Communications to the editor

ANTRIMYCIN, A NEW PEPTIDE ANTIBIOTIC

Sir:

In the course of screening for new antibiotics, a new peptide antibiotic named antrimycin, which showed antibacterial activity against *Mycobacterium smegmatis* ATCC 607, has been isolated. The structure was determined (Fig. 1) by chemical study as discussed in the following paper.[1] In this communication, the production, isolation, and chemical and biological properties of antrimycin are reported.

The antrimycin-producing strain (strain number in Institute of Microbial Chemistry, MG125-CF1) was isolated from a soil sample collected at Okunikko, Tochigi Prefecture, Japan, and classified as *Streptomyces xanthocidicus* MG125-CF1 (unpublished). The strain was precultured in a 500 ml Erlenmeyer flask containing 120 ml of medium [galactose 2%, dextrin 2%, Bactosoytone (Difco) 1%, corn steep liquor (Ajinomoto) 0.5%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2%, pH 7.4 before sterilization] on a rotary shaking machine (180 rpm) at 28°C for 2 days. The cultured broth (3 ml) was inoculated into a 500 ml flask containing 120 ml of the same medium. It was cultured under the conditions described above for 6 days.

The filtrate (9.7 liters) of the cultured broth thus obtained was passed through a carbon column (550 ml), and the adsorbed material was eluted by a linear gradient of acetone. The eluate showing antibacterial activity was dried under reduced pressure to give 21.32 g of crude material. It was adsorbed on a column of Dowex 50W-X4 (H^+ form, 550 ml) from 2% aqueous solution, and eluted with 1 N NH_4OH to give 6.08 g of brownish powder after evaporation. It was purified by column chromatography on Dowex 50W-X4 (600 ml), previously equilibrated with pyridine - acetate buffer of pH 4.65, developed with 0.4 M pyridine - acetate buffer of pH 4.65. The bioactive eluate was adsorbed on a column of Dowex 50W-X4 (H^+ form, 500 ml), and eluted with 1 N NH_4OH to give 739.4 mg of crude antrimycin after evaporation. It was further purified by column chromatography (800 ml) of SP-Sephadex C-25, pretreated with 0.05 M sodium citrate buffer on pH 4.6, developed with a linear gradient of sodium chloride (0.05 M→0.5 M). The bioactive eluate was adsorbed on a column of Diaion HP-40 (360 ml), and eluted with 50% acetone containing 0.001 N HCl to give 247.3 mg of pure antrimycin after evaporation.

Antrimycin is a colorless amorphous powder melting at 183°C (decomp.), $[\alpha]_D^{28}$ −74.0° (*c* 0.5, H_2O). The molecular formula was established as $C_{28}H_{47}N_9O_{11}$ (MW 685.75) by elemental analysis and field desorption mass spectrometry (Calcd. for $C_{28}H_{47}N_9O_{11}\cdot H_2O$: C, 47.79; H, 7.02; N, 17.91; O, 27.28. Found: C, 47.37; H, 6.77; N, 17.64; O, 27.23. M^+ of monomethyl ester, *m/z* 699). Antrimycin shows a UV absorption maximum at 229 nm (ε 21,400) in aqueous solution. The IR spectrum shown in Fig. 2 suggests the presence of peptide bonds [1650 cm^{-1} (broad) and 1530 cm^{-1} (broad)]. Antrimycin shows positive reactions with ninhydrin, RYDON-SMITH and iodine tests, but negative with SAKAGUCHI, DRAGENDORFF and PAULI reactions. It is soluble in water, methanol and dimethylsulfoxide, but hardly soluble in ethanol, acetone, ethyl acetate, chloroform and benzene. Acid hydrolysis of

Fig. 1. Structure of antrimycin.

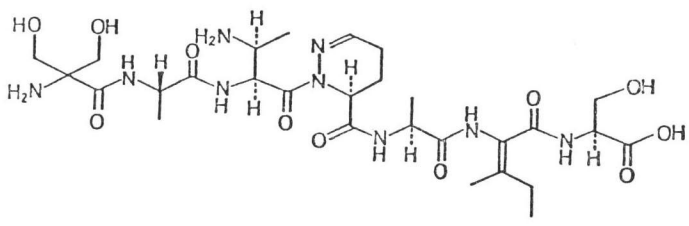
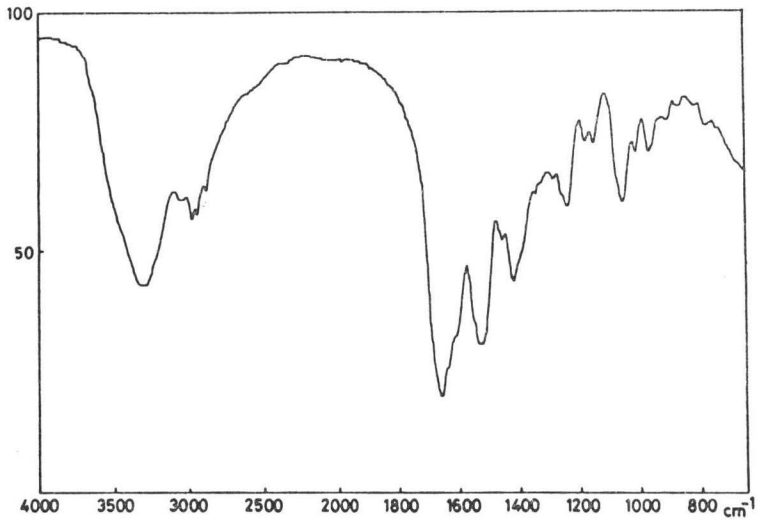


Fig. 2. IR spectrum of antrimycin (KBr).



antrimycin with 6 N HCl at 105°C for 16 hours yielded two moles of alanine and one mole each of serine, 2,2-bis(hydroxymethyl)glycine and 2,3-diaminobutanoic acid.[1] These chemical properties described above indicate that antrimycin is a new peptide antibiotic containing novel amino acids.

Antrimycin showed antibacterial activity against *Mycobacterium smegmatis* ATCC 607 (MIC 12.5 mcg/ml) on nutrient agar. *Mycobacterium tuberculosis* $H_{37}Rv$ was also inhibited at 50 mcg/ml on Kirchner semi-solid medium containing 10% calf serum after 3 weeks culture at 37°C. Interestingly, a rifampicin-resistant strain (3.1 mcg/ml) and capreomycin-resistant strain (12.5 mcg/ml) were more susceptible than the original strain. Other bacteria and fungi so far tested were not inhibited at 100 mcg/ml. Intravenous injection of 8 mg of antrimycin to mice (*ca.* 400 mg/kg) did not show any signs of toxicity.

Added in proof: Several minor congeners of antrimycin have been isolated recently. The structures were determined by using molecular secondary ion mass spectrometry. The results will be published in this Journal soon.

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Reference

1) MORIMOTO, K.; N. SHIMADA, H. NAGANAWA, T. TAKITA & H. UMEZAWA: The structure of antrimycin. J. Antibiotics 34: 1615~1618, 1981