Communications to the Editor

A NEW ANTIBIOTIC, RK-699A

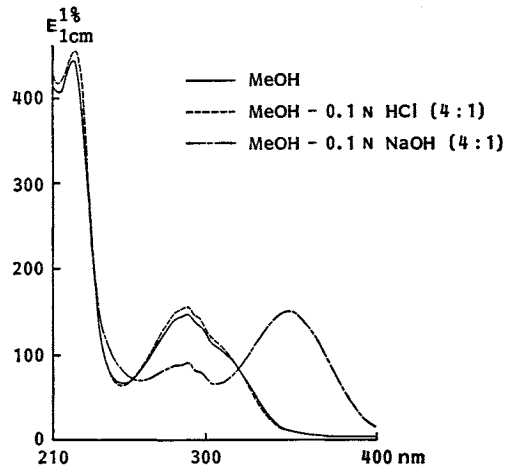
Sir:

We wish to report a new peptide antibiotic which shows protective effect on cucumber anthracnose in pot test. The antibiotic was isolated from a culture broth of a streptomycete, which was isolated from a soil sample collected in Kurihashi, Saitama Prefecture, Japan. Taxonomic study showed that the strain is most similar to *Streptomyces purpurascens*.

This strain (strain RK-699) was cultured at 28°C for 72 hours in a jar fermenter containing 18 liters of a medium consisting of glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, sodium chloride 0.2%, and dipotassium hydrogen phosphate 0.005%. The pH was adjusted to 7.0 before sterilization.

The culture broth (40 liters) was filtered, and the mycelial cake was extracted with 80% aqueous acetone. After removal of acetone, the extract was combined with the filtrate. It was adjusted to pH 3 and extracted twice with 15 liters of butanol. The upper layer was washed twice with each 5 liters of water at pH 8. The butanol extracts were combined and concentrated *in vacuo* to dryness. The residue was applied on a silica gel column prepared with chloroform. The elution was made stepwise by chloroform - methanol (50 : 1 → 1 : 1). Active fractions were combined and concentrated *in vacuo* to dryness affording 0.8 g of a crude powder. It was further purified by chromatography on a Sephadex LH-20 column with the solvent system, butanol - methanol - water (3 : 1 : 2), and then on TSK-Gel Toyopearl HW-40F with the same solvent system. Final purification was achieved by preparative HPLC on a column (20×300 mm) of Nucleosil $5C_{18}$ with methanol - 0.25% diethylamine - formic acid buffer (pH 5.3) (71 : 29). The active fractions were combined, concentrated *in vacuo* and lyophylized to give a colorless powder. The inorganic salts were removed by dissolving the powder in 30% aqueous methanol then passage through a Amberlite IRC-50 (H^+) column eluting with 0.5 N pyridine. The eluate was concentrated *in vacuo* and lyophilized, affording 120 mg of RK-699A as a white powder.

Fig. 1. UV absorption spectra of RK-699A.



The antibiotic decomposes gradually above 245°C. It is optically active, $[\alpha]_D^{20}$ −31° (*c* 0.5, MeOH). It is an amphoteric compound with a *pKa'* of 2.7, 4.1, 5.4, 6.7 in 70% Methyl Cellosolve. It is soluble in lower alcohols, sparingly soluble in water, hardly soluble in acetone and ethyl acetate.

The high-resolution fast atom bombardment mass spectrometry (FAB-MS) gave $(M+H)^+$ *m/z* 1,729.826. Calcd for $C_{83}H_{113}N_{18}O_{23}$: 1,729.821. Elemental analysis gave C 56.02, H 6.49, N 13.84. Calcd for $C_{83}H_{112}N_{18}O_{23}\cdot 3H_2O$: C 55.88, H 6.67, N 14.13.

