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Arborcandins A, B, C, D, E and F, Novel 1,3-β-Glucan Synthase Inhibitors: Production and Biological Activity

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Arborcandins A, B, C, D, E and F, which possess potent 1,3-β-glucan synthase inhibitory activity, were isolated from the culture broth of a filamentous fungus, strain SANK 17397. Arborcandins are novel cyclic peptides, that are structurally different from known glucan synthase inhibitors such as echinocandins. The 1,3-β-glucan synthases of *Candida albicans* and *Aspergillus fumigatus* were inhibited by arborcandins with IC_{50} ranging from 0.012 to 3 μg/ml. The apparent *Ki* value of arborcandin C for *C. albicans* and *A. fumigatus* were 0.12 μM and 0.016 μM, respectively. The inhibition against these two 1,3-β-glucan synthases by arborcandin C was noncompetitive. These compounds exhibited potent fungicidal activity against *Candida* spp. with MIC ranging from 0.25 to 8 μg/ml. The growth of *A. fumigatus* was suppressed by arborcandins with concentrations ranging from 0.063 to 4 μg/ml.

The incidence of invasive fungal infections has increased in the past 20 years[1,2]. These infections are often life-threatening and a major problem particularly in immunocompromised patients[2]. The most common drugs for the treatment of fungal infections are azole antifungals. However, they have a limited spectrum of activity, and the long-term treatment of candidiasis and other fungal infections leads to the emergence of resistant strains[1,3]. Amphotericin B, the most conventional drug to treat fungal infections, is mostly effective but rather toxic causing renal failures[1,2]. New fungicidal drugs with less side effect are expected to be developed. The 1,3-β-glucan polymer, an essential component of the fungal cell wall, is not present in mammalian cells[4~7]. Therefore 1,3-β-glucan synthase is expected to be an ideal target for specific fungicidal agents[5]. In the course of our screening program for 1,3-β-glucan synthase inhibitors, we found that a filamentous fungus, strain SANK 17397, produced novel antifungal antibiotics, arborcandins A, B, C, D, E and F (Fig. 1). In this paper we describe the fermentation and biological activities of arborcandins. The isolation and structural elucidation as well as physico-chemical properties of these compounds will be described elsewhere.

Materials and Methods

Producing Organism

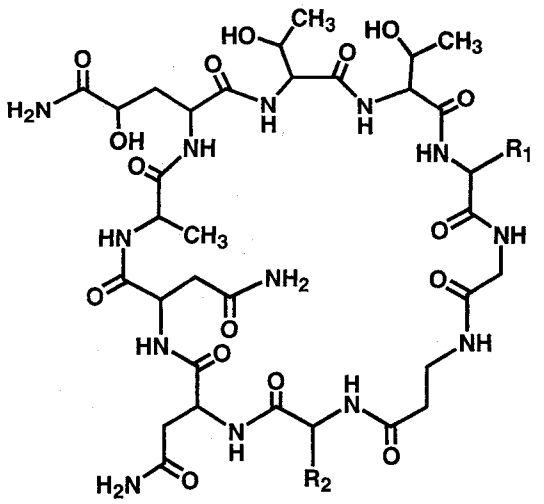
The producing fungus was isolated from a plant sample collected at Ube, Yamaguchi prefecture, Japan. It was deposited at the National Institute of Bioscience and Technology, Japan as SANK 17397 under the accession number of FERM BP-6123.

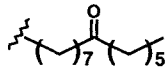
Fermentation

FFA-1 medium consisted of glucose 3%, glycerol 3%,

Dedicated to the memory of Sir EDWARD ABRAHAM.

Fig. 1. Structures of arborcandins.



