A NOVEL ANTIBIOTIC, SOHBUMYCIN

TAXONOMY, FERMENTATION, ISOLATION AND PHYSICO-CHEMICAL AND BIOLOGICAL CHARACTERISTICS

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A new antibiotic sohbumycin, was isolated from the culture broth of *Streptomyces* sp. No. 82-85. It appeared to belong to the peptide lactone type of antibiotics from physico-chemical studies and has an empirical formula of $C_{31}H_{47}N_3O_{10}Cl$.

In *in vitro* studies, the antibiotic was found to possess potent cytocidal activity against HeLa S3 cells and antimicrobial activities against Gram-positive bacteria with MIC values about 0.3~0.6 μg/ml, but showed no activity on the Gram-negative bacteria, yeast and fungi tested.

In the course of a screening program for novel antibiotics showing antitumor activity, sohbumycin was isolated from the fermentation broth of *Streptomyces* sp. No. 82-85 which had been isolated from a soil sample collected in Kanagawa Prefecture, Japan. The antibiotic shows strong activity against HeLa S3 cells and against Gram-positive bacteria *in vitro*.

The present paper deals with the taxonomy of the producing strain and with the production, isolation and biological and physico-chemical properties of the new antibiotic, sohbumycin.

Materials and Methods

Taxonomic Studies

For taxonomic studies, most cultures were grown in accordance with methods adopted by the International Streptomyces Project[1]. For experiments on cultural properties, all cultures were incubated at 27°C and were observed for 15~20 days. The colors recorded for mature cultures are described according to the "Color Harmony Manual"[2]. Physiological properties including utilization of carbon sources were examined by the method of PRIDHAM and GOTTLIEB[3]. Diaminopimelic acid in the cell wall was analyzed by the method of BECKER *et al.*[4].

Fermentation and Isolation of Sohbumycin

A stock culture of the producing organism was inoculated into a 500-ml Sakaguchi flask containing 100 ml of seed medium consisting of starch 1.5%, glucose 0.2%, peptone 0.25%, yeast 0.15%, meat extract 0.3% and $CaCO_3$ 0.25% (pH 7.0 before sterilization). The flasks were incubated at 27°C for 72 hours on a reciprocal shaker. Then 6 liters of the resulting culture were transferred to a 600-liter fermentor containing 300 liters of the same medium described above, and fermentation was carried out at 28°C for 72 hours using an agitation rate of 160 rpm and an aeration rate of 60 liters/minute.

The fermentation broth of *Streptomyces* sp. No. 82-85 was mixed with 15 kg of Hyflo Super-Cel (Johns-Manville Co., USA) and then filtered with a filter press. The brown filtrate (260 liters) was adjusted to pH 6.0 and extracted with EtOAc (2×150 liters). The combined EtOAc layers were concd to about 10 liters, washed with H_2O (5 liters) and dried over Na_2SO_4 (anhydrous). Concen-

tration of the EtOAc layer resulted in a brown oil.

The brown oil was chromatographed over Silica gel 60 (Merck) using $CHCl_3$ - MeOH as solvent. Fractions exhibiting antimicrobial activity against *Micrococcus luteus* ATCC 9341 were collected and rechromatographed over Silica gel 60 (Merck) using $CHCl_3$ - MeOH as the solvent to give crude sohbumycin. Sohbumycin was further purified through preparative HPLC (YMC packed column, Yamamura Kagaku Kenkyusho; $\phi 10 \times 300$ mm; MeOH - H_2O as solvent) or through preparative TLC (Kieselgel 60 F_{254} Merck; $CHCl_3$ - MeOH (19: 1) as solvent).

Effect of Sohbumycin on HeLa S3 Cells

HeLa S3 cells were maintained in monolayers in EAGLE's minimum essential medium (MEM) supplemented with 10% bovine serum and an antibiotic (60 μg/ml of kanamycin) at 37°C.