It has a characteristic UV absorption spectra as shown in Fig. 1. UV λ_{max}^{MeOH} nm ($E^{1\%}_{1cm}$) 222 (445), 284 (sh, 144), 290 (148), 295 (sh, 137), 305 (sh, 109); $\lambda_{max}^{MeOH-0.1N\,HCl\,(4:1)}$ 222 (461), 284 (sh, 153), 290 (160), 295 (sh, 146), 305 (sh, 119); $\lambda_{max}^{MeOH-0.1N\,NaOH\,(4:1)}$ 222 (447), 282 (sh, 89), 289 (91), 295 (sh, 81), 348 (150). The IR spectrum, ^1H NMR, and ^{13}C NMR are shown in Figs. 2, 3, and 4, respectively. ^{13}C NMR data in DMSO-d_6 indicated the resonance of 81 ~ 83 carbons.

Amino acid analysis of the acid hydrolysis products gave the following amino acids; aspartic acid (0.9), serine (1.0) glutamic acid (2.2), glycine (3.2), alanine (2.1), isoleucine (1.8), lysine (1.0), and tryptophane (0.3). The numbers

Fig. 2. IR spectrum of RK-699A (in KBr).

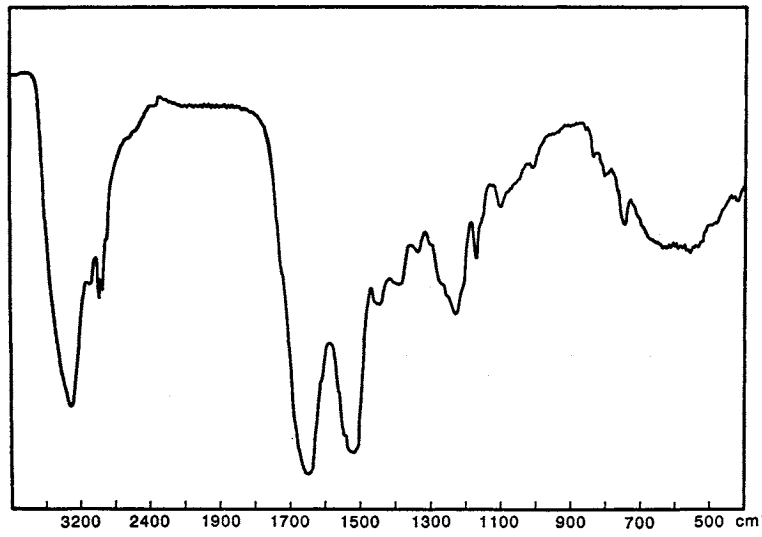
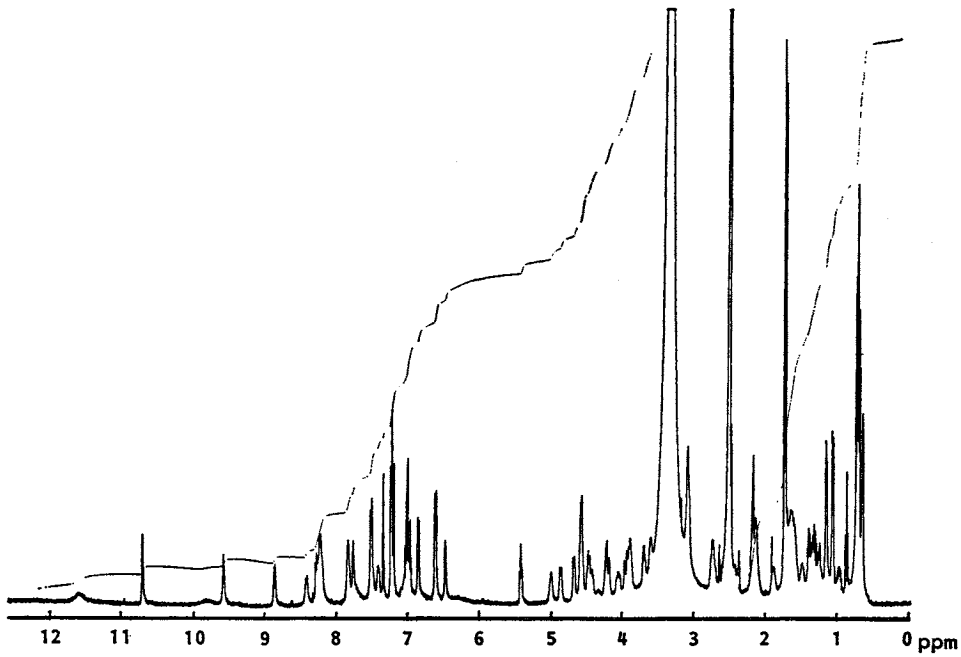


