AABOMYCIN A, A NEW ANTIBIOTIC. I

PRODUCTION, ISOLATION AND PROPERTIES OF AABOMYCIN A

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Aabomycin A is a new antifungal antibiotic produced by a hitherto unidentified species of the genus *Streptomyces*. The active substance in fermented mash may be purified by solvent extraction followed by the chromatography on alumina column and then crystallization. Aabomycin A is easily soluble in many organic solvents, but is insoluble in water. It exhibits inhibitory activity against many fungi, especially against *Piricularia oryzae*, and has limited antiviral activity. The toxicity of aabomycin A for higher plants and animals is very low. Aabomycin A is similar in some properties to a few known antifungal antibiotics but differs from them in certain special characteristics.

Streptomyces sp. No. 325-17, an organism isolated from a soil sample collected at Bibai City, Hokkaido, Japan, was found to produce an antifungal antibiotic. The active principle was isolated, and found to be a new antibiotic, and was named aabomycin A. This substance was extractable with organic solvents such as ethyl acetate, acetone, or alcohols. It resembled the known organic solvent-extractable antifungal antibiotics such as antimycin, oligomycin, cycloheximide, or venturicidin. Differentiation has been established by comparison of physico-chemical properties. This communication describes the fermentation production, isolation procedures, and characterization of aabomycin A. The taxonomic studies of the producing organism will appear in a subsequent paper of this series.

Methods, Materials and Results

I. Production and Isolation of Aabomycin A

(1) Fermentation production.

Preculture of *Streptomyces* sp. No. 325-17 was carried out in K-1 flasks[1] on a rotary shaking machine at 27°C for 72 hours. The preculture (300 ml) was used to inoculate to a secondary preculture in a jar fermentor containing 15 liters of medium, to culture at 27°C for 48 hours with adequate aeration and agitation. The culture mash in the jar fermentor was then transferred to a tank fermentor containing 400 liters of the medium to culture at 27°C for approximately 70 hours under the usual conditions of aeration and agitation.

The medium used throughout was of the following composition (g per liter): soy

Fig. 1. Fermentation of aabomycin A.
(I) Shake-flask culture

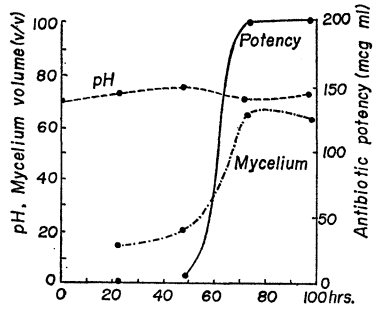
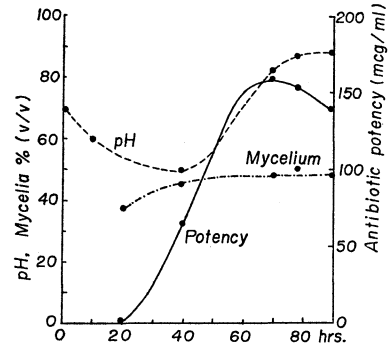


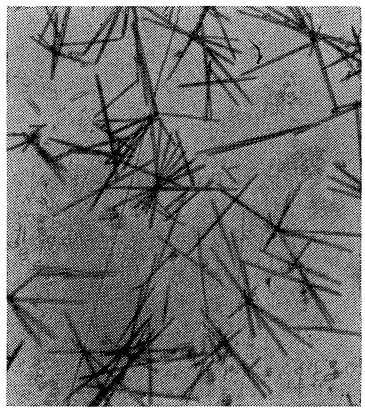
Fig. 2. Fermentation of aabomycin A.
(II) Tank culture



bean flour, 25; peptone, 5; glycerol, 40; NaCl, 5; pH 7.0.

Antibiotic activity was checked by paper-disc bioassay with *Piricularia oryzae* as an indicator organism. Chemical changes during aabomycin A fermentation are shown in Figs. 1 and 2.

Fig. 3.