Arborcandins	R_1	R_2
A	m=8	n=3
B	m=8	n=4
C	m=8	n=5
D	m=8	
E	m=9	n=5
F	m=10	n=5

starch 2%, soybean meal 1%, gelatin 0.25%, yeast extract 0.25% and NH_4NO_3 0.25%, and used as both the seed and production media for arborcandins. Slant cultures of strain SANK 17397 grown on Potato dextrose agar (Nissui PDA powder 3.9% and agar 0.5%) for 7 days at 23°C were inoculated into each of four 2-liter Erlenmeyer flasks containing 450 ml of FFA-1 medium. The flasks were incubated on a rotary shaker at 23°C, 210 rpm for 4 days. Nine hundred ml of the seed culture was transferred into each of two 60-liter fermentors containing 30 liters of FFA-1 medium. This preculture was grown at 23°C for 24 hours. Then, 9 liters of the culture was transferred into each of two 600-liter fermentors containing 300 liters of FFA-1 medium. The fermentation was carried out at 23°C for 8 days with an aeration of 300 liters/minute. The dissolved oxygen concentration was kept at 5 ppm by agitation. The production of arborcandin C was monitored by HPLC (Waters Symmetry ODS, 4.6×150 mm, Waters; 45% CH_3CN in 0.2% triethylamine phospate buffer (pH 3.0), 1.0 ml/minute; UV at 210 nm). Under these conditions, arborcandin C was eluted at a retention time of 17.6 minutes.

Isolation

The 600 liters of the 8-day cultured broth and the 310 liters of fermentor-wash were combined and an equal volume of acetone - MeOH (50 : 50) was added, followed by stirring for 1 hour. The filtrate of the mixture (1675 liters) was concentrated *in vacuo* to 930 liters of aqueous residue, which was adjusted to pH 7.0 with 6 N NaOH. The pH-adjusted extract was then diluted with MeOH to 1725 liters and subjected to a 60-liter Diaion HP-20 column equilibrated with MeOH - H_2O (50 : 50). After washing the column with 300 liters of MeOH - H_2O (60 : 40), the active materials were eluted with 280 liters of CH_3CN - H_2O (40 : 60). Two hundred fifty liters of the eluate was diluted with H_2O to 400 liters and subjected to a 5-liter Diaion HP-20 column equilibrated with MeOH - H_2O (50 : 50). After washing the column with 40 liters of MeOH - H_2O (65 : 35), the active fraction was eluted with 29 liters of MeOH - H_2O (90 : 10). Twenty liters of the active fraction was concentrated *in vacuo* to yield a crude powder, which was then dissolved in 550 ml of dimethyl sulfoxide (DMSO). The final step of the purification was carried out by preparative HPLC (YMC YMC-Pack-ODS, 100×500 mm; CH_3CN - H_2O (45 : 55, from 0 to 41.5, and 50 : 50, from 41.6 minutes); 220 ml/minute; UV at 210 nm). Arborcandin A was eluted at a retention time of 31.9 minutes. Arborcandins B, C, D, E and F were eluted at retention times of 74.3, 79.3, 92.2, 126.6 and 139.3 minutes, respectively. Arborcandin C (6.45 g) was obtained through direct concentration *in vacuo* of the fraction. Arborcandins A (382 mg), B (162 mg), D (34 mg) and E (326 mg) were obtained after re-purification of the fraction by the same preparative HPLC. Arborcandin F fraction was diluted with water and subjected to a 1.5-liter Cosmosil column (Nakarai Tesque) equilibrated with CH_3CN - H_2O (30 : 70). After washing the column with CH_3CN - H_2O (30 : 70) and CH_3CN, arborcandin F was eluted with CH_3CN - DMSO (90 : 10), which was concentrated *in vacuo* and lyophilized to yield the powder of arborcandin F (711 mg).

Arborcandins A, B, C, D, E and F were eluted with retention times of 3.6, 5.0, 7.4, 9.2, 11.6 and 18.8 minutes, respectively by HPLC (Waters Symmetry ODS, 4.6× 150 mm, Waters; CH_3CN - H_2O (50 : 50), 1.0 ml/minute; UV at 210 nm).

