HERBICIDINS F AND G, TWO NEW NUCLEOSIDE ANTIBIOTICS

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A mutant of *Streptomyces saganonensis* No. 4075, obtained with N-methyl-N′-nitro-N-nitrosoguanidine treatment, produced herbicidins F and G without herbicidins A and B. Isolation of the antibiotics was performed by adsorption on resinous adsorbent followed by elution with aqueous MeOH. Herbicidin F was obtained as colorless needles after extraction of the eluate using methylene dichloride. Purification of herbicidin G was completed with silica-gel chromatography to give a crystalline powder. Physico-chemical characterization revealed that herbicidins F and G were new nucleoside antibiotics having an adenine moiety in their structures. There was no inhibition activity at 100 μg/ml of herbicidins F and G against all of bacteria and yeast tested. Herbicidin F, as well as herbicidin G, are inhibitory activity against some of fungi such as *Tricophyton rubrum* (MIC; 6.25 μg/ml), *T. asteroides* (6.25 μg/ml), *T. mentagrophytes* (6.25~12.5 μg/ml), *Botrytis cinerea* (12.5 μg/ml), *Blastomyces brasiliensis* (12.5~25 μg/ml).

Streptomyces saganonensis No. 4075 produced new antibiotics, herbicidins A, B[1,2], C and E[3] with 9-β-D-arabinofranosyl adenine and deoxycoformycin. We isolated some mutants of the organism by means of the treatment with N-methyl-N′-nitro-N-nitrosoguanidine (NTG). A strain of these mutants produced herbicidins F and G, new nucleoside antibiotics, without the production of herbicidins A and B.

The present paper describes the fermentation, isolation, physical and chemical properties and biological properties of herbicidins F and G. Structural elucidation of the antibiotics will be reported elsewhere.

Materials and Methods

Mutagenesis and selection of the mutants

A strain of *S. saganonensis* was cultivated for 10 days on a slant YM agar medium containing per liter; 4 g yeast extract (Difco Lab., Detroit, U.S.A.), 10 g malts extract (Difco Lab.), 4 g glucose and 20 g agar powder (Eikenkagaku, Tokyo, Japan) (pH 6.5 before sterilization). Spores of the organism grown on the slant were suspended in 1/15 M phosphate buffer (pH 7.0), and treated with N-methyl-N′-nitro-N-nitrosoguanidine (NTG) at 2,000 μg/ml at 27°C for 2 hours (killing rate 99.7%). After washing twice with saline, the mutagenized spores were spread on the YM agar plates and incubated at 27°C for 3 days.

Colonies, well separated, on the plates were transferred to the fresh slants and incubated at 27°C for 10 days. Each strains on the slants were inoculated into 500-ml shake flasks containing 50 ml of HC-4 medium consisting per liter; 20 g soybean meal, 10 g glucose, 20 g soluble starch, 20 g fresh baker's yeast, 10 g KH_2PO_4, 3 g $CaCO_3$, 5 g NH_4Cl, 1 g $ZnSO_4 \cdot 7H_2O$ and 0.2 g $MgSO_4 \cdot 7H_2O$ (pH 6.5 before sterilization). Each of the culture broths was assayed by the method described in the previous paper[3].

Fermentation

The fermentation of a mutant strain of the organism was performed in 30-liter jar fermentors

containing 15 liters of the HC-4 medium. The preculture was conducted at 27°C for 2 days in 2-liter Erlenmeyer flasks containing 500 ml of the HC-4 medium. Each flask of a good preculture was then inoculated into each jar fermentor (inoculum size; 3%). During 120-hour incubation period of the main culture at 24°C, dissolved oxygen concentration in the broth was held at more than 2 ppm by agitation and aeration.