To determine the cytotoxicity of sohbumycin on mammalian cells, HeLa S3 cells (5×10^4) in 2 ml of medium were placed in a 30-mm Petri dish and incubated for 48 hours at 37°C in a 5% CO_2 - 95% air atmosphere. Each culture dish was filled with fresh medium containing a different concentration of sohbumycin. After the incubation for 72 hours, the HeLa S3 cells were trypsinized to form a single cell suspension, and cells were counted in hemocytometer.

Results

Taxonomic Studies

The chain of the mature spores consisted of more than 20 spores and was almost straight to flexuous. Most of the spores were short and ovoid ($0.6 \sim 1.1 \times 0.9 \sim 1.4$ μm) and possessed a smooth surface as seen by electron microscopy (Fig. 1). Color characteristics of aerial mycelia of strain No. 82-85, when grown on various standard media, are shown in Table 1. Physiological properties and utilization of carbon sources of strain No. 82-85 are shown in Tables 2 and 3 respectively. Cell wall analysis showed the presence of LL-diaminopimelic acid and it was classified as type I. Microscopic studies and cell wall type indicated that strain No. 82-85 belongs to the genus *Streptomyces* and it was designated as *Streptomyces* sp. No. 82-85. Further studies are in progress.

Fig. 1. Electron micrograph of the spores of strain No. 82-85.
(Bars in the photo represent 1 μm).

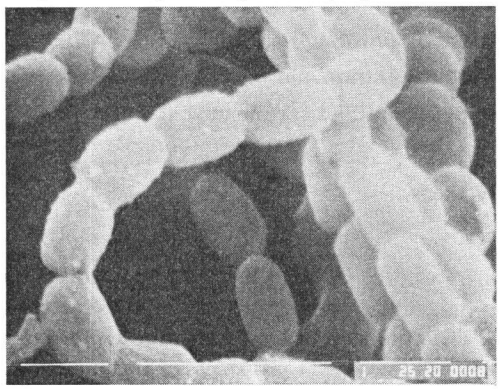

Table 1. Cultural properties of strain No. 82-85.

Medium	Growth	Aerial mycelium	Reverse	Soluble pigment
Yeast extract - malt extract agar	Good	Pale orange	Pinkish white	Yellowish brown
Oatmeal agar	Good	Pale orange	Light brown	Yellowish brown
Inorganic salts - starch agar	Good	Pale orange	Pale orange	Pale yellow brown
Glycerol - asparagine agar	Good	White	Pale yellow orange	Pale yellow
Peptone - yeast extract - iron agar	Good	Dark covert gray	Grayish yellow brown	Chocolate brown
Tyrosine agar	Good	Pale orange	Brownish black	Yellowish brown

Table 2. Physiological characteristics.

Nitrate reduction	Positive
Liquefaction of gelatin	Negative
Coagulation of milk	Negative
Hydration of starch	Positive
Melanin formation	Positive
Production of H_2S	Negative

Table 3. Utilization of carbon sources.

Responses	Carbon source
Positive	L-Arabinose, D-xylose, sucrose, D-fructose, inositol, L-rhamnose, D-mannitol, D-glucose
Negative	Raffinose

Table 4. Physico-chemical properties of sohbumycin.

Appearance	Colorless powder
Molecular formula	$C_{31}H_{47}N_8O_{10}Cl$
MW	726.5
MP	162~167°C
$[\alpha]_D^{20}$ (c 0.24, MeOH)	−56°
UV absorption in MeOH	210 (sh), 226 (sh), 234 nm
Solubility: Soluble in	MeOH, EtOH, $CHCl_3$, CH_2Cl_2, EtOAc
Insoluble in	H_2O, n-hexane
TLC (Kieselgel 60 F_{254})	
$CHCl_3$ - MeOH (19: 1)	Rf 0.48
$CHCl_3$ - CH_3CN (17: 3)	Rf 0.07

Physico-chemical Properties of Sohbumycin

The physico-chemical properties of sohbumycin are summarized in Table 4. UV and IR absorption spectra and the ^1H NMR spectrum are shown in Figs. 2, 3 and 4 respectively. This antibiotic was soluble in chloroform, dichloromethane, ethyl acetate, methanol and ethanol but practically insoluble in water and n-hexane. Soh-

Fig. 2. UV spectrum of sohbumycin (MeOH).

