NOTE

ANTIBIOTIC AB-65, A NEW ANTIBIOTIC FROM *SACCHAROMONOSPORA VIRIDE*

ATSUSHI TAMURA and IMAO TAKEDA

Research Laboratories
Dainippon Pharmaceutical Co., Ltd.,
Suita, Osaka, Japan

(Received for publication February 21, 1975)

In a continuing search for less known genera of the Actinomycetales as producers of new antibiotics, a new antibiotic, named antibiotic AB-65, was isolated from the cultured broth of *Saccharomonospora viride* T-80, which was isolated from soil and described by NONOMURA and OHARA[1].

The culture of strain T-80 was maintained on yeast extract-malt extract agar (ISP medium 2). Fermentation condition suitable for the production were studied and the following media were found to be useful. Vegetative medium of slant (g/liter): soluble starch, 15; yeast extract, 0.5; pyridoxine · HCl, 0.5; inositol, 0.5; Ca-pantotenate, 0.5; *p*-aminobenzoic acid, 0.5; biotin, 0.25; agar, 20; distilled water, 1 liter; pH 7.5. Vegetative medium of flask (g/liter): malt extract, 10; glucose, 4; yeast extract, 4; distilled water, 1 liter; pH 7.0. Fermentation medium (g/liter): dextrin, 30; defatted soybean meal, 15; yeast extract, 5; NaCl, 3; Silicon KM-70, 1 ml; kaolin 102, 0.1 ml; pH 6.4 (adjusted to pH 7.9 prior to sterilization).

The vegetative agar slant was seeded with strain T-80. The strain T-80 was incubated for 2~3 days at 37°C, and then the bacteria-like mycelia used to inoculate 70 ml of a vegetative medium contained in 500-ml Sakaguchi flask. The flask was cultivated for 3 days at 37°C on a reciprocal shaker. The seed culture (70 ml) was inoculated into a secondary preculture in a 3-liter Sakaguchi flask containing 700 ml of the same medium. The flask was incubated for 3 days at 37°C. The vegetative culture (2.5 liters) was then transferred to a 100-liter fermentor containing 50 liters of the medium, and incubated aerobically (1 v.v.m.) under stirring (250 r.p.m.) 3~5 days at 37~38°C.

Antimicrobial activity was checked by a paper-disc bioassay with *Staphylococcus aureus* Terajima as a test organism. Maximum antibiotic activity was obtained after 72 hours of fermentation.

The cultured broth (50 liters) was separated continuously in an S-type ultracentrifuge at 10,000 r.p.m. The mycelial cake (2.7 kg, wet) was extracted with acetone (10 liters). The extract was evaporated to dryness *in vacuo*. The residue was dissolved in water (10 liters) and adjusted to pH 3.0 with conc. HCl and extracted two times with 10-liter portion of *n*-butanol. The supernatant broth (50 liters) was adjusted to pH 3.0 and extracted two times with 50-liter portions of *n*-butanol. The butanol extracts from the mycelial cake and the broth were combined and washed two times with a 120-liter portion of 0.05 M carbonate buffer of pH 10.0. The butanol layer was evaporated to dryness *in vacuo*. The residue was dissolved in 1 liter of methanol and precipitated by addition of ethyl acetate. The crude substance (4.5 g) of AB-65 was obtained. The crude substance (500 mg) was dissolved in 500 ml of *n*-butanol - acetone - water (1:1:1, v/v) and purified with the same solvent mixture on a column of cellulose (Avicel). The active fractions were combined and concentrated *in vacuo* to 300 ml and precipitated by addition of 500 ml of *n*-butanol. The white powder was further purified by reprecipitation as aforesaid, and washed with acetone and ethyl acetate, and filtered off and dried *in vacuo*. From 50 liters of the cultured broth, 3.6 g of pure white powder (AB-65) was isolated.

Antibiotic AB-65 is a white powder, with physico-chemical and biological properties as follows:

(1) Solubility: Soluble in dimethylsulfoxide and pyridine. Slightly soluble in *n*-butanol - methanol - water (1 : 1 : 1), *n*-butanol - methanol - benzene - water (2:1:1:1). Very slightly soluble in water, methanol, ethanol and *n*-butanol. Insoluble in ethyl acetate, chloroform, benzene, acetone and ethyl ether.