1,3-β-Glucan Synthase Activity

The membrane fraction containing 1,3-β-glucan synthase

was prepared from *C. albicans* ATCC 90028 according to the procedure described by CABIB *et al.*[8] with some modification. Cells were grown at 30°C in 2.5 liters of 1/2 YPD medium (peptone 1%, yeast extract 0.5% and glucose 2%), harvested in the early logarithmic phase (A_{600}=0.6) by centrifugation at 3,000×*g* for 5 minutes at 4°C, and washed with Buffer A (50 mM Tris-HCl pH 7.5, 1 mM EDTA and 1 mM 2-mercaptoethanol) to yield 3 g of wet cells, which were then stored at −80°C. The following operations were performed at 4°C. The frozen cells were suspended in Buffer A containing 1 mM phenylmethanesulfonyl fluoride (PMSF) and disrupted with acid-washed glass beads. The crude homogenate was centrifuged at 20,000×*g* for 10 minutes to precipitate cell wall and unbroken cells. The membrane fraction was obtained from the supernatant by centrifugation at 100,000×*g* for 1 hour, and suspended in 5 ml of Buffer A containing 33% glycerol.

1,3-β-Glucan synthase from *A. fumigatus* was prepared according to the procedure described by BEAULIEU *et al.*[9] with some modification. Spores of *A. fumigatus* SANK 10662 were transferred at an inoculum size of 2×10^6 spores/ml into a 10-liter jar fermentor containing 5 liters of Sabouraud medium (glucose 4% and peptone 1%) supplemented with 0.5% yeast extract. The culture was grown at 26°C for 24 hours with aeration of 1.5 liters/minute and agitation of 150 rpm. The hyphae were then harvested on a cheesecloth, washed with L/G solution (lactose 5% and glycerol 10%) to yield 40 g of the hyphae. The washed hyphae were disrupted at 4°C in a Dynomill (300-ml grinding container) with 150 ml of acid-washed glass beads and 150 ml of extraction buffer (50 mM HEPES; pH 7.7, 1 M sucrose, 50 mM NaCl, 5 mM EDTA, 10 mM NaF, 1 mM DTT and 0.1 mM GTP). The crude homogenate was centrifuged at 9,000×*g* for 10 minutes at 4°C to precipitate unbroken cells. Membrane fraction was obtained by centrifugation at 100,000×*g* for 1 hour at 4°C, and suspended in 40 ml of Buffer A containing 33% glycerol. The 1,3-β-glucan synthase was solubilized from the membrane fraction by addition of the detergent W-1 to a final concentration of 0.4%. This suspension was left at 4°C for 16 hours, followed by centrifugation at 100,000×*g* at 4°C for 60 minutes.

Glucan synthase activity was measured according to the procedure described by CABIB *et al.*[8] with some modification. The reaction mixture (final volume of 100 μl) was composed of 75 mM Tris-HCl pH 7.5, 0.75 mM EDTA, 25 mM KF, 0.75% BSA, 20 μM GTP[γ-S], 1 mM UDP-glucose and 0.016 μCi of UDP-[*U*-^{14}C] glucose (519 mCi/mmol, Amersham Pharmacia Biotech). The amount of enzyme in the reaction mixture was less than 5 μl, depending on the purity of the enzyme. After incubation at 30°C for 60 minutes, the reaction was terminated by the addition of 100 μl of ice-cold 20% TCA. The precipitate was harvested on a filter (Multiscreen-DVplate, Millipore), washed with 100 μl each of 10% TCA and 95% EtOH, and dried, followed by the addition of 100 μl of scintillation cocktail (Microscint-20, Packard). Radioactivities were measured by a microplate scintillation counter (TopCount, Packard). Each assay was run in duplicate.

Antifungal Activity

MIC was determined by the microbroth dilution method according to the procedure proposed in Japanese Journal of Medical Mycology, Vol. 36, 62~64 (1995), using RPMI 1640 medium (ICN Biomedicals Inc.) buffered with 0.165 M MOPS (pH 7.0). Microplates (96-well) were inoculated at a cell (or spore) concentration of $1\sim5\times10^3$ cells (spores)/ml, and incubated at 37°C for 24 or 48 hours. As proposed by KURTZ *et al.*[10], the minimum effective concentration (MEC) for *A. fumigatus* was defined as the lowest concentration at which any morphological changes occurred.

