SAMAROSPORIN, A NEW PEPTIDE ANTIBIOTIC

I. FERMENTATION, ISOLATION AND CHARACTERIZATION

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A new antibiotic, samarosporin, was isolated in a crystalline state from a mycelial extract of a strain of *Samarospora* species (an ascomycete). Samarosporin is a neutral cyclopeptide and has a molecular formula of $C_{72}H_{111}N_{15}O_{19}$. Samarosporin shows a broad antimicrobial activity against various test microorganisms.

A fungal strain No. F-7762 isolated from a soil sample was found to belong to the genus *Samarospora* of *Ascomycetes*. A cyclopeptide antibiotic was produced in the mycelium of the strain, and the compound was named samarosporin. The samarosporin-producing strain has been deposited in the Fermentation Research Institute, Chiba, Japan, with accession number FERM-P 224. The taxonomic study of the producing strain of the fungus will be reported in a separate paper.

The fermentation, isolation and characterization of samarosporin are described in this paper.

Fermentation

The inoculum of strain No. F-7762 was prepared by suspending the aerial mycelia and spores from a slant culture in sterile distilled water. To obtain the preculture each ml of the suspension was inoculated into 500-ml SAKAGUCHI-flasks each containing 100 ml of medium consisting of 2.0% glucose, 0.3% meat extract, 0.5% peptone, 0.3% yeast extract, 0.5% sodium chloride and 0.3% calcium carbonate, and the flasks were incubated at 28°C for 48 hours on a reciprocal shaker. Two liters of the preculture gathered from 20 flasks incubated as described above were transferred to 100 liters of the same constituent medium in a 150-liter stainless steel fermentor, and the fermentation was conducted at 28°C under aeration of 50 liters per minute with stirring at 250 rpm.

Soybean oil was employed as an antifoaming agent. The time course of pH change, level of residual glucose, mycelial growth and antibiotic activity are shown in Table 1. The accumulation of

Table 1. Typical pattern of the fermentation

Time (hours)	pH	Glucose* (mg/ml)	Mycelium** ratio (%)	Samarosporin*** (mcg/ml)
16	6.0	8.4	10	300
24	6.4	3.8	35	420
32	7.0	0	45	490
40	8.0	0	45	600

* The level of glucose was measured by SHAFFER-HARTMANN method.[1)]

** The mycelium ratio was measured by centrifuging a 10 ml sample of the whole broth for 15 minutes at 3,000 rpm. The percentage of the solid (vol/vol) was described.

*** Antibiotic activity was assayed by a paper disc method using *Staph. aureus* FDA 209 P as test organism. Samples tested were prepared by extracting the mycelium with methanol.

samarosporin was in parallel with mycelial growth and increasing pH. Glucose was consumed completely in the period from 24 to 32 hours of fermentation, but further accumulation of samarosporin succeeded after glucose was exhausted.

Isolation and Purification

Samarosporin was extracted from the mycelium of strain No. F-7762 but was not extracted from the broth-filtrate. The isolation and purification procedures are summarized in Fig. 1.

Fig. 1. Isolation and purification process of samarosporin

Cultured broth (100 liters)
↓ filtered with filter-aid
Mycelium cake
↓ extracted with methanol
Methanol extract
↓ concentrated *in vacuo*
Precipitate
↓ dissolved in methanol at 45°C and decolorized with activated carbon
Methanol solution
↓ added 3-fold volumes of acetone and kept overnight at room temperature
Crude crystals (70 g)
↓ recrystallized from methanol
Pure crystals (40 g)

Physico-chemical Properties

Samarosporin recrystallized from MeOH is colorless crystalline needles, which melt at 255~256.5°C. The optical rotation shows $[\alpha]_D^{20}+16.5°$ (*c* 1, MeOH). The elemental analysis gave C 57.73, H 7.67, N 14.03%, no halogen nor sulfur. The molecular weight of 1,480±20 was measured by vapour pressure method. Samarosporin is easily soluble in methanol, soluble in ethanol, propylene glycol, dimethylsulfonamide, pyridine and glacial acetic acid, and insoluble in ethyl acetate, chloroform, benzene, petroleum ether and *n*-hexane. Furthermore, samarosporin is soluble in neither acetone nor water, but soluble in the mixture of acetone and water in the ration of 5: 5 to 8: 2.

