A New Antibiotic, AM-2504

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Received June 29, 1977

In the course of screening for new alkaloids from microorganisms,¹⁾ a basic compound was isolated from the culture broth of the soil isolate, *Streptomyces* sp. strain No. AM-2504. Later, it was found that this compound is a new peptide antibiotic. The sporophore of strain No. AM-2504 was spiral, representative of the section *Spirales* of Pridham *et al.*²⁾ and the spore was oval with a smooth surface. The aerial mass color was gray and melanoid pigment did not form.

A 48-hr culture of strain No. AM-2504 was transferred into 75 liters of medium in a 100-liter fermentor and the fermentation was carried out for 2 days at 27°C. The composition of the seed medium was 2% glucose, 0.5% peptone, 0.5% meat extract, 0.3% dried yeast, 0.3% CaCO₃ (pH 7 before sterilization). The production medium contained 1% glucose, 2% starch, 0.5% yeast extract, 0.5% peptone and 0.4% CaCO₃ (pH 7 before sterilization). The titer of antibiotic AM-2504 was determined as the maximum dilution number of a Dragendorff positive solution.¹⁾

The culture broth was adjusted to pH 10 with aqueous ammonia. The antibiotic AM-2504 produced was extracted with 18 liters *n*-butyl acetate and then transferred into 1.5 liters 0.1 N hydrochloric acid. The water layer was subsequently adjusted to pH 10 with aqueous ammonia and extracted twice with 1.2 liters of ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and concentrated *in vacuo* to dryness to yield 3.6 g of a yellowish brown powder.

The crude powder was dissolved in chloroform and then chromatographed on silica gel (100 g) eluting with a solvent mixture of chloroform and methanol (100:1, v/v). The active fractions were concentrated *in vacuo* to yield 1.3 g of a yellowish powder. The yellowish powder was again chromatographed using silica gel (50 g) eluting with ethyl acetate and 780 mg of antibiotic AM-2504, a pale yellow powder was isolated from the active fractions.

Antibiotic AM-2504 is a basic compound whose mp 209°C (dec.) and $[\alpha]_D^{25} - 5.4$ ($c = 0.5$, CH₃OH). It gives a positive test on Dragendorff, ninhydrin and Rydon-Smith reactions, but negative on anisaldehyde-sulfuric acid and aniline phthalate tests. It is soluble in alcohols, ethyl acetate, acetone, chloroform, slightly soluble in benzene, and sparingly soluble in water, petroleum ether, *n*-hexane. The elemental analysis is as follows: C, 57.35; H, 6.47; N, 9.45%. Its molecular weight is 1225 as determined by Rast method. The UV spectra (Fig. 1) show maxima at $\lambda_{\max}$ 280 nm ($E_{1\%}^{1\text{cm}}$ 70), 300 (sh. 57) in methanol and *N*/20 NaOH-MeOH and $\lambda_{\max}$ 287 nm (73), 305 (75), in *N*/20 HCl-MeOH. The IR spectrum shows amide bonds ($\nu_{\max}^{\text{KBr}}$ cm⁻¹: 1660~1630) as shown in Fig. 2. The *R_f* values of antibiotic AM-2504 are as follows: 0.70 with CHCl₃-MeOH (10:1), 0.58 with benzene-acetone (1:1) and 0.10 with ethyl acetate on silica gel thin layer chromatography (Merck Kieselgel G). Antibiotic AM-2504 is hydrolyzed with 6 N HCl within 20 hr at 110°C. The amino acid analysis of its acid hydrolyzate shows the following amino acid (molar ratio): proline (5.7), valine (1.0) and two unidentified amino acids. Considering these constituent amino acid, it can most reasonably be concluded that antibiotic AM-2504 is a novel peptide antibiotic.

Antamanid³⁾ and an ionophoretic peptide⁴⁾ from beef heart mitochondria are known to be rich in proline content. From this point of view, it is intriguing that proline content is high in antibiotic AM-2504 as well.

The antimicrobial spectrum of antibiotic AM-2504 determined by agar dilution method

FIG. 1. UV Spectra of Antibiotic AM-2504.

FIG. 2. IR Spectrum of Antibiotic AM-2504 (KBr).

is shown in Table I. The antibiotic is active against *Bacillus* sp., *Corynebacterium paurometabolum* and *Clostridium* sp., but inert to *Staphylococcus aureus* and Gram-negative bacteria. No acute toxicity of the antibiotic was observed in mice after intraperitoneal (200 mg/kg) and intravenous (100 mg/kg) injection.

Since production of antibiotic AM-2504 is low in yield and accompanied with that of two known antibiotics, aureothricin and thiolutin, it would be difficult to find the antibiotic in the fermentation broth by ordinary screening method. The screening based on the chemical assay with Dragendorff's reaction led to the

TABLE I. ANTIBACTERIAL SPECTRUM OF ANTIBIOTIC AM-2504

Minimal inhibitory concentrations (MIC) were determined by the agar dilution method. Anaerobic culture of *Clostridium* sp. was carried out by steel wool method.

Test organism	MIC (mcg/ml)
<i>Staphylococcus aureus</i> FDA 209P	>100
<i>Bacillus subtilis</i> PCI 219	6.25
<i>Bacillus cereus</i> IFO 3001	1.56
<i>Bacillus cereus</i> var. <i>mycoides</i>	1.56
<i>Bacillus agri</i>	6.25
<i>Bacillus anthracis</i>	100
<i>Bacillus megaterium</i> IFO 12108	25
<i>Bacillus pumilis</i> IFO 12092	50
<i>Sarcina lutea</i> PCI 1001	>100
<i>Mycobacterium avium</i>	25
<i>Corynebacterium paurometabolum</i>	0.39
<i>Escherichia coli</i> NIHJ	>100
<i>Pseudomonas aeruginosa</i> P-3	>100
<i>Clostridium perfringens</i> ATCC 3624	6.25
<i>Clostridium acetobutylicum</i> IFO 3346	3.12
<i>Clostridium botulinum</i> IFO 3733	3.12
<i>Clostridium kintanotoi</i> IFO 3353	1.56
<i>Clostridium kluyveri</i> IFO 12016	3.12

finding of such a new antibiotic.

Acknowledgements. The authors wish to thank Kyowa Hakko Co., Ltd. for analysis of amino acids, in particular Mr. R. Masuma, Miss R. Gomyo and Miss H. Honda for their assistance.

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