CHRYSCANDIN†, A NOVEL PEPTIDYL NUCLEOSIDE ANTIBIOTIC

I. TAXONOMY, FERMENTATION, ISOLATION AND CHARACTERIZATION

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A novel antifungal antibiotic, chryscandin was found in the culture broth of a strain of fungi. The producing organism was subsequently identified as *Chrysosporium pannorum*. The antibiotic obtained as colorless needle crystals ($C_{20}H_{23}N_7O_6$, MW 457) is active against *Candida albicans* and Gram-positive bacteria, but it is ineffective against filamentous fungi and Gram-negative bacteria. Acute toxicity is very low in mice.

In the course of screening for new antifungal antibiotics, we found a novel antibiotic, chryscandin. In this paper, we describe taxonomic studies on the producing strain, fermentation, isolation procedures, and physico-chemical and biological properties of chryscandin.[1]

Studies in our laboratories[2,3] have shown that chryscandin has a unique structure, which possesses a 3-aminoribofuranuronic acid and an adenine nucleus in the molecule. Details of structure determination and synthesis of chryscandin will be reported in the succeeding paper.[2]

Taxonomy

The strain No. 4629 producing chryscandin was originally isolated from Otaru City, Hokkaido. The mycological characters of the strain are as follows.

The hyaline conidiophores are erect, up to 30 μm long and 1~2 μm thick. They are verticillately branched at nearly right to acute angles, and produce hyaline conidia at the tips or in intercalary position of the laterals. This conidiogenesis is holoblastic. The conidia are solitary, pyriform or subglobose, slightly roughened, 3~4.5×2~4 μm in size and 1~1.5 μm wide at the truncate base (Fig. 1).

The colonies on malt extract agar (Difco) grow very restrictedly, attaining 1~2 cm in diameter after two weeks at 25°C, and the surface is raised, dense and pale yellow orange to pale red. The conidial formation is very limited and the reverse is yellow brown. This strain can grow at temperature range from 1 to 29°C with the growth optimum at 17°C.

From comparing these characteristics with the descriptions of CARMICHAEL[4] and MATSUSHIMA,[5] the strain No. 4629 was identified as one strain of *Chrysosporium pannorum* (Link) Hughes, and named *Chrysosporium pannorum* No. 4629 (ATCC 20617).

† A part of this paper was presented at the Annual Meeting of Agricultural Chemical Society of Japan, No. 1V-11. Tokyo, April 1~4, 1984. Chryscandin was originally designated as WF-4629 (FR-48736).

Fig. 1. Photographs of *C. pannorum* No. 4629. (Scale: 20 μm)

A. Conidia produced from a tree-like conidiophore. B. Mature conidia and conidiophores.

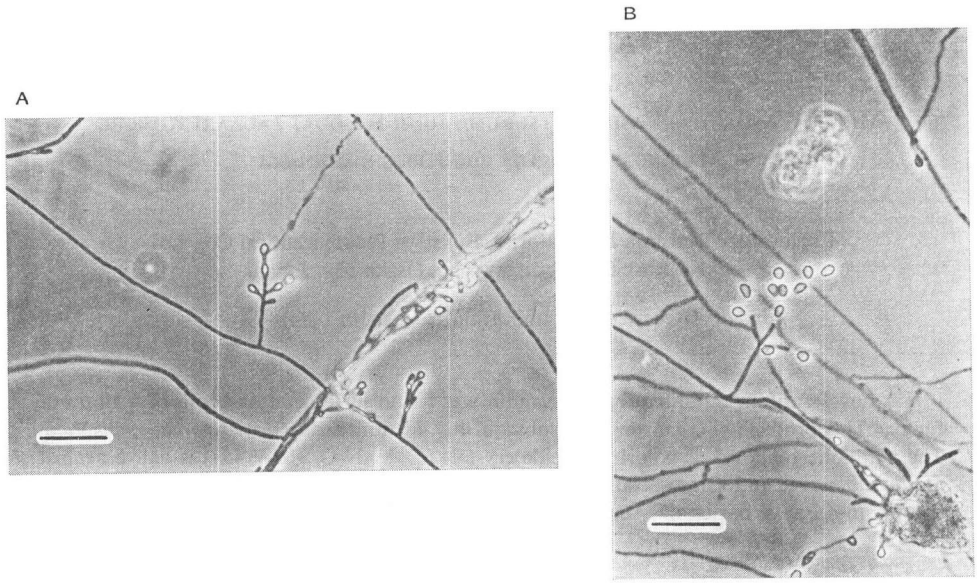


Table 1. Media used for production of chryscandin.