Samarosporin is positive in RYDON-SMITH and negative in MOLISCH, anthrone, ninhydrin, biuret, ferric chloride, benzidine and 2,4-dinitrophenylhydrazine reactions. The ultraviolet absorption

Fig. 2. Ultraviolet absorption spectrum of samarosporin

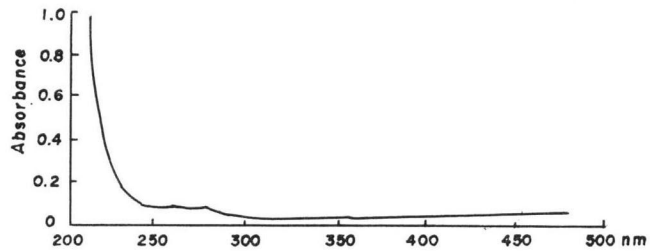
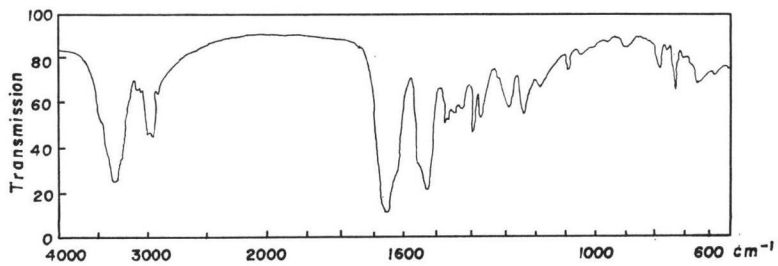


Fig. 3. Infrared absorption spectrum of samarosporin (KBr)

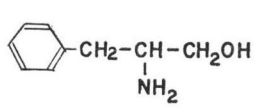


spectrum of samarosporin shows no characteristic absorption in methanol as shown in Fig. 2. The infrared absorption spectrum in KBr tablet (Fig. 3) exhibits characteristic bands for hydroxyl (3300 cm^{-1}) and amide carbonyl (1650 cm^{-1}).

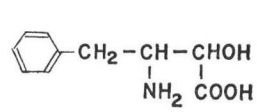
Amino acid analysis of the acid hydrolyzate of samarosporin indicated the molar ratio of phenylalanine (1 mole), hydroxyproline (2 moles), glutamic acid (1 mole), valine (1 mole), glycine (1 mole), leucine (1 mole), α-amino-*iso*-butyric acid (6 moles), ammonia (1 mole) and one unknown compound (1 mole).

A pale yellow substance was obtained from the $CHCl_3$ extract of the hydrolyzate. Recrystallization of the substance from ether gave colorless crystals of m.p. 91~93°C, $[\alpha]_D^{20}$ −25.5° (*c* 1, EtOH) and analytical data C 71.86, H 8.68, N 9.26%, which were supposed to be phenylalaninol. So, L-phenylalaninol was synthesized following the method by SEKI *et al.*[2] The above crystalline substance was identical with this phenylalaninol in mixed melting point and IR spectra.

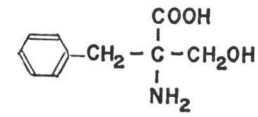
From the results of IR spectrum and ninhydrin reaction a cyclopeptide structure could be proposed for samarosporin. It can therefore be presumed that the unknown compound may exist in a form of 1-hydroxy-2-amino-3-phenylbutyric acid or 1-hydroxymethyl phenylalanine in the antibiotic, and L-phenylalaninol obtained above may have resulted from decarboxylation of one of them.



