A NEW PEPTIDE ANTIBIOTIC COMPLEX S-520. I

ISOLATION AND CHARACTERIZATION

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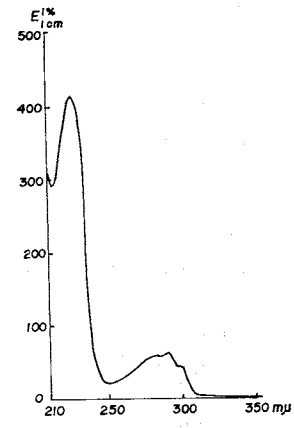
An antibiotic named S-520 was isolated from a strain S-520 which was identified as *Streptomyces diastaticus*. The antibiotic is primarily active against gram-positive bacteria. The antibiotic is suggested to be a complex which consists of several peptides closely related to each other. It has λ_{max}^{MeOH}: 227 mμ, 283.5 mμ, 290 mμ and 299.5 mμ and $[\alpha]_D$ +13.2° (in methanol). Analytical data corresponds to $C_{40}H_{59\sim60}O_{10}N_{8\sim9}Cl$. Glycine, valine, isoleucine, ornithine, lysine and some unknown amino acids are produced on hydrolysis.

In the course of searching for new antibiotics, a streptomyces strain S-520 was found to produce an antibiotic which has a protective effect against mice infected with *Diplococcus pneumoniae*. Morphological, cultural and physiological characteristics of the strain were studied, and the results indicated that the strain is the same species as *Streptomyces diastaticus*.

The antibiotic named S-520 which was later recognized to be a complex of closely related peptides, was isolated from the mycelium mass by solvent extraction and purified by gel-filtration.

The hydrochloride of the antibiotic was obtained as a colorless amorphous powder, which has no definite melting point and softens at 191~198°C. Elemental analysis and molecular weight determination correspond to $C_{40}H_{59\sim60}O_{10}N_{8\sim9}Cl\cdot HCl$. The hydrochloride is soluble only in aqueous lower alcohols but hardly soluble or insoluble in water and other organic solvents. It has the following UV-maxima in methanol: 227 mμ ($E_{1cm}^{1\%}$ 416), 283.5 mμ ($E_{1cm}^{1\%}$ 59.4), 290 mμ ($E_{1cm}^{1\%}$ 61.8) and 299.5 mμ ($E_{1cm}^{1\%}$ 43.5) as illustrated in Fig. 1. The infrared absorption spectrum shows peptidic nature (Fig. 2). It is optically active: $[\alpha]_D^{23°}$ +13.2°±1.2° (*c*, 0.461, MeOH). The antibiotic is positive to ninhydrin and DRAGENDORF reagents and decolorizes potassium permanganate solution.

Fig. 1. Ultraviolet absorption spectrum of S-520 hydrochloride in methanol.



The preparation as described above showed a single spot on the thin-layer chromatography: Silica gel GF, *n*-butanol-acetic acid-water (3:1:1), Rf 0.58±0.05. How-

Fig. 2. Infrared absorption spectrum of S-520 hydrochloride (KBr).

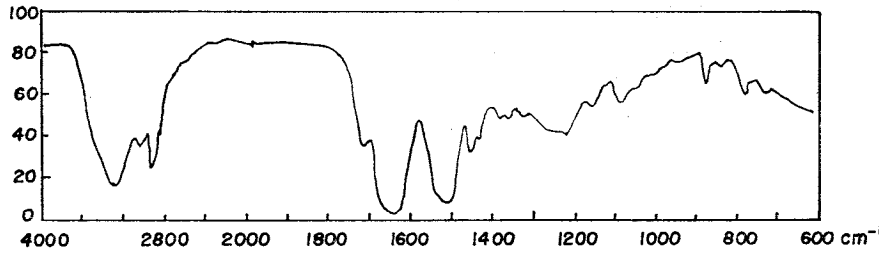


Table 1. Antimicrobial spectrum of S-520

Test organism	MIC (mcg/ml)
Escherichia coli JC-2	>200
Klebsiela pneumoniae	100
Salmonella typhimurium	>200
Pseudomonas aeruginosa	>200
Proteus vulgaris	>200
Shigella dysenteriae	100
Bacillus subtilis	6.25
Bacillus anthracis	6.25
Staphylococcus aureus FDA 209P	6.25
Staphylococcus aureus 60658	6.25
Streptococcus pyogenes C-203	25
Diplococcus pneumoniae type I	25
Sarcina lutea	10
Corynebacterium diphtheriae	20
Mycobacterium 607	100
Mycobacterium smegmatis	10
Mycobacterium phlei	5

Table 2. *In vivo* activity of S-520 against *Diplococcus pneumoniae*

Dose	Survivors
10 mg/kg×4	8/10
5 mg/kg×4	6/10
1 mg/kg×4	1/10
0	0/10

Mice were intraperitonealy inoculated with 100 LD_{50} of viable cells per mouse. Drug was intraperitonealy administered each dose four times (2, 24, 48 and 72 hours after infection).

ever, the acid hydrolysate gave the following amino acids by an automatic amino acid analyzer in moles per mole of the preparation: glycine (1.0), valine (*ca.* 0.79), isoleucine (*ca.* 0.21), ornithine (*ca.* 0.80), lysine (*ca.* 0.25) and four unknown amino acids.

These data suggest that the antibiotic preparation is a complex of several peptides, certain parts of which are composed of one of pair amino acids which are replaceable by one another; such as valine and isoleucine, and ornithine and lysine.

This antibiotic is active against gram-positive bacteria as shown in Table 1. Toxicity to mice by intraperitoneal injection was given as LD_{50} 130 mg/kg (Fiducial limit, 110~134). Protective effect to mice infected with *Diplococcus pneumoniae* was observed (Table 2). The effectiveness *in vivo* in contradiction to its relatively higher MIC *in vitro* is considered to be characteristic of this antibiotic.

Many peptide antibiotics have been isolated from streptomycetes strains. However, the antibiotic S-520 is considered to be a new one from its characteristics.

Further characterization of this antibiotic complex and studies on the degradation product will be published in following papers[1].

Experimental

Production: The medium used was composed of starch 1.0%, glycerine 0.5%, soybean meal 1.0%, corn steep liquor 0.5%, NaCl 0.3% and $CaCO_3$ 0.35%, pH 7.0. The streptomyces strain S-520 was inoculated on 500 ml of the medium in 2-liter Erlenmeyer flask, which was cultured at 27°C for 48 hours on a rotary shaking machine. The culture was then transferred to a 30-liter jar fermentor containing 15 liters of the same medium. Fermentation was carried out at 28°C for 4 days under aeration of 20 liters per minute and agitation of 250 r.p.m.

Isolation: About 60 liters of the cultured broth obtained as above was filtered at pH 3.0 using filter-aid. The mycelial cake was extracted twice with methanol (5 and 3 liters), the pH being kept at 2.0 by HCl. The extract was evaporated at *ca.* 30°C to a nearly aqueous solution, in which gel-like precipitate was formed. The aqueous solution containing the gel-like mass was extracted with *n*-butanol (500 ml), and the butanol layer was washed with dilute hydrochloric acid. When the butanol extract was washed with 2% $NaHCO_3$, gel-like precipitate was formed again. After the washing was repeated, the butanol layer was then acidified by shaking with dilute hydrochloric acid to dissolve the gel-like mass. Then it was concentrated to a small volume, to which acetone was added to yield a slightly brown solid (27 g). The solid was dissolved in methanol containing hydrogen chloride and a small amount of residue was filtered. Addition of ethyl acetate afforded a crude preparation of S-520 hydrocloride (22 g).

Purification of the antibiotic complex S-520: Some 1.0 g of the crude preparation was dissolved in methanol (5 ml) and placed on a Sephadex LH-20 column (2.5×85 cm). The column was eluted with methanol. After elution of brownish pigments, the active fraction, as tested on a *Bacillus subtilis* assay plate, was eluted. Concentration of the eluate and addition of ethyl acetate gave a colorless powder (*ca.* 830 mg). For an analytically pure preparation, the above procedure was repeated.

S-520 hydrochloride:

Anal. Found: C 53.70, 53.20; H 7.16, 7.19; N 13.27, 13.75; Cl 7.87, 8.25; MW 985 (osmometry in pyridine).

Calcd. for $C_{40}H_{60}O_{10}N_9Cl \cdot HCl$: C 53.45, H 6.79, N 14.03, Cl 7.91, MW 898

$C_{40}H_{59}O_{10}N_8Cl \cdot HCl$: C 54.36, H 6.80, N 12.68, Cl 8.04, MW 883.

Reference

1) SHOJI, J. & R. SAKAZAKI: A new peptide antibiotic complex S-520. II. Further characterization and degradative studies. J. Antibiotics 23: 432~436, 1970