NOTES

A NEW PEPTIDE ANTIBIOTIC KM-8

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(Received for publication July 14, 1975)

In the course of screening for new antibiotics, a strain of a new species belonging to *Streptoverticillium* was found to produce a new peptide antibiotic.

Most of the procedure used in the taxonomic study of the strain were carried out in accordance with the method adopted by the International Streptomyces Project (ISP).[1] Additional media recommended by WAKSMAN[2,3] were also used. From the morphological characteristics, the strain KM-8 was shown to belong to the genus *Streptoverticillium*. That is, several short branches are formed in a whorl at regular intervals along the aerial mycelium, and about 10~50 oval-shaped spores with smooth surface formed a chain. The color of the colony was white to yellow, and that of the reverse side of the colony was light yellow on glycerol-asparagine agar and pale yellowish brown on glucose-nitrate agar. A soluble yellow pigment formed on oat meal agar and a pale brown pigment formed on glycerol-asparagine agar, while melanoid pigments were not formed. This strain was examined in detail to be compared to the strains, *Streptomyces morookaensis* ISP 5503, *Streptoverticillium griseoverticillatum* ISP 5507 and *Streptomyces kobenensis* KA-410, which most resemble the characteristics of the strain KM-8. From the result of these taxonomic studies, this strain was found not to belong to any known species, and named *Streptoverticillium taitoensis* since it was isolated from the soil sample collected in Taito-ku, Tokyo.

The antibiotic KM-8 was produced by shaked flask or submerged cultures in the medium containing 3.0 % starch, 0.5 % ammonium sulfate, 0.5 % yeast extract and 0.3 % calcium carbonate for 150~170 hours at 27°C. The cultured broth was filtered and passed through a column of Amberlite XAD-2. The resin column was washed with water and 10 % aqueous acetone. The active substance was eluted with 50 % aqueous acetone, and concentrated *in vacuo* to an aqueous solution. The active substance was then adsorbed on the Amberlite XAD-7 column and also eluted with 50 % aqueous acetone after the resin had been washed with water and 10 % aqueous acetone. The eluate was concentrated *in vacuo* and adsorbed on a silica gel dry column. The column was washed with water and then chromatographically developed with 40 % aqueous ethanol. The active fractions were collected and concentrated to be aqueous solution. KM-8 was obtained as a white powder by lyophilizing the concentrated solution. It was observed to be a single spot on thin-layer chromatography in several solvent systems.

The KM-8 substance is a white powder having a basic nature (pKá 8.7 in H_2O), and decomposes at 240°C. It is soluble in water, slightly soluble in methanol and ethanol, and insoluble in acetone, ethylacetate, chloroform, benzene, ether and hexane. The UV spectrum in water shows four maxima at 252 nm ($E^{1\%}_{1cm}$ 5.13), 258 nm ($E^{1\%}_{1cm}$ 5.20), 264 nm ($E^{1\%}_{1cm}$ 4.51) and 268 nm ($E^{1\%}_{1cm}$ 3.79) as shown in Fig. 1, which may result from phenylalanine detected in the hydrolyzate of this antibiotic.

The IR spectrum in KBr disc is shown in Fig. 2. From the absorption maxima at 3300,

Fig. 1. UV spectrum of KM-8 (H_2O)

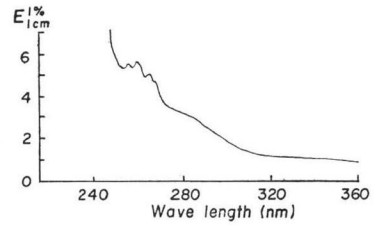
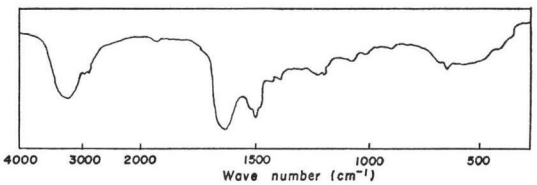


Fig. 2. IR spectrum of KM-8 (KBr)



1650 and 1510 cm^{-1}, KM-8 is suggested to be a peptide antibiotic. The specific rotation $[\alpha]_D^{23}$ is $-91.7°$ (*c* 0.42, H_2O). The elemental analysis is as follows: C 47.54 %, H 5.82 %, N 15.00 %. The antibiotic is positive to ninhydrin, RYDON-SMITH, PAULY, and biuret reactions, and negative to SAKAGUCHI, EHRLICH, MOLISCH, ELSON-MORGAN and anthron reactions. Rf values of KM-8 was shown as follows: 0.35 with $CHCl_3$ - MeOH - H_2O (5 : 4 : 2), 0.70 with $CHCl_3$ - MeOH - 1% CH_3COOH in H_2O (5 : 4 : 2) on silica gel thin-layer chromatography (Merck Kieselgel G) and 0.33 with *n*-BuOH - CH_3COOH - H_2O (3 : 1 : 1), 0.71 with *n*-PrOH - pyridine - CH_3COOH - H_2O (15 : 10 : 3 : 12) on cellulose powder thin-layer chromatography (Asahi Kasei, Avicel SF) detected by bioautography and ninhydrin reaction.

The antimicrobial spectrum is shown in Table 1. This antibiotic is principally active against gram-positive bacilli and weakly active against some fungi.

LD_{50} of KM-8 to mice was 22 mg/kg by intravenous injection. While no changes were observed by oral administration of 1,000 mg/kg.

Table 1. Antimicrobial activity of KM-8

Test organism	MIC (mcg/ml)
Staphylococcus aureus FDA 209 P	>200
Sarcina lutea PCI 1001	>200
Bacillus subtilis PCI 219	0.78
Bacillus cereus var. *mycoides*	<0.20
Bacillus agri	<0.20
Bacillus anthracis	>200
Mycobacterium smegmatis ATCC 607	100
Corynebacterium paurometabolum	0.39
Escherichia coli NIHJ	200
Klebsiella pneumoniae PCI 602	>200
Shigella flexneri	200
Xanthomonas oryzae	25
Candida albicans	100
Aspergillus niger	>200
Alternaria kikuchiana	25
Botrytis cinerea	100
Sclerotinia cinerea	25
Trichophyton interdigitale	25

Agar dilution method
Bacteria: Nutrient agar, 37°C, 24 hours
Fungi: Potato agar, 27°C, 48 hours

From the hydrolysis of KM-8 with 6 N HCl for 24 hours at 140°C, nine ninhydrin-positive substances were detected with an amino acid analyzer. They were glycine (2.4), aspartic acid (1.2), glutamic acid (1.3), valine (1.2), lysine (1.2), phenylalanine (5.4) and three other substances which are going to be studied. The figures in the respective parentheses show the molar ratios found from the result of the analysis. The amino acid composition was compared with that of known basic peptide antibiotics. However, any substance containing these six amino acids altogether has not been reported.

Leucopeptin most resembles KM-8 in physical and chemical properties, while glycine, aspartic acid, glutamic acid, valine, lysine, proline and other five components were detected in the hydrolyzate of it. Phenylalanine is not in leucopeptin and proline is not in KM-8. The sulfur content of leucopeptin was reported to be 4.80 %, but that of KM-8 was found to be zero from the result of the elemental analysis.

From the physical, chemical and biological properties described above, we concluded that KM-8 is a new antibiotic.

Acknowledgement

We wish to thank Dr. Y. WAKISAKA (Shionogi Co., Ltd.) for supplying the strain, *Streptomyces morookaensis* ISP 5503, and Mr. K. KOMIYAMA for the determination of LD_{50}. We also thank Dr. T. HATA for taking a warm interest in this study.

References

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