Seed medium	% (W/V)
Corn starch	2
Glucose	1
Corn steep liquor	1
Peanut powder	1
Dried yeast	0.5
Calcium carbonate	0.5
pH	6.0
Production medium	**% (W/V)**
Modified starch	2
Glucose	1
Beast bone extract	3
Calcium carbonate	0.5
pH	6.0

Fig. 2. Isolation procedure of chryscandin.

Filtrate 40 liters
↓
Diaion HP-20
→ eluted with 40% aq MeOH
concentrated
↓
CM-Sephadex C-25 (H^+ form)
→ eluted with 0.1 N HCl
concentrated
adjusted to pH 7.0 with 6 N NaOH
↓
Crude crystals
↓
Dissolved in 0.1 N HCl
→ decolorized with activated carbon
↓
Colorless needles 1.28 g (Dihydrochloride)

Fermentation

A loopful of *C. pannorum* No. 4629 on mature slant culture was inoculated to each of twenty one 500-ml Erlenmeyer flasks containing 100 ml of sterile seed medium shown in Table 1. The flasks were shaken on a rotary shaker (220 rpm, 5.1 cm throw) for 96 hours at 25°C. The content of seven flasks was inoculated to 18 liters of sterile fermentation medium shown in Table 1, in each of three stainless steel fermentors. The fermentation was carried out at 28°C for 160 hours, with aeration of 18 liters/minute, agitation of 250 rpm and inner pressure of 1.0 kg/cm^2. *Candida albicans* was used as a test organism with malt extract agar for the bioassay.

Isolation

A procedure for isolation of chryscandin is outlined in Fig. 2. The culture broth (48 liters) was filtered with the aid of filter aid (Radiolite). The filtrate (40 liters, pH 7.2) was passed through a

column (8 liters) of Diaion HP-20 (Mitsubishi Chemical Industries Ltd.). The column was washed with water and 20% aqueous methanol and then eluted with 40% aqueous methanol (16 liters). The eluate was concentrated to remove methanol *in vacuo* and the concentrate (5 liters) was chromatographed on CM-Sephadex C-25 (H^+ form, Pharmacia Fine Chemicals) column (1 liter). The column was washed with water (1 liter) and then eluted with 0.1 N HCl (2 liters). The eluate was concentrated to 500 ml and adjusted to pH 7.0 with 6 N NaOH. The concentrate was left to stand overnight in a refrigerator. The yellow needle crystals (1.85 g) were collected by filtration. The crystals were dissolved in 0.1 N HCl (100 ml) and the solution was decolorized with activated carbon (Sirasagi: Wako Pure Chemicals Co.). After the carbon was filtered off, the filtrate was left to stand overnight in the cold. Colorless needle crystals were collected to give dihydrochloride monohydrate of chryscandin (1.28 g).

Physico-chemical Properties

Chryscandin is an amphoteric colorless substance which decomposes at 215~233°C. It is slightly soluble in water and methanol, and insoluble in acetone, ethyl acetate and chloroform. The optical rotation is $[\alpha]_D^{22}+34°$ (c 1.0, 1 N HCl). Color reactions are as follows: Positive to ninhydrin, iodine and cerric sulfate, negative to Molish and ferric chloride, The molecular formula was determined as $C_{20}H_{23}N_7O_6 \cdot 2HCl \cdot H_2O$ by FD-MS (m/z 458 ($M^+ +1$)) and elementary analysis which gave the following data:

Anal Calcd for $C_{20}H_{23}N_7O_6 \cdot 2HCl \cdot H_2O$: C 43.80, H 4.96, N 17.88, Cl 12.93.
Found: C 43.49, H 4.82, N 18.25, Cl 13.04.

Rf values of chryscandin on TLC are summarized in Table 2. The UV spectra of chryscandin showed absorption maxima at 260 nm (ε 32,500) in H_2O, 258 nm (ε 30,600) in 0.1 N HCl and 260 nm (ε 33,400) in 0.1 N NaOH. The IR and NMR spectra of chryscandin are shown in the succeeding paper.[2]

Biological Properties

The antibacterial spectrum of chryscandin is shown in Table 3. This test was conducted by the serial agar dilution method. For bacteria, one loopful of an overnight culture (about 10^8 viable cells/ml) of each test strain in nutrient broth was streaked on nutrient agar containing the graded

Table 2. Chromatographic behavior of chryscandin.