The minimum fungicidal concentration (MFC) was determined according to the method described by BARTIZAL *et al.*[11] with some modification. Microplates, after the MIC was determined, were shaken by a platemixer to resuspend the cells, and 10 μl aliquots from the wells were spreaded onto a 1/2 YPD agar plate. Colonies were counted after incubation at 37°C for 24 or 48 hours. MFC was defined as the lowest concentration at which the number of colonies decreased to <10% of the initial number.

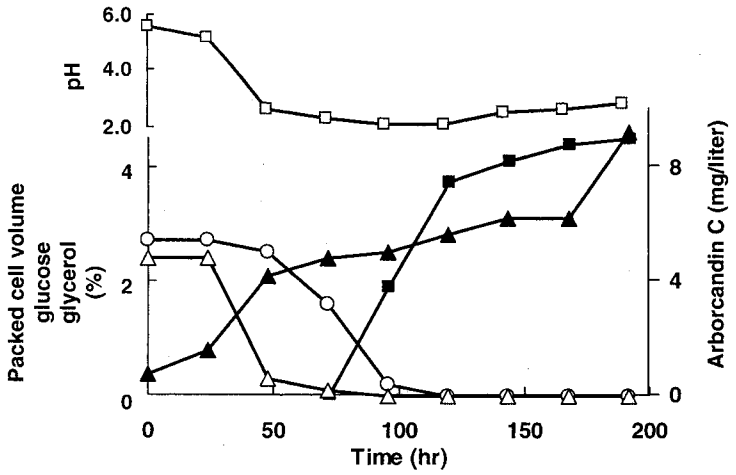
Fungicidal Activity

A liquid culture of *C. albicans* ATCC 90028 was diluted with RPMI1640 medium buffered with 0.165 M MOPS (pH 7.0) to a concentration of 1×10^4 cells/ml and preincubated at 37°C for 1 hour. Antifungal agents were then added at varying concentrations. The number of colonies was determined at 24 hours after the addition of antifungal agents.

Macromolecular Synthesis

Logarithmic phase cells of *C. albicans* ATCC 90028 were grown in RPMI 1640 medium buffered with 0.165 M MOPS (pH 7.0) and dispensed (100 μl) into 96-well plates. Then antifungal agents were added at varying concentrations. After preincubation of the mixture for 10 minutes at 37°C, 0.5 μCi of L-[^{3}H]-leucine (72 Ci/mmol, Amersham Pharmacia Biotech) or 0.05 μCi of [^{14}C]-

Fig. 2. Time course of fermentation of the fungal strain SANK 17397.



□, pH; ▲, packed cell volume; △, glucose; ○, glycerol; ■, arborcandin C.

adenine (287 mCi/mmol, Amersham Pharmacia Biotech) was added to measure protein synthesis or polynucleotide synthesis, respectively, and the mixtures were further incubated for 30 minutes at 37°C. An equal volume of 20% TCA was then added and the precipitates were harvested on a glass filter (Multiscreen FC plate, Millipore). The precipitates were washed with 300 μl of 10% TCA twice and dried, followed by the addition of 100 μl scintillation cocktail (Microscint-20, Packard). Radioactivities were measured by a microplate scintillation counter.

Results

Fermentation

The time course of arborcandin C production in the 600-liter fermentor is shown in Fig. 2. The cell growth continued over the course of the fermentation as evidenced by an increase in the packed cell volume. The pH of the culture broth decreased sharply from 24 to 48 hours in correlation with the consumption of glucose. Production of arborcandin C was first determined on day 4, and seemed to depend on the consumption of glycerol. Accumulation of arborcandin C continued for the next 3 to 4 days until the fermentation was terminated on day 8 with an average titer of 9.1 mg/liter.