Isolation

Fermentation broth (30 liters) containing herbicidins F and G was filtered with the aid of infusorial earth (Celite 545 from Johne-Manville Products Corp., Calif., U.S.A.) The filtrate (20 liters) was adsorbed on 2 liters of Diaion HP-20 (Adsorbent resin from Mitsubishi Co., Ltd., Japan) column. The spent broth and water washes (10 liters) were discarded. The adsorbate was washed with 10 liters of 40% aqueous MeOH again, and eluted with 20 liters of 45% aqueous MeOH to obtain herbicidin G. Herbicidin F was then eluted from the adsorbate (Diaion HP-20) with 25 liters of 70% aqueous MeOH.

Fifteen liters of the eluate containing herbicidin G was concentrated *in vacuo* at 45°C to 3 liters of aqueous solution, which was extracted twice with 3 liters of EtOAc. The extract was concentrated to dryness to yield 5.8 g of crude solid. The solid was dissolved in 10 ml of MeOH, and placed on a 700 ml of Sephadex LH-20 column to be developed by MeOH. Three hundred milliliters of the fraction containing herbicidin G was concentrated to dryness *in vacuo* at 45°C. One hundred ninety milligrams of herbicidin G was obtained as a crystalline powder and recrystallized from EtOAc.

Twenty liters of the eluate containing herbicidin F was concentrated *in vacuo* at 45°C to 3 liters of aqueous solution, which was extracted twice with 3 liters of CH_2Cl_2. The extract was concentrated to dryness *in vacuo* at 45°C. The crude solid thus obtained was dissolved in 500 ml of MeOH and decolorized by means of 5 g of active carbon. Twelve grams of needle crystals of herbicidin F were obtained from the decolorized MeOH solution after concentration *in vacuo* at 45°C.

Results and Discussion

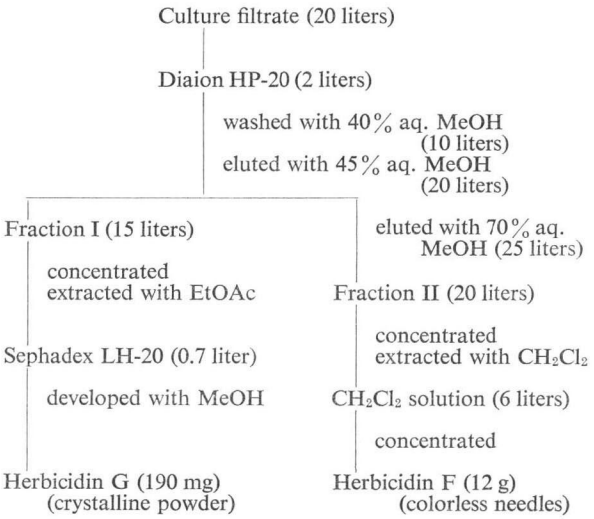
Mutagenesis and Fermentation

A mutant of *S. saganonensis* No. 4075, m 17-F strain, which mainly produce herbicidins F and G without herbicidins A, B, C and E, was obtained by mutagenesis using N-methyl-N′-nitro-N-nitrosoguanidine (NTG) at 2,000 μg/ml for 2 hours. Large-scale fermentations of the mutant were conducted in 30-liter jar fermentor containing 15 liters of the HC-4 medium. After 120-hour incubation, the fermentation broth contained herbicidins per liter; 1,100 mg F, and 30 mg G. However, herbicidins A, B, C and E was not detected in the final broth.

Isolation

The isolation method used for herbicidins F and G is outlined in Fig. 1. For the first step purification, the resinous adsorbent, Diaion HP-20 was selected, because it gave a satisfactory separation of herbicidins each other and satisfactory re-

Fig. 1. Isolation and purification of herbicidins F and G.



covery of the antibiotics in their eluates. Herbicidin F was extracted by CH_2Cl_2 from the eluate, and obtained as colorless needles. Purification of herbicidin G was completed on Sephadex LH-20 column after extraction with EtOAc from the eluate.

Table 1. Physico-chemical properties of herbicidins F and G.