TLC	Solvent system	Rf
Cellulose[a]	2-Propanol - H_2O (75: 25)	0.11
	1-BuOH - AcOH - H_2O (4: 1: 2)	0.78
Silica gel[b]	1-BuOH - AcOH - H_2O (4: 1: 2)	0.21

[a] Eastman cellulose sheet No. 6065.
[b] Merck Kieselgel 60 F_{254}, Art 5735.

Table 4. Protective efficacy in experimental infection in mice of chryscandin.

Compound	Route	ED_{50} (mg/kg)
Chryscandin	sc	10
	po	25
5-Fluorocytosine	po	20

Table 3. Antibacterial spectrum of chryscandin.

Test organism	MIC (μg/ml)
Escherichia coli NIHJ JC-2	>100
Staphylococcus aureus 209P JC-1	12.5
Candida albicans FP-614	0.8
〃 〃 FP-616	0.2
〃 〃 FP-618	0.8
〃 〃 FP-620	0.2
〃 〃 FP-622	3.1
〃 〃 FP-633	1.6
C. tropicalis FP-583	>100
C. krusei FP-585	>100
C. parakrusei FP-586	>100
Aspergillus niger	>100
Trichophyton mentagrophytes	>100

Agar dilution method (nutrient agar for bacteria, malt extract agar for fungi and yeasts).

concentration of drug and the minimal inhibitory concentration (MIC) was determined after incubation at 37°C for 24 hours. For fungi and yeasts, one loopful of an overnight culture of each test strain in malt extract broth was streaked on malt extract agar containing the graded concentration of drug and the MIC was determined after incubation at 30°C for 3 days.

Chryscandin shows antibacterial activity against *C. albicans* and *Staphylococcus aureus* 209P weakly, but it has no activity against Gram-negative bacteria and filamentous fungi.

A single intraperitoneal administration of 1 g/kg of chryscandin into *dd*Y mice did not result in any toxic symptom for 2 weeks after injection. The *in vivo* activity of chryscandin against experimental infections due to *C. albicans* was examined. One hour after the intravenous injection of 3.0×10^6 cells of *C. albicans* FP-633 to each *dd*Y mouse (5 weeks, ♂) the drug solutions were administered subcutaneously or orally. A group of five mice was used for each dosage level with animal being observed for seven days to determine the median effective dose (ED_{50}).

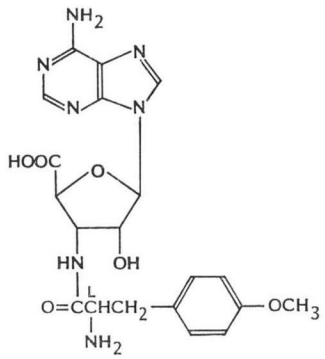
As shown in Table 4, chryscandin has a protective efficacy against an experimental infection of *C. albicans* which is almost the same as the efficacy of 5-fluorocytosine.

Discussion

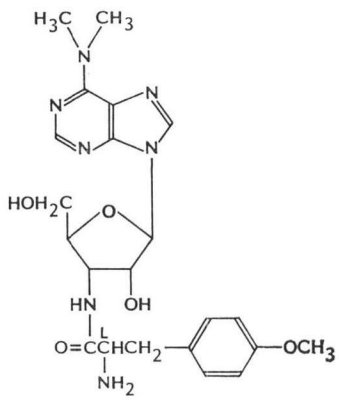
Chryscandin shows antibacterial activity against *C. albicans* and has low toxicity in mice. Chryscandin is an amphoteric, water-soluble antibiotic and its molecular formula has been determined to be $C_{20}H_{23}N_7O_6$. As we described in succeeding paper,[2] chryscandin has an adenine nucleus. Although several adenine-containing nucleoside antibiotics have been reported, chryscandin is distinct from any antibiotics so far reported in its chemical and biological properties.

From the point of chemical structure, chryscandin (Fig. 3) resembles puromycin, an adenine-containing peptidyl nucleoside antibiotic. However, distinct differences were found in biological properties of the two antibiotics. Chryscandin has an activity against *C. albicans* and has low toxicity in mice as mentioned above, whereas puromycin is only active against bacteria and fairly toxic in mice.

Fig. 3. The structures of chryscandin and puromycin.



Chryscandin



Puromycin

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