Inhibitory Activity Against Glucan Synthase

As shown in Table 1, arborcandins and pneumocandin A_0[12] exhibited potent glucan synthase inhibitory activities against both *C. albicans* and *A. fumigatus* glucan synthases. Among the arborcandins, arborcandins C, E and F were more potent than pneumocandin A_0. Arborcandin F, the strongest glucan synthase inhibitor among the arborcandins, was 3- to 4-fold more active than pneumocandin A_0. Arborcandin D, in which the hydroxyl residue in the alkyl side chain of arborcandin C is replaced with a ketone, showed much weaker activity. The inhibitory activity of arborcandin D was 10-fold lower than that of arborcandin C. All the compounds showed more potent inhibitory activity against the enzyme of *A. fumigatus* than *C. albicans*.

Fig. 3 shows the Dixon plots for the *C. albicans* and *A. fumigatus* glucan synthases. The apparent inhibition constant (Ki_{app}) values for *A. fumigatus* and *C. albicans* were determined to be 0.016 and 0.12 μM, respectively. Arborcandin C was a noncompetitive inhibitor for both glucan synthases as shown by the convergence of the curves at the x-axis in Fig. 3.

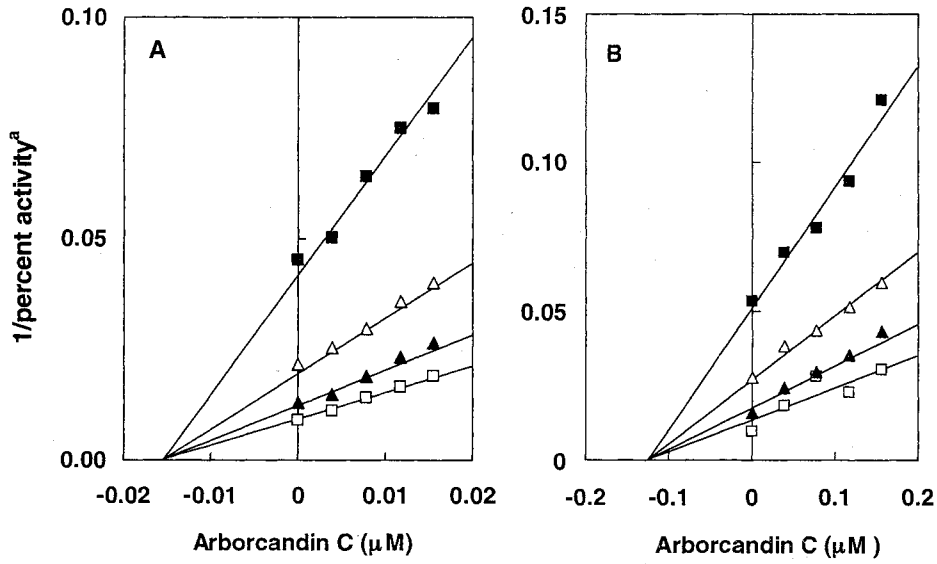
In Vitro Growth Inhibitory Activity

In vitro growth inhibitory activity of arborcandins against human pathogenic yeasts, *Candida* spp. and *Cryptococcus neoformans*, and a filamentous fungus,

Table 1. Inhibition of 1,3-β-glucan synthase by arborcandins.

Sample	IC_{50} (μg/ml)	
	Candida albicans ATCC 90028	*Aspergillus fumigatus* SANK 10662
Arborcandin A	0.25	0.05
Arborcandin B	0.30	0.025
Arborcandin C	0.15	0.015
Arborcandin D	3	0.35
Arborcandin E	0.1	0.012
Arborcandin F	0.08	0.012
Pneumocandin A_0	0.35	0.035