(2) Extraction and purification

The harvested mash (400 liters) was filtered using diatomaceous earth. Preliminary tests suggested that the active substance could be extracted from both the mycelia and filtrate using an organic solvent. The mycelial cake was extracted with acetone, concentrated *in vacuo*, and further extracted with ethyl acetate. The broth filtrate was extracted with ethyl acetate. These ethyl acetate extracts were combined, washed with distilled water, and then concentrated under reduced pressure. Addition of hexane to the concentrated extract precipitated about 120 g of crude powder. The crude powder was extracted with a small amount of ethyl acetate which was passed through a neutral alumina column. The column was successively washed with benzene and chloroform. Development and elution was carried out with ethyl acetate. The eluted active fractions were combined and concentrated to a small

Fig. 4. Extraction of aabomycin A

Culture broth 400 liters, pH 7.6
| filtration
Mycelial cake
| extracted with acetone
Extract
| concd. *in vacuo*
| extracted with EtOAc
EtOAc layer
| washed with water
| concd. *in vacuo*
Syrup
| washed with hexane
Crude product 120 g

Fig. 5. Purification of aabomycin A

Crude product 25 g
| dissolved in EtOAc
Alumina column chromatography
| washed with benzene and chloroform
| developed with EtOAc
Active fractions
| concd. *in vacuo*
| added with benzene
Crystal
| recrystallization from chloroform-benzene
White needle crystal 4 g

volume. Addition of benzene to the concentrated extract precipitated crude crystalline aabomycin A, which was further purified by recrystallization from several solvent systems including chloroform-benzene, ethyl acetate-benzene, acetone-water, and methanol-water systems. About 20 g of white needle crystals of aabomycin A were obtained (Fig. 3). The isolation process is outlined in Figs. 4 and 5.

II. Physico-chemical Properties of Aabomycin A

Aabomycin A is a white needle crystal, with physical and chemical properties as follows:

(1) Solubility: Soluble in ethyl ether, chloroform, acetone, ethyl acetate, methyl alcohol, ethyl alcohol, pyridine. Sparingly soluble in benzene, toluene, carbon tetrachloride. Insoluble in hexane, petroleum ether, pure water.

(2) Melting point: 144~145°C.

(3) Optical rotation: $[\alpha]_D^{26}$ +93.5° (*c* 1, chloroform).

(4) Color reaction: Positive FEHLING and TOLLENS reactions. Negative biuret, SAKAGUCHI, ninhydrin, 2, 4-dinitrophenylhydrazine reactions.

(5) Thermostability: When aabomycin A was dissolved in methanol-water mixture and held at 100°C for 10 minutes, it was stable at neutral and basic pH, unstable at acidic pH.

(6) Stability to ultraviolet irradiation: When aabomycin A was dissolved in methanol and irradiated with ultraviolet emission lamp (Toshiba's chemical lamp of 15 watt) at 20 cm distance for 150 hours, no loss of antibiotic activity was observed.

(7) Elementary analysis: C 63.67, H 9.17, N 1.95, O 23.51; indicated a molecular formula, $C_{39\sim40}H_{65\sim67}NO_{11}$ (MW 723.918~737.944). Molecular weight determinations by the vapor pressure equilibrium method (Model 115 Hitachi) gave value of 770.

(8) Ultraviolet absorption spectrum (Figs. 6 and 7):

max: 280 mμ, $E_{1cm}^{1\%}$ 1.67

(in neutral or acidic methanol)

Fig. 6.

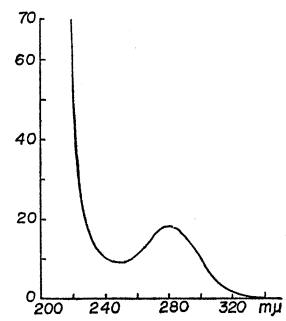


Fig. 7.

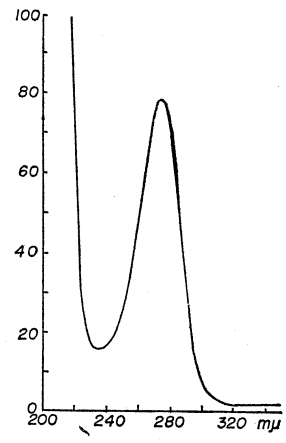


Table 1. Thin-layer chromatography of aabomycin A

Solvent systems	Rf value on	
	Silica gel	Alumina
Ethyl acetate	0.82	0.70
Benzene	0.00	0.00
Ethyl acetate, benzene (1 : 1)	0.35	0.10
Chlorform	0.00	0.00
Ethyl acetate, benzene (2 : 1)	0.47	0.23
Ethyl acetate, benzene (1 : 2)	0.10	0.00
Ethyl ether	0.80	0.79
Methanol	0.96	0.93
Acetone	1.00	1.00

The movement of aabomycin A was indicated both by bioautography against *Piricularia oryzae*, and by heating with conc. H_2SO_4.

max: 273 mμ, $E^{1\%}_{1cm}$ 65.0 (in 0.1 N NaOH - 90 % methanol)

(9) Infrared absorption spectrum (Fig. 8): The presence of -OH or -NH (3480 cm^{-1}), and carbonyl (1720 cm^{-1}) groups was indicated.

