VOL. 56 NO. 12, DEC. 2003 THE JOURNAL OF ANTIBIOTICS pp. 1024 — 1032

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

Physico-chemical Properties and Structure Elucidation

TAKAO OHYAMA[a], YUKO IWADATE-KURIHARA[b], TOMIO ISHIKAWA[c], SHUNICHI MIYAKOSHI[a], KIYOSHI HAMANO[d] and MASATOSHI INUKAI[a,*]

[a] Lead Discovery Research Laboratories, [b] Exploratory Chemistry Research Laboratories, [c] Biomedical Research Laboratories, [d] Research Information Department, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

(Received for publication August 27, 2003)

Arborcandins A, B, C, D, E and F, which possess potent 1,3-β-glucan synthase inhibitory activity, were isolated from the cultured broth of a filamentous fungus, strain SANK 17397. The structures of arborcandins A, B, C, D, E and F were elucidated by a combination of NMR and mass spectrometry, and established to be novel cyclic peptides containing uncommon amino acid residues.

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[3]. 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]. As described in a previous report, we found that a filamentous fungus, strain SANK 17397, produced novel antifungal antibiotics, arborcandins A, B, C, D, E and F[8]. Arborcandins A, B, C, D, E and F possess potent inhibitory activity against 1,3-β-glucan synthase from *Candida albicans* and *Aspergillus fumigatus*, and also possess growth inhibitory activity. In this paper, we report the structure elucidation of arborcandins A, B, C, D, E and F.

Results

Physico-chemical Properties

The physico-chemical properties of arborcandins A, B, C, D, E and F are summarized in Table 1. The molecular formulae of arborcandins A, B, C, D, E and F were determined to be $C_{57}H_{101}N_{13}O_{18}$, $C_{58}H_{103}N_{13}O_{18}$, $C_{59}H_{105}N_{13}O_{18}$, $C_{59}H_{103}N_{13}O_{18}$, $C_{60}H_{107}N_{13}O_{18}$ and $C_{61}H_{109}N_{13}O_{18}$, respectively, by HRFAB-MS and NMR spectral analysis (Table 2). The IR spectra of all arborcandins showed the same absorption bands corresponding to the hydroxyl groups at approximately 3310 cm^{-1} and amide groups at approximately 1660 cm^{-1} and 1540 cm^{-1}. The UV spectra of all arborcandins did not show characteristic absorption.

Structure of Arborcandin C