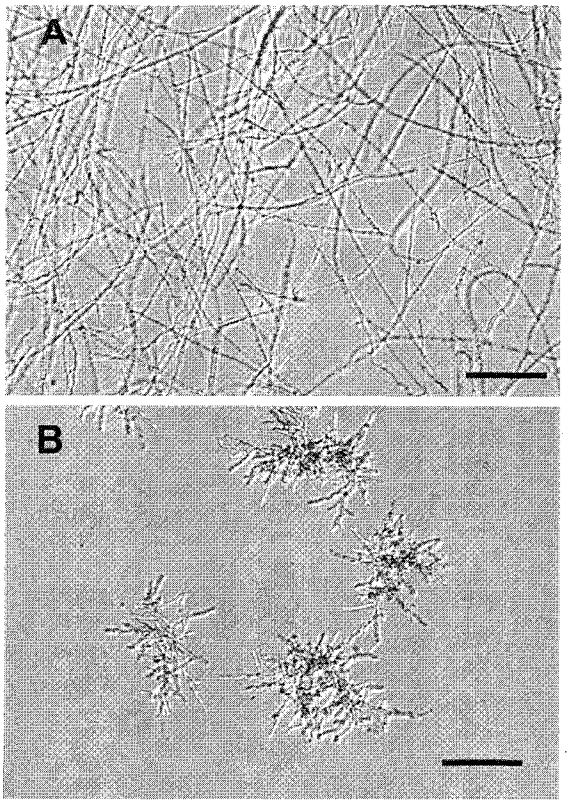
Fig. 3. Kinetics of arborcandin C inhibition against 1,3-β-glucan synthase.



Glucan synthase reactions were performed with different concentrations of UDP-glucose (■, 0.25 mM; △, 0.5 mM; ▲, 1 mM; □, 2 mM) and arborcandin C. [^{14}C]-labeled product was collected, and the relative reaction velocity was calculated. The Dixon plots of the results produced lines for each substrate concentration which converged at the x-axis. Panels: A, glucan synthase from *A. fumigatus*; B, from *C. albicans*. a) percent activity, percent of activity without inhibitors in the presence of 2 mM UDP-glucose.

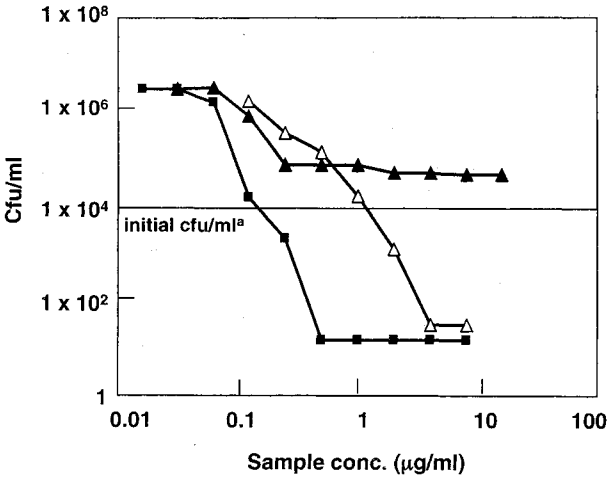
A. fumigatus, were evaluated by the microbroth dilution method as shown in Table 2. Arborcandins exhibited complete growth inhibitory activity against *Candida* spp., and among the arborcandins, arborcandin C, E and F had stronger activities as it was shown for the glucan synthase inhibition activities. The MICs of arborcandins C, E, and F ranged from 0.25 to 2 μg/ml, which were comparable to those of fluconazole, but were 2- to 8-fold higher than those of amphotericin B and pneumocandin A_0. All arborcandins did not show complete growth inhibition against *C. neoformans* and *A. fumigatus* at the concentrations tested (MIC>64 μg/ml).

Fig. 4. Morphological change of arborcandin C-terated *A. fumigatus* SANK 10662.



Panels: A, DMSO solvent control; B, 2 μg/ml of arborcandin C. All bars represent 0.1 mm.

Fig. 5. Fungicidal activity against *C. albicans*.



Cfu/ml of *C. albicans* ATCC 90028 after a 24-hour culture in RPMI 1640 medium containing arborcandin C (△), amphotericin B (■), or fluconazole (▲) were determined. [a] Initial cfu/ml, the cfu/ml just before the 24-hour culture.

Although arborcandin C did not inhibit the growth of *A. fumigatus* completely, it suppressed the hyphal elongation (Fig. 4). To evaluate the growth suppression activities of arborcandins against *A. fumigatus*, the MECs were determined according to the definition described previously[10]. As shown in Table 3, the MECs of arborcandins ranged from 0.063 to 4 μg/ml, and were 4- to 60-fold lower than that of pneumocandin A_0.