(10) N. M. R. spectrum (Fig. 9): It shows two signals corresponding to $N-CH_3$ and $C-CH_3$ respectively at 7.8 and 8.4~9.2 ($CDCl_3$, 100 MC). No aromatic hydrogen absorptions are presented.

Fig. 8. I. R. absorption spectrum of aabomycin A (in Nujol)

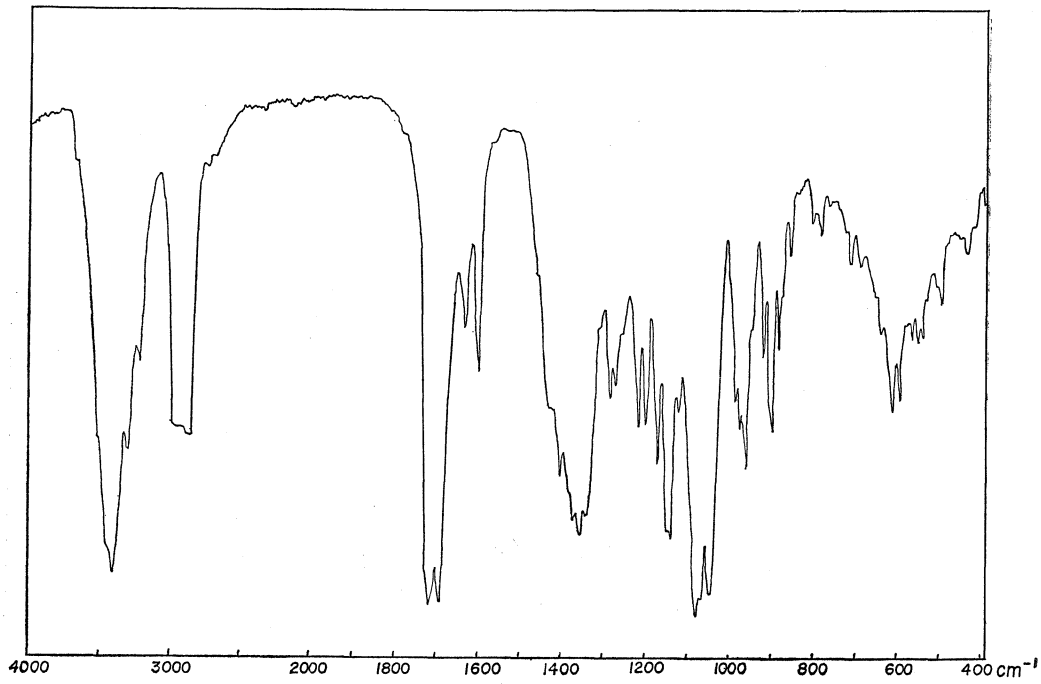
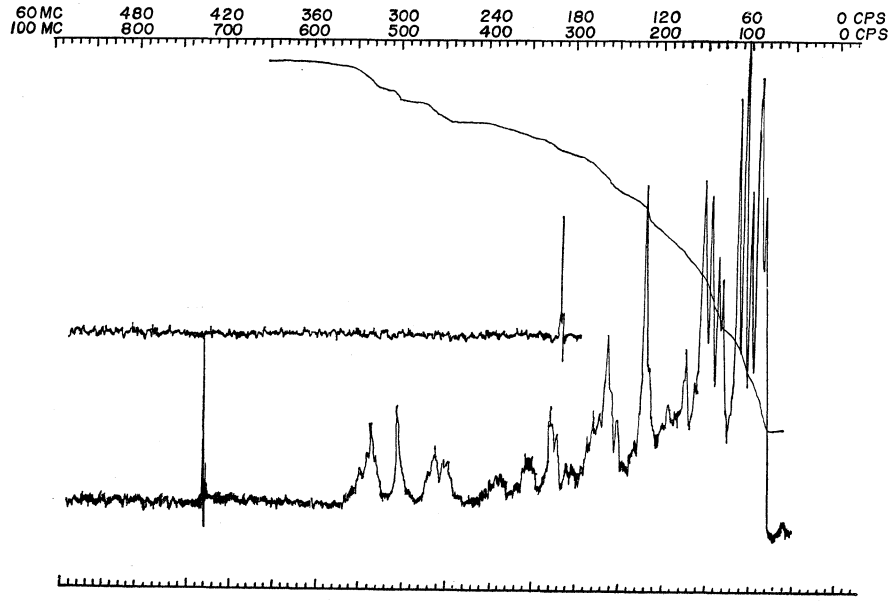


Fig. 9. N. M. R. spectrum of aabomycin A.