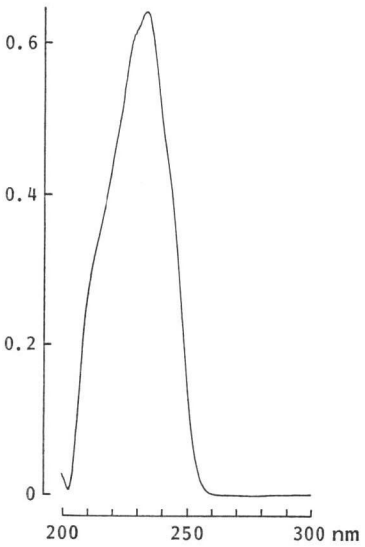

Fig. 3. IR spectrum of sohbumycin (KBr).

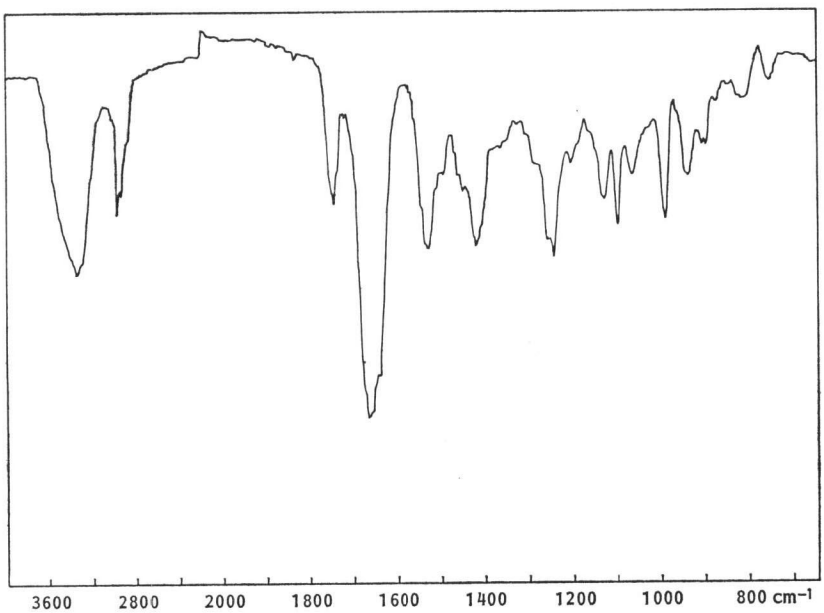

Fig. 4. ^{1}H NMR spectrum of sohbumycin (250 MHz in DMSO-d_6, δ ppm).