	Herbicidin F	Herbicidin G
Nature	Colorless needle crystals	White crystalline powder
Solubility	Soluble in H_2O, MeOH, EtOH, acetone, EtOAc, CH_2Cl_2	Soluble in MeOH, EtOH, acetone EtOAc.
Melting point	150~154°C (dec.)	142~146°C (dec.)
M. W.	535	
Elementary analysis	C, 51.58; H, 5.46; N, 13.08%	C, 50.67; H, 5.22; N, 13.43%
	Calcd for $C_{23}H_{29}N_5O_{10}$ (535.51) C, 51.59; H, 5.46, N, 13.08%	
Rf*	0.71	0.53
UV_{max} ($E^{1\%}_{1cm}$)	260 nm (245)	260 nm (200)

* Merck, DC-Ferting platten Kieselgel 60 F_{254} ($CHCl_3$ - MeOH, 7 : 3)

Physical and Chemical Properties

Physico-chemical data of herbicidins F and G are listed in Table 1. Herbicidin F is readily soluble in MeOH, EtOH and acetone, soluble in H_2O, EtOAc and CH_2Cl_2 but insoluble in *n*-hexane. Herbicidin G is readily soluble in MeOH, soluble in EtOH, acetone and EtOAc, but insoluble in *n*-hexane. Molecular weight of herbicidin F was derived from analysis of its FD mass spectrum. The IR and NMR spectra of herbicidins F and G are shown in Figs. 2, 3, 4 and 5, respectively. Signals at δ 2.05 ppm in Fig. 4 and at δ 3.18 in Fig. 5 were due to $(CD_3)_2CO$ and CD_3OD used as a solvent, respectively.

Their physico-chemical properties, such as UV maxima at 260 nm, elementary analysis, two singlets due to heteroaromatic protons at δ 7.8~8.3 ppm and doublet due to one proton at δ 5.9~6.2 ppm corresponding to the chemical shift of anomeric proton of usual nucleosides in NMR spectra, and fragment ion peak at 135 in FD mass spectrum, suggested adenine nucleoside moiety in the structures of both herbicidins F and G. From the results mentioned above and characteristic biological activities described below, one can conclude that herbicidins F and G are two new nucleoside antibiotics

Fig. 2. Infrared spectrum of herbicidin F (KBr).

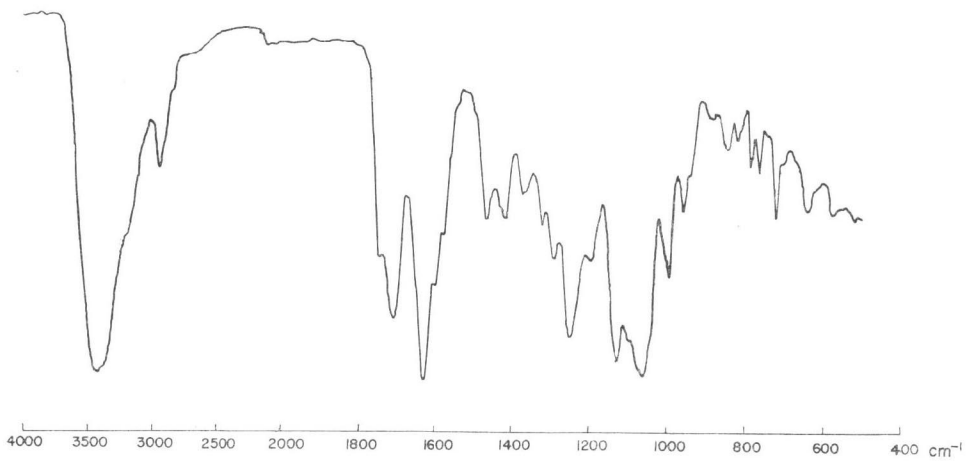


Fig. 3. Infrared spectrum of herbicidin G (KBr).