(11) Electrophoretic chromatography: Aabomycin A was applied for electrophoresis on a Sephadex sheet with the phosphate buffer at pH 7.0. No movement of the antibiotic zone occurred (15 mA for 30 minutes). However, the antibiotic zone moved slightly to the anode, when a buffer at pH 10.5 was used.

(12) Chromatography of aabomycin A: The Rf values of aabomycin A on thin-layer chromatography using silica gel and alumina and developed by bioautography and heating with conc. H_2SO_4 are shown in Table 1.

Table 2. Antimicrobial spectrum of aabomycin A (serial dilution method)

Test organisms	Minimum inhibition concentration (mcg/ml)	Test organisms	Minimum inhibition concentration (mcg/ml)
Piricularia oryzae	0.001	*Trichophyton rubrum*	0.8
Cochliobolus miyabeanus	3.0	*Trichophyton mentagrophytes*	1.5
Gloeosporium lacticolor	10.0	*Candida albicans* 57	12.0
Gloeosporium kaki	1.0	*Staphylococcus aureus* FDA 209 P	>100
Pellicularia filamentosa	10.0	*Pseudomonas aeruginosa*	>100
Glomerella cingulata	>100	*Mycobacterium smegmatis* ATCC 607	>100
Cladosporium fulvum	100	*Escherichia coli* NIHJ	>100
Macrosporium batapicola	10.0	*Xanthomonas oryzae*	>100
Trichophyton asteroides	1.5	*Shigella dysenteriae*	>100

Table 3. Comparison of summarized properties of aabomycin A, venturicidin and flavucidin

Property	Antibiotic		
	Venturicidin	Flavucidin	Aabomycin A
Isolation	extracted from mycelium with acetone		
Nature	colorless needle		
m. p.	142~143	144~145	144~145
$[\alpha]_D^{20}$ (c 1, chloroform)	114°	94°	93.5°
Analysis C	64.6~65.1	65.33~65.06	61.94~64.95
H	8.8~9.0	9.11~9.14	9.00~9.19
N	1.75	1.78~2.32	1.76~2.01
Formula	$C_{43}H_{71}O_{12}N$	$C_{34}H_{55\sim59}O_9N$	$C_{39\sim40}H_{65\sim67}O_{11}N$
UV Max. (in methanol) mμ	none	275	280 (neutral, acidic) 273 (alkaline)
Antibiotic activity against fungi	+	−	+
M. flavus	−	+	±
IR absorption at; 1580~1600 cm^{-1}	−	−	+
1620~1640 cm^{-1}	+	+	+
Color reaction with 2, 4-dinitrophenylhydrazine	+		−
Existence of phenolic hydroxyl group		+ (a)	− (b)

(a) Suggested by color reaction with vanillin. (b) Suggested by n. m. r. spectrum.

Table 4. Acute toxicity of aabomycin A to mice

Dose (mg/kg)	Solvent	Days after injection										
		0	1	2	3	4	5	6	7	8	9	10
100	10% ethanol aq.	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
500	Olive oil	0/4	0/4	0/4	1/4	1/4	2/4	2/4	2/4	2/4	2/4	2/4

Female mice of *ddN* strain were injected intraperitoneally.

III. Biological Properties of Aabomycin A

(1) Antimicrobial spectrum: The minimum inhibitory concentration of aabomycin A against a variety of microorganisms was examined by a serial agar dilution method. The results are given in Table 2. Aabomycin A inhibited the growth of some fungi, especially of *Piricularia oryzae* and the genus *Trichophyton*, whereas no activity against bacteria was demonstrable.

(2) Toxicity of aabomycin A: The acute toxicity in mice by intraperitoneal injection was examined (Table 4). Toxicity to fish was examined with five killifish. No fish died after over seven days in 1 liter of water containing 1,000 mg of aabomycin A, whereas all fish in an equal volume of water containing only 0.1 mg of antimycin A died in 2 hours.

Discussion

There are many antifungal antibiotics which can be extracted with organic solvents. Among them, venturicidin[2] is the most closely related compound based on physico-chemical properties. However, several characteristics indicated a difference between the two; namely, elementary analysis (molecular formula), ultraviolet and infrared absorption characteristics, and color reactions.

Flavucidin, a solvent-extractable antibiotic discovered by M. SHIBATA *et al.*[3] is another compound closely related physico-chemically to aabomycin A. Melting point and optical rotatory values of the two antibiotics are almost the same values. However, flavucidin is active against *Micrococcus flavus*, whereas aabomycin A is active only against some fungi or yeasts. The summarized comparison of these three antibiotics is shown in Table 3.

Acknowledgement

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