermiculine and vermistatin, inhibit tumor cells, they differ from vermicillin. Talaron inhibits the growth of fungi, vermiculine inhibits Gram-positive bacteria, and vermistatin is effective only against tumor cells. In contrast, vermicillin inhibits tumor cells and Gram-negative bacteria.

Vermicillin is not identical with any one of the known microbial metabolites inhibiting the growth and biochemical functions of tumor cells[15].

Because many maxima of vermicillin in infrared absorption spectrum are identical or very similar to those of vermiculine, one can speculate that vermicillin is a derivative of vermiculine.

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