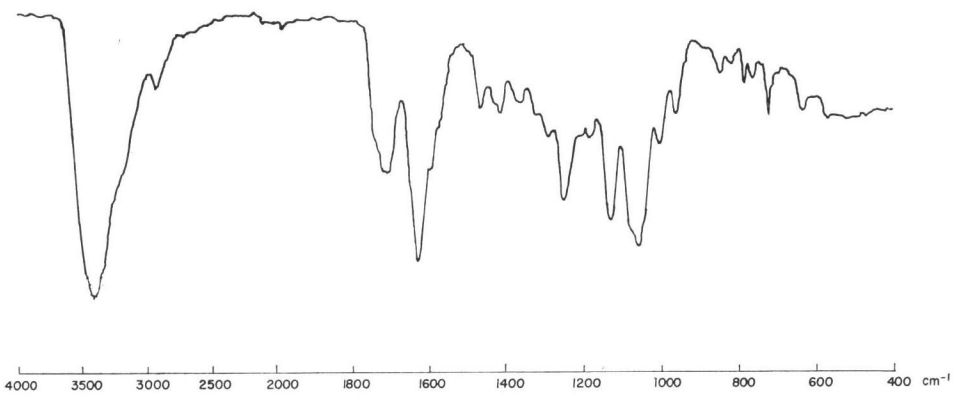
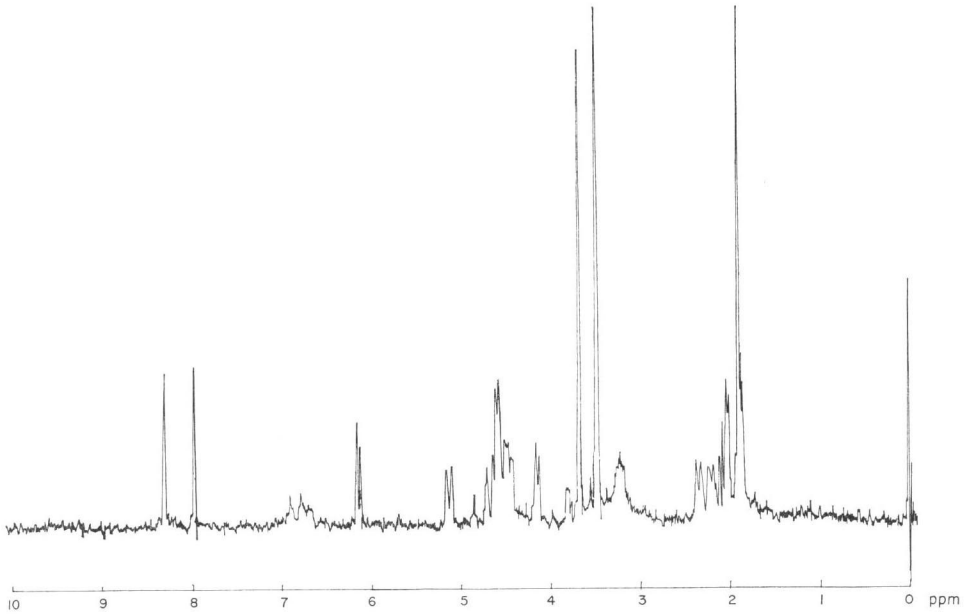


Fig. 4. NMR spectrum of herbicidin F in $(CD_3)_2CO$ at 60 MHz.



with very closely related structures.

Biological Properties

Antimicrobial activities of herbicidins F and G were tested with the method reported from our laboratories[1]. There were no activity at 100 μg/ml of herbicidins F and G against all of the bacteria tested such as *Staphylococcus aureus* FDA 209P (JC-1), *Escherichia coli* NIHJ (JC-2), *Mycobacterium smegmatis* ATCC 607, *Pseudomonas aeruginosa* SANK 73575, *Proteus rettgeri* SANK 73775, *Klebsiella pneumoniae* PCI 602, *Serratia marcescens* SANK 73060, *Bacteroides fragilis* SANK 71176, *Bacillus subtilis* PCI 219, and all of the yeast tested such as *Candida albicans* SANK 50157, *Cryptococcus neoformans* SANK 57963, *Saccharomyces cerevisiae* SANK 50170. However, growth of *Trychophyton mentagrophytes* SANK 11868 was inhibited by herbicidin F at 6.25~12.5 μg/ml and herbicidin G at

Fig. 5. NMR spectrum of herbicidin G methyl ester in $CDCl_3$ and CD_3OD at 60 MHz.