In Vitro Fungicidal Activity

As it was reported that 1,3-β-glucan synthase inhibitors possess fungicidal activities against *Candida* spp.[11,13,14], the fungicidal activity of arborcandin C as well as amphotericin B and fluconazole, which were reported to have a strong fungicidal and a fungistatic activity, respectively[14], were determined. As shown in Fig. 5, arborcandin C exhibited as strong a fungicidal activity as amphotericin B, and decreased the number of viable cells of *C. albicans* to <0.1% of the initial number. As arborcandin C was fungicidal, MFCs of arborcandins against human pathogenic yeasts and pathogenic fungi were determined according to the method described by BARTIZAL *et al.*[11]. As shown in Table 4, arborcandins showed fungicidal activity against all of the *Candida* spp. tested. Arborcandin C was 4- to 8-fold less active than amphotericin B and pneumocandin A_0. Arborcandins, like pneumocandin A_0, did not show fungicidal activity against *C. neoformans* and *A. fumigatus* at the tested concentrations (MFCs>64 μg/ml), but amphotericin B was fungicidal against *C. neoformans* and *A. fumigatus* (MFC of 0.5 and 1 μg/ml, respectively).

Arborcandin C did not significantly inhibit protein or polynucleotide synthesis at the concentrations at which glucan synthase was inhibited (data not shown).

Discussion

We discovered novel cyclic peptide antibiotics, arborcandins A to F, as 1,3-β-glucan synthase inhibitors. For the identification of the producing fungal strain SANK 17397, we attempted to produce fruiting structures by exposure to black light, use of various media, and incubation of the strain for up to one year, but all these attempts failed. At this stage, the strain has not yet been

Table 2. MICs of arborcandins, compared with other antifungal agents.

Strain	MIC (μg/ml)								
	Arborcandins						$PncA_0$[a]	AMPH[b]	FLCZ[c]
	A	B	C	D	E	F			
Candida albicans ATCC 90028	4	2	2	4	2	1	0.5	0.5	1
Candida albicans ATCC 90029	4	2	1	4	1	0.5	0.25	0.25	1
Candida parapsilosis IFO 1396	4	2	1	4	0.5	0.25	0.25	0.125	0.5
Candida tropicalis SANK 59263	8	4	2	4	2	2	0.5	0.125	0.5
Cryptococcus neoformans SANK 59863	>64	>64	>64	>64	>64	>64	>64	0.25	4
Aspergillus fumigatus SANK 10662	>64	>64	>64	>64	>64	>64	>64	1	16

a) $PncA_0$, pneumocandin A_0; b) AMPH, amphotericin B; c) FLCZ, fluconazole

identified.

Among arborcandins, arborcandin C, D and F exhibited stronger glucan synthase inhibitory activity (Table 1). It seems that arborcandins which have longer alkyl side chains, have stronger activity. Arborcandin D, in which the hydroxyl residue in the alkyl side chain of arborcandin C is replaced with a ketone, showed a much weaker activity. This suggests that the hydroxyl residue is related to the activity of arborcandins. The inhibitory activity of arborcandins C, D and F against *C. albicans* and *A. fumigatus* glucan synthase was stronger than that of pneumocandin A_0 (Table 1), and comparable to that of pneumocandin B_0[10]. The Ki_{app} of arborcandin C for *C. albicans* and *A. fumigatus* was almost the same as that of FK463[16]. Arborcandins showed more potent inhibitory activity against the glucan synthase of *A. fumigatus* than that of *C. albicans* (Table. 1). This may be due to the difference in the method of glucan synthase preparation, but it is more likely to be due to the difference in their direct inhibitory activity against glucan synthases. Indeed, arborcandins exhibited antifungal activity at lower concentrations against *A. fumigatus* than *C. albicans* (Table 2, and 3).