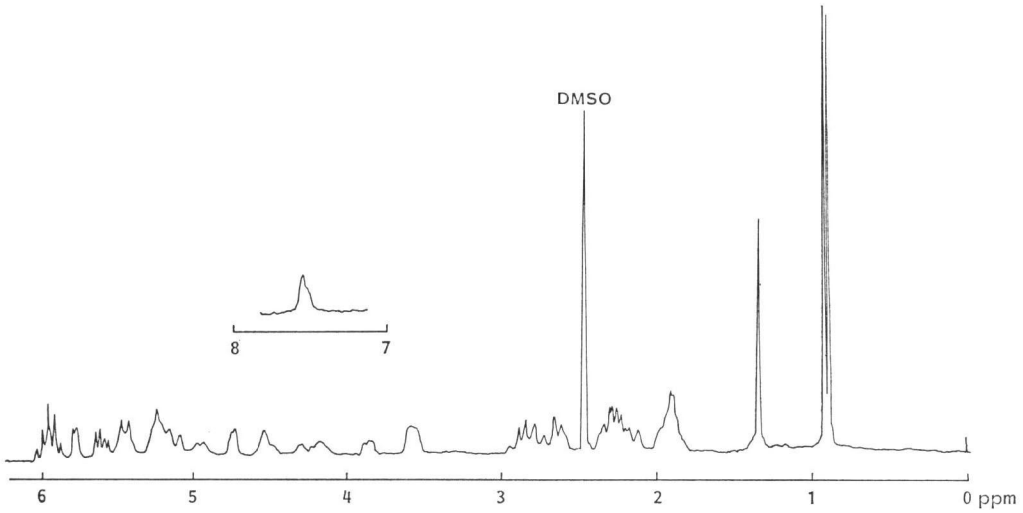

Table 5. Antimicrobial spectrum of sohbumycin.

Organism	MIC (μg/ml)
Bacillus subtilis PCI 219	0.3
B. cereus IFO 3001	0.6
Micrococcus luteus ATCC 9341	0.3
Staphylococcus aureus FDA 209P	0.3
Salmonella typhimurium KB 20	>100
Shigella flexneri E 20	>100
S. sonnei E-33	>100
Escherichia coli NIHJ	>100
Klebsiella pneumoniae PCI 602	>100
Proteus vulgaris IFO 3167	>100
Candida albicans KF 1	>100
Saccharomyces sake KF 26	>100
Schizosaccharomyces pombe IAM 4863	>100
Rhizopus javanicus IAM 6241	>100
Aspergillus niger ATCC 6275	>100
Alternaria kikuchiana KF 185	>100
Mucor racemosus IFO 5403	>100

Table 6. Cytocidal activity of sohbumycin on HeLa S3 cells.

Dose (μg/ml)	Cell No./plate ($\times 10^4$)	Control (%)
Control	67.5	100
0.74	0	0
0.37	46.5	69
0.18	68.5	101

HeLa S3 cells were exposed to sohbumycin for 72 hours.

Table 7. Comparison of molecular formulas and molecular weight between sohbumycin and monamycins.

Compound	Molecular formula	MW
Sohbumycin	$C_{31}H_{47}N_3O_{10}Cl$	726.5
Monamycin G_1	$C_{34}H_{54}N_7O_8Cl$	723.5
G_2	$C_{33}H_{54}N_7O_8Cl$	711.5
G_3	$C_{33}H_{54}N_7O_8Cl$	711.5
H_1	$C_{34}H_{56}N_7O_8Cl$	725.5
H_2	$C_{34}H_{56}N_7O_8Cl$	725.5
I	$C_{35}H_{58}N_7O_8Cl$	739.5

bumycin gave positive color reaction with iodine, 50% sulfuric acid and DRAGENDORFF's reagent, and was negative to ninhydrin and 3% ferrous chloride/EtOH solution.

Biological Properties of Sohbumycin

Sohbumycin showed antimicrobial activity against Gram-positive bacteria but did not show activity against the Gram-negative bacteria, yeast and fungi tested (Table 5). The effect of sohbumycin on asynchronous exponentially growing HeLa S3 cells was determined. As shown in Table 6, sohbumycin showed cytocidal activity at concentrations above 0.37 μg/ml.

Discussion

A new antibiotic, sohbumycin, was isolated

from the culture filtrate of *Streptomyces* sp. No. 82-85. This antibiotic showed antimicrobial activities against Gram-positive bacteria and cytocidal activity against HeLa S3 cells.

From the IR absorption bands at 1748 cm^{-1} (lactone) and the established molecular formula ($C_{31}H_{47}N_8O_{10}Cl$) of this compound, it is suggested that this antibiotic belongs to the group of peptide lactone antibiotics with a chlorine atom in the molecule.

Among the peptide lactone antibiotics, monamycins G_1, G_2, G_3, H_1, H_2 and I are known to have a chlorine atom in the molecule as in the case of sohbumycin, but the molecular formulas of these compounds are different from that of sohbumycin (Table 7)[5~12].

From the accumulated data described above it was suggested that sohbumycin is a new antibiotic which belongs to the peptide lactone type of antibiotics. Studies on other biological activities and structural elucidation will be reported elsewhere.

Acknowledgment

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