Fig. 3. ^{1}H NMR spectrum of RK-699A (Jeol GX-400, in DMSO-d_6).



in parenthesis indicate molar ratios. RK-699A showed positive tests to permanganate, 2,4-dinitrophenylhydrazine and Rydon-Smith reagents but was negative to ferric chloride spray.

The antibiotic showed weak activity against *Mycobacterium phlei* and fungi as shown in Table 1. Nevertheless, it showed a preventive value of 88% against cucumber anthracnose when administered at a concentration of 125 ppm in pot test. It was not toxic to mice when 200 mg/kg was injected intraperitoneally.

Among the known peptide antibiotics containing tryptophane, antibiotics A21978C series[1] have some resemblance to RK-699A but

Fig. 4. ^{13}C NMR spectrum of RK-699A (Jeol GX-400, in DMSO-d_6).

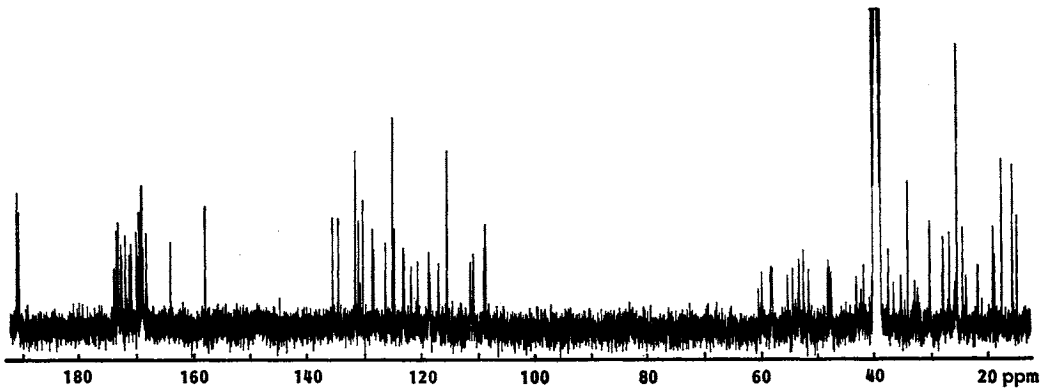


Table 1. Antimicrobial Activity of RK-699A.

Test organism	MIC (μg/ml)
Staphylococous aureus FDA 209P IFO 12732	>100
Escherichia coli K-12 IFO 3301	>100
Salmonella typhimurium TA1535	>100
Mycobacterium phlei IFO 3158	100, 12.5[a]
Xanthomonas oryzae IFO 3312	100
Rhizoctonia solani IFO 6258	100[a]
Colletotrichum lagenarium IFO 7513	>100
Pyricularia oryzae IFO 5994	>100
Botrytis cinerea IFO 5365	100[a]

[a] Partial inhibition was observed at this concentration.

they are distinctly different in molecular weights and the compositions of amino acids.

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Reference

1) DEBONO, M.; M. BARNHART, C. B. CARRELL, J. A. HOFFMANN, J. L. OCCOLOWITZ, B. J. ABBOTT, D. S. FUKUDA, R. L. HAMILL, K. BIEMANN & W. C. HERLIHY: A21978C, a complex of new acidic peptide antibiotics: Isolation, chemistry, and mass spectral structure elucidation. J. Antibiotics 40: 761~777, 1987