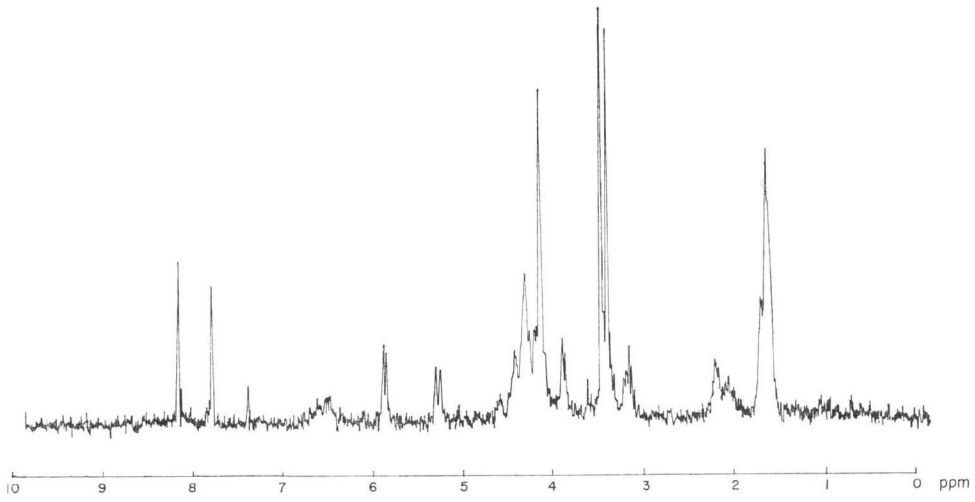


Table 2. Antifungal spectrum of herbicidin F.

Test organism	MIC (μg/ml)		
	2 days	4 days	7 days
Trichophyton mentagrophytes SANK 11868	6.25	12.5	12.5
Trichophyton rubrum SANK 11768	6.25	6.25	6.25
Trichophyton tonsurans SANK 30970	25	50	50
Trichophyton asteroides SANK 10362	6.25	6.25	6.25
Trichophyton interdigitale SANK 10469	50	50	50
Trichophyton megninii SANK 30870	12.5	12.5	12.5
Microsporum gypseum SANK 11668	12.5	50	50
Sporothrix schenckii SANK 30166	100	>100	>100
Aspergillus oryzae SANK 11262	>100	>100	>100
Hormodendrum pedrosoi SANK 11758	100	100	100
Mucor mucedo IFO 5776	>100	>100	>100
Coccidioides immitis IFO 5960	25	50	100
Blastomyces brasiliensis SANK 20567	12.5	25	25
Alternaria solani SANK 16676	50	100	100
Botrytis cinerea SANK 16976	12.5	12.5	12.5
Cochliobolus miyabeanus SANK 16476	>100	>100	>100
Colletotrichum lagenarium SANK 16576	>100	>100	>100
Fusarium oxysporum SANK 17076	100	>100	>100
Pellicularia filamentosa SANK 16376	>100	>100	>100
Penicillium chrysogenum SANK 12768	>100	>100	>100
Pyricularia oryzae SANK 14758	100	>100	>100

100 μg/ml. The inhibitory activities of herbicidins F and G were not affected by addition of 10% volume of horse serum. As shown in Table 2, herbicidin F also inhibited some of fungi, such as *T. rubrum* SANK 11768, *T. asteroides* SANK 10362, *T. megninii* SANK 30870, *T. tonsurans* SANK 30970, *Microsporum gypseum* SANK 11668, *Blastomyces brasiliensis* SANK 20567, *Botrytis cinerea*

SANK 16976, *Coccidioides immitis* IFO 5960.

The acute toxicity of herbicidin F, LD_{50}, administered intraperitonealy to mice, was more than 600 mg/kg. Herbicidal activities of the antibiotics will be reported elsewhere.

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