L-Phenylalaninol



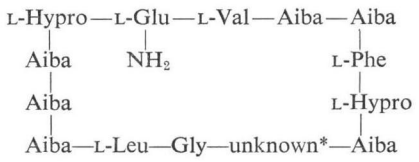
1-Hydroxy-2-amino-3-phenylbutyric acid



1-Hydroxymethyl phenylalanine

The molecular formula $C_{72}H_{111}N_{15}O_{19}$ was tentatively indicated for samarosporin from the results of elemental analysis, molecular weight and amino acid analysis. The molecular weight and elemental analysis calculated for the molecular formula are 1,489 and C 57.90, H 7.58, N 14.03%, respectively.

Fig. 4. Tentative amino acid sequence of samarosporin



*: found as phenylalaninol in the hydrolyzate
Aiba: α-Amino-*iso*-butyric acid

The studies on the chemical structure of the antibiotic will be reported in this journal upon its completion, however, from the results of the studies, the amino acid sequence is presumed as shown in Fig. 4.

Biological Properties

Table 2 shows the antimicrobial spectrum of samarosporin when assayed by two-fold agar dilution method. Samarosporin is active against Gram-positive and Gram-negative bacteria, yeasts, fungi and protozoa, but its activity is generally moderate.

The effect of serum on the antimicrobial activity of the antibiotic was determined by the bouillon dilution method in the presence of 0, 5, 10 and 20% (v/v) of bovine serum using *Staphylococcus aureus* FDA 209P as the test organism. As the result, increase in the minimum inhibitory concentration was observed when serum was added (Table 3).

The LD_{50} values of the antibiotic in mice were as follows: 27 mg/kg intraperitoneally and 750 mg/kg orally when it was administered in the form of aqueous suspension with 0.5% carboxymethyl cellulose.

Table 2. Antimicrobial spectrum of samarosporin

Test organisms	Minimum inhibitory concentration (mcg/ml)	Medium
Staphylococcus aureus FDA 209 P	15.6	N
Staphylococcus albus	31.2	N
Corynebacterium sepedonicum	15.6	N
Sarcina lutea	15.6	N
Bacillus subtilis PCI 219	31.2	N
Micrococcus flavus	15.6	N
Serratia marcescens	31.2	N
Aerobacter aerogenes	125.0	N
Escherichia coli B	62.5	N
Salmonella enteritidis No. 11	62.5	N
Shigella flexneri	31.2	N
Erwinia carotovora	15.6	GP
Xanthomonas oryzae	15.6	GP
Mycobacterium 607	15.6	G
Candida albicans YU-1200	62.5	S
Cryptococcus neoformans	62.5	S
Saccharomyces sake	31.2	S
Trichophyton interdigitale	500.0	S
Sporotricum gougeroti	15.6	SY
Penicillium chrysogenum	62.5	GP
Piricularia oryzae	31.2	GP
Tetrahymena geleii W	15.6	*

N: Nutrient agar. GP: Glucose-potato agar. G: Nutrient agar+glycerin. S: SABOURAUD agar. SY: SABOURAUD agar+yeast extract.
* Proteose-peptone bouillon dilution.

Table 3. Effect of serum on samarosporin

Serum (%)	Minimum inhibitory concentration (mcg/ml)
0	15.6
5	500
10	500
20	500

Discussion

Of the peptide antibiotics produced by fungi, antibiotic I.C.I. 13959,[3] enniatin,[4] suzukacillin,[5,6] and radicicolon[7] are known. However, the above mentioned physico-chemical properties of samarosporin, especially its amino acid constitution are different from any of these antibiotics and also from any other known peptide antibiotics. As a peptide antibiotic containing phenylalaninol antiamoebin[8,9] was reported, but the physico-chemical properties and amino acid constitutions of antiamoebin and samarosporin are different from each other.

Thus, samarosporin can be considered to be a new antibiotic.

Acknowledgement

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