Though arborcandins exhibited potent antifungal activities against both *Candida* spp. and *A. fumigatus*, they were fungicidal only against *Candida* spp. and only the growth of *Candida* spp. was completely inhibited (Table 4, Fig. 5). As shown in Table 4, fluconazole exhibited fungicidal activity against *C. parapsilosis*. It was reported that fluconazole showed fungicidal activity against a small

Table 3. MECs of arborcandins against *Aspergillus fumigatus*.

Sample	MEC (μg/ml)
Arborcandin A	1
Arborcandin B	0.5
Arborcandin C	0.063
Arborcandin D	1
Arborcandin E	0.063
Arborcandin F	0.063
Pneumocandin A_0	4

population of *Candida* spp.[17]. The tested strain may belong to this group. The MFCs of arborcandins were almost the same as their MICs (Table 2 and 4). Furthermore, arborcandin C did not affect the macromolecular synthesis (data not shown). Therefore, it is suggested that the antifungal activities of these compounds against *Candida* spp. were the result of the inhibition of glucan synthase. Among arborcandins, arborcandins C, D and F exhibited stronger antifungal activity as shown in Table 2. The antifungal activity of these compounds against *Candida* spp. was weaker than that of pneumocandin A_0, although the glucan synthase inhibitory activity of these compounds was stronger. This discrepancy may be explained by the difference in the penetration of these compounds through

Table 4. MFCs of arborcandins.

Strain	MFC (μg/ml)								
	Arborcandins						$PncA_0$	AMPH	FLCZ
	A	B	C	D	E	F			
Candida albicans ATCC 90028	4	4	2	8	4	2	0.5	0.5	>64
Candida albicans ATCC 90029	4	4	1	4	1	1	0.25	0.25	>64
Candida parapsilosis IFO 1396	8	2	1	4	1	1	0.25	0.25	4
Candida tropicalis SANK 59263	8	4	2	4	2	2	0.5	0.25	>64
Cryptococcus neoformans SANK 59863	>64	>64	>64	>64	>64	>64	>64	0.5	64
Aspergillus fumigatus SANK 10662	>64	>64	>64	>64	>64	>64	>64	1	>64

the membrane. The difference between the antifungal activity and the enzyme inhibitory activity was reported for other glucan synthase inhibitors[11].

Arborcandins did not completely inhibit the growth of *A. fumigatus*. KURTZ *et al.* reported that pneumocandin B_0 (or A_0) caused a change in the hyphal growth of *A. fumigatus*, although it did not inhibit its growth completely[10]. We tested the effect of arborcandins on the hyphal growth of *A. fumigatus*, and the same major morphological change, the inhibition of hyphal elongation, was observed (Fig. 4). The MECs of arborcandins C, E and F were comparable or superior to pneumocandin A_0 (Table 3) and B_0[10]. Known glucan synthase inhibitors did not exhibit complete growth inhibitory activity against *A. fumigatus*[10,17] and arborcandins, which are structurally different from known glucan synthase inhibitors, also did not inhibit the growth of *A. fumigatus* completely. Therefore it is suggested that 1,3-β-glucan synthase inhibitors may not have complete growth inhibitory activity against *A. fumigatus*. However, this does not mean that 1,3-β-glucan synthase inhibitors are not potent antifungal agents against *A. fumigatus*.

Arborcandins were not active against *C. neoformans* as reported for other glucan synthase inhibitors. It is not shown why known glucan synthase inhibitors are ineffective against *C. neoformans*. However, it was recently reported that glucan synthase was essential for the growth of *C. neoformans*, and the resistance of *C. neoformans* against glucan synthase inhibitors might be due to the resistance of glucan synthase itself[16]. We have not yet tested the effect of arborcandin C against *C. neoformans* glucan synthase. Compounds that possess potent inhibitory activities against the glucan synthase of *C. neoformans* will be promising candidates as the glucan synthase inhibitors with a superior antifungal spectrum.

The inhibitory activity of arborcandin C against glucan synthase was as strong as that of the echinocandin group of antifungal agents, although the MIC against *Candida* spp. was much weaker. Therefore it is expected that arborcandin C could be improved further through chemical or microbial modification.

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