(2) Melting point: 262~263°C (dec.).

(3) Optical rotation: $[\alpha]_D^{20}$ −65.3° (*c* 1.0, dimethylsulfoxide).

(4) Elementary analysis: C 53.04, H 6.52, N 8.92, Cl 5.03 (%); indicated an empirical formula, $C_{62}H_{91}N_9O_{23}Cl_2$. The molecular weight of AB-65 was not directly obtained owing to its low solubility in common organic solvents.

(5) UV spectrum (Fig. 1): $\lambda_{max}^{0.1N\ NaOH}$ nm ($E_{1cm}^{1\%}$) 225 (221.6), 280 (33.6), 289 (32.0), 298~299 (sh).

(6) IR spectrum (Fig. 2): The presence of amide (1660, 1550, 1530 cm^{-1}), —OH (1060 cm^{-1}) and =C—O (1230 cm^{-1}) group were indicated.

(7) Chromatography of AB-65: AB-65 on cellulose TLC (Merck, 5552/0025) detected with iodine. Rf 0.17 (*n*-butanol - ethanol - water, 4:1:1), 0.37 (*n*-butanol - methanol - benzene - water, 2 : 1 : 1 : 1), 0.64 (*n*-butanol - acetic acid - water, 4:1:5), 0.77 (*n*-butanol - acetone - water, 1:1:1).

Fig. 1. UV spectra of antibiotic AB-65

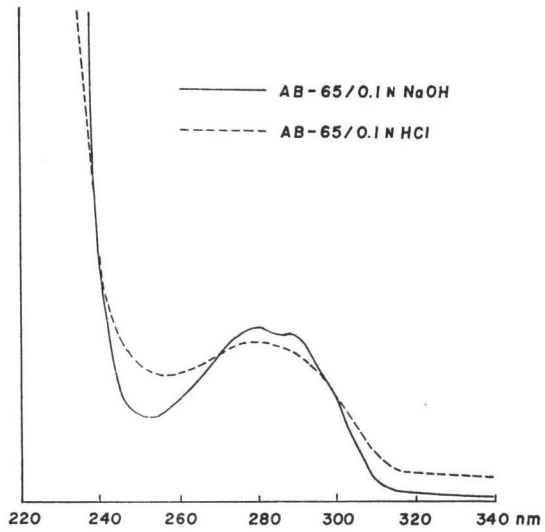
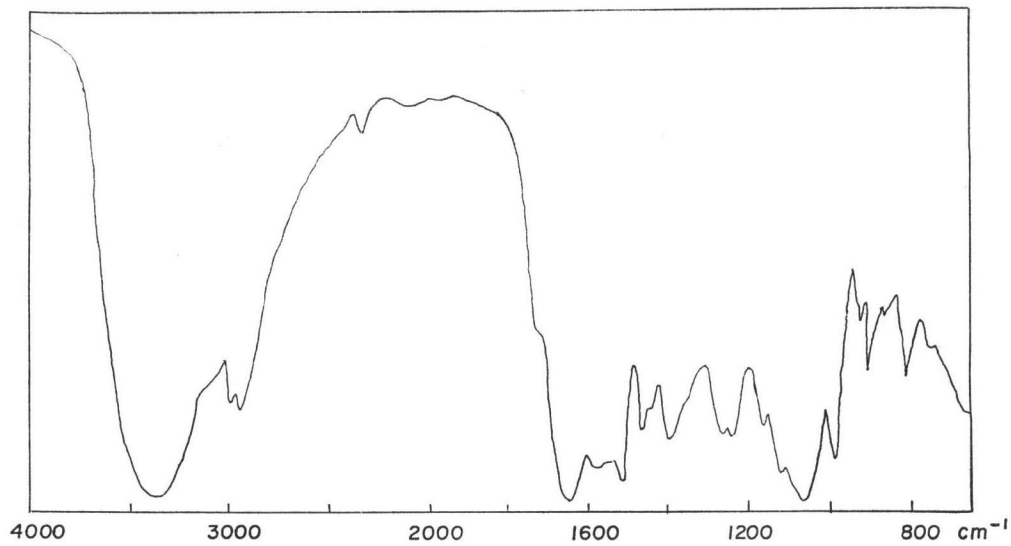


Fig. 2. IR spectrum of antibiotic AB-65 (KBr)



(8) Hydrolysate of AB-65: The acid hydrolysate contained at least five ninhydrin-positive products on cellulose TLC, high voltage paper electrophoresis and Amberlite CG-120 column chromatography, and one sugar reaction-positive product on cellulose TLC (aniline phthalate-positive). But these products were not identical with general known amino acids and sugars.

(9) Antimicrobial spectrum (Table 1): AB-65 was effective against gram-positive bacteria, *Mycobacteria*, *Candida*, *Trichophyton* and *Trichomonas*. However, AB-65 was not effective against gram-negative bacteria.

(10) Acute toxicity (LD_{50}) in mice: <12.5 mg/kg (i.p.), 18 mg/kg (s.c.), >1,000 mg/kg (p.o.).

Table 1. Antimicrobial spectrum of antibiotic AB-65

Test organisms	MIC (mcg/ml)
Staphylococcus aureus Terajima	0.1～0.03
S. aureus Miyamoto	1*
S. aureus ATCC 6538	1*
S. aureus S-21	1*
S. aureus S-23	1*
S. aureus No. 5	0.3*
S. aureus No. 6	0.3*
S. aureus P-7c (SM,PC,TC-R)	0.3*
S. aureus P-32 (SM,CP,TC,EM-R)	0.3*
S. aureus P-213 (PC-R)	0.3*
S. aureus FDA 209P (SM,STH-R)	1*
S. aureus FDA 209P JC-1 (KM,STH-R)	1*
S. aureus FDA 209P JC-1 (SM, STH-R)	1*
S. epidermidis No. 8	1*
S. albus AKM	1*
Diplococcus pneumoniae I	10
Bacillus subtilis PCI 219	0.1
Listeria monocytogenes	0.3
Escherichia coli K-12	>100
Shigella flexneri 2a EW 10	10～30
S. sonnei EW 33	>100
Pseudomonas aeruginosa Tsuchijima	>100
Salmonella typhimurium S-9	>100
Proteus vulgaris OX-19	>100
Klebsiella pneumoniae No. 13	>100
Brucella abortus	30
Mycobacterium tuberculosis $H_{37}Rv$	10
Aspergillus fumigatus	10
A. terreus	3
Trichophyton mentagrophytes	3
T. interdigitale	30
Microides gypseum	30
Epidermophyton floccosum	3
Candida albicans	3～10
Cryptococcus neoformans	1
Trichomonas vaginalis 4F	30
Sporotrichum schenkii	10
Nocardia asteroides	3

No asterics MIC: dilution method
* MIC: streaking method

On the basis of the investigation described above, antibiotic AB-65 is a member of sugar-peptide antibiotics and related to known nitrogen and chlorine-containing peptide or polysaccharide antibiotics such as vancomycin[2] and everninomicin[3].

From the viewpoint of elemental analysis, melting point, optical rotation, UV and IR spectra and degradation products, it seems reasonable to conclude that antibiotic AB-65 is a new antibiotic.

Acknowledgements

The authors express deep thanks to Dr. H. NONOMURA, Faculty of Engineering, Yamanashi University, for the supply of strain T-80 and his helpful advices, and Drs. H. TAKAMATSU, M. SHIMIZU and K. YOKOGAWA, Research Laboratories, Dainippon Pharmaceutical Co., Ltd., for their encouragement throughout this work. Thanks are also due to the members of Analytical Center and Chemotherapy Department of our Research Laboratories for microanalyses, MIC and acute toxicity testings respectively, and Mr. Y. SONPACHI of our laboratory, for his cooperation during this work.

References

1) NONOMURA, H. & Y. OHARA: Distribution of actinomycetes in soil. X. New genus and species on monosporic actinomycetes. J. Ferment. Tech. (Japan) 49: 895～903, 1971
2) MCCORMICK, M. H.; W. M. STARK, G. E. PITTENGER, R. C. PITTENGER & J. M. MCGUIRE; Vancomycin, a new antibiotic. I. Chemical and biologic properties. Antibiotic Annual 1955/1956: 606～611, 1956
3) WEINSTEIN, M. J.; G. M. LUEDEMANN, E. M. ODEN & G. H. WAGMAN: Everninomicin, a new antibiotic complex from *Micromonospora carbonacea*. Antimicr. Agents and Chemoth.-1964: 24～32, 1965