Communications to the editor

A NEW ANTIBIOTIC, ANTLERMICIN A

Sir:

We wish to describe here isolation and characterization of a new antibiotic from cultures of a strain No. T-90 which was classified as a subspecies of *Micromonospora chalcea* and designated as *Micromonospora chalcea* subsp. *kazunoensis*. The strain was isolated from a soil sample collected in Kazuno-shi, Akita Prefecture, Japan. Taxonomic studies will be reported in a separate paper.

The strain was cultured at 28°C for 140 hours in a fermentation tank under agitation (250 rpm) and aeration (400 liters per minute) in 400 liters of a medium containing 1.5% dextrin, 3% soluble starch, 0.3% meat extract, 0.3% Polypeptone, 0.3% dry yeast, 1% soybean flour, 0.3% $CaCO_3$, and 0.3% NaCl. Fermentation broth (pH 7.1) was filtered with the aid of 5% Celite and the filtrate was extracted twice with ethyl acetate (200 and 80 liters). Mycelium was extracted with methanol (200 liters) and the extract was concentrated *in vacuo* to give an aqueous solution (40 liters), which was then extracted twice with ethyl acetate (30 and 10 liters). All the ethyl acetate extracts were combined and concentrated *in vacuo* to dryness. The syrupy residue was purified with successive silicic acid chromatographies using three different solvent systems: (1) Benzene - methanol (10: 1→7: 3), (2) ethyl acetate - acetone (7: 1→2: 1), and (3) chloroform - methanol (15: 1). Purified powder thus obtained (180 mg) contained a considerable amount of metals, which were removed by the following procedure. The powder was dissolved in ethyl acetate and washed with 0.1 N HCl followed by water. The organic layer was dehydrated with anhydrous sodium sulfate and concentrated to dryness. The residue was dissolved in a small amount of ethyl acetate and the antibiotic was precipitated by adding hexane. After filtration, pure antlermicin A free acid (150 mg) was obtained as white powder.

Antlermicin A melted at 199~204°C with decomposition. It is optically active, $[\alpha]_D^{28}$ −67.6° (*c* 1, methanol). Elementary analysis was C 59.33, H 7.20, N 1.98%. The antibiotic is a monobasic acid with pKa′ of 3.9 (in 70% methyl cellosolve). A titration equivalent was 1,365. Molecular weight determined by a vapor pressure osmometry in ethyl acetate was 1,306. From these data, a tentative molecular formula, $C_{64\sim67}H_{90\sim98}N_2O_{25\sim27}$ was proposed for this antibiotic. It has a characteristic UV absorption spectrum, λ_{max} nm ($E_{1cm}^{1\%}$): in 90% methanol, 232 (154), 265 (95), 278sh (78); in 1 N HCl - methanol (1: 9), 253~262 (63); in 1 N NaOH - methanol (1: 9), 235 (132), 265 (90), 275sh (85). The spectra are shown in Fig. 1. It is soluble in lower alcohols, acetone and ethyl acetate, sparingly soluble in ether and chloroform, and hardly soluble in petroleum ether and water. It produces a blue color with concentrated sulfuric acid and reduces permanganate solution. It is positive to periodate-benzidine, 2,4-dinitrophenylhydrazine, *o*-dianisidine, RYDON-SMITH, ferric chloride and ELSON-MORGAN tests but negative to FEHLING, MOLISCH and anthrone tests. The IR spectrum and ^{13}C NMR spectrum are shown in Fig. 2 and Fig. 3, respectively. In the ^{13}C NMR spectrum, at least 64 carbon signals are observed. Presence of an aldehyde group is indicated by NMR spectra (δ_H 9.62 and δ_C

Fig. 1. UV absorption spectra of antlermicin A.

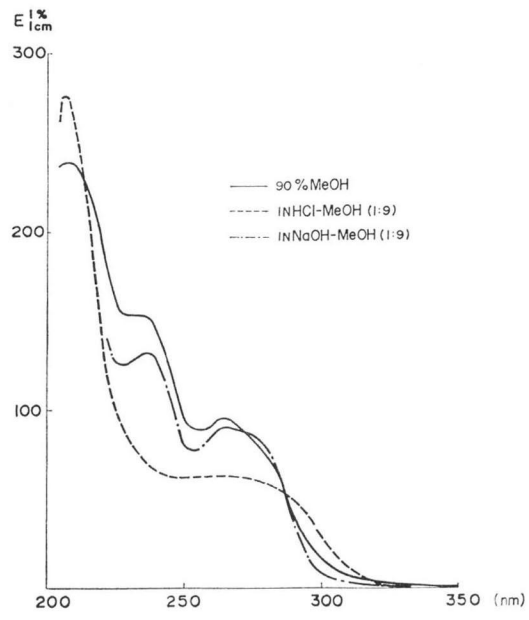

Fig. 2. IR absorption spectrum of antlermicin A (in KBr).

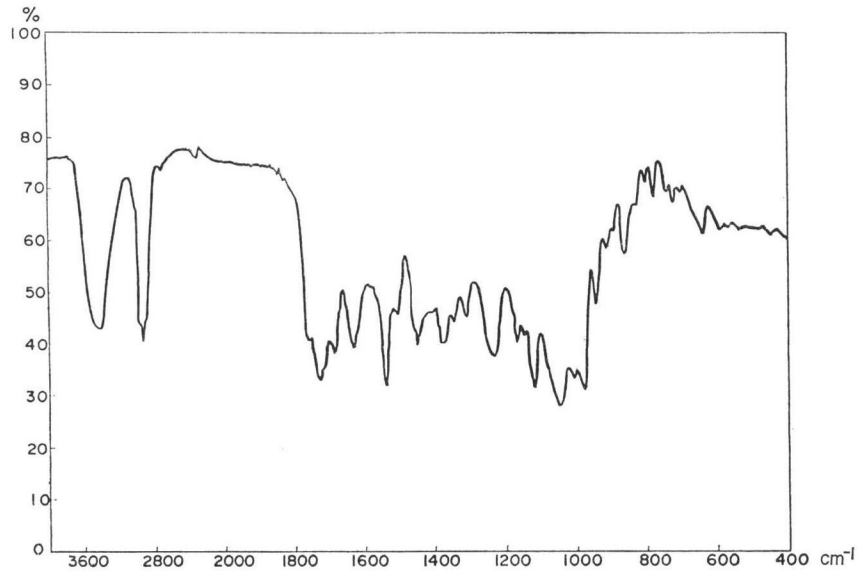

Fig. 3. ^{13}C NMR spectrum of antlermicin A (JEOL FX 100, in acetone-$^{12}C_3$, d_6).

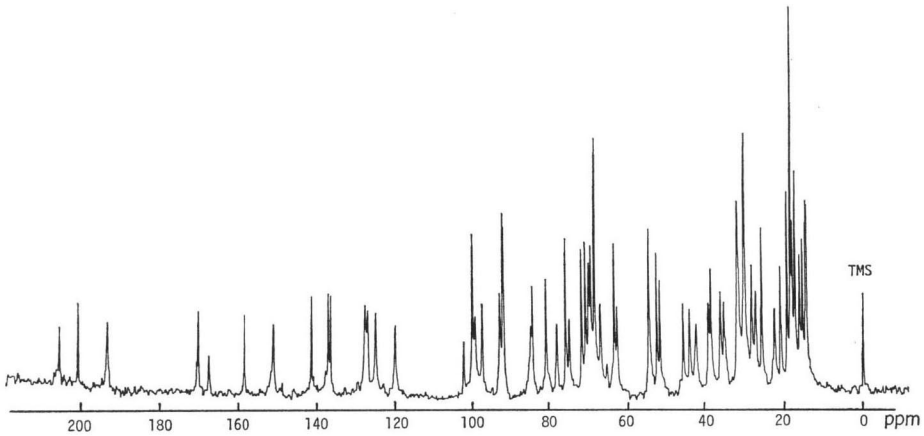

Table 1. Antimicrobial spectrum of antlermicin A.

Microorganism	MIC (μg/ml)
Bacillus subtilis PCI 219	0.015
Bacillus subtilis H17 (rec$^+$)	<0.015
Bacillus subtilis M45 (rec$^-$)	<0.015
Bacillus cereus var. *mycoides* ATCC11778	0.5
Bacillus agri	2
Micrococcus luteus	2
Staphylococcus aureus FDA 209P	>50
Escherichia coli	>50
Salmonella typhimurium TV 119	>50
Mycobacterium phlei	>50

Conventional agar-dilution method was employed using bouillon agar.

193.5 ppm).

Antlermicin A showed strong inhibitory activity against *Bacillus subtilis* (Table 1). Other Gram-positive bacilli and cocci are less sensitive. It showed no activity against Gram-negative bacteria, mycobacteria, yeasts and fungi tested. The antibiotic showed cytotoxicity against YOSHIDA sarcoma cells (I_{50} 1 μg/ml). Mice tolerated intraperitoneal injection of 25 mg of per kg of body weight. A dose of 50 mg/kg was lethal.

Antlermicin A seems to belong to an ionophore antibiotic group because the metal salt is soluble in organic solvents. However, it is different in its molecular formula and the UV absorption spectrum from all the polyether and peptide antibiotics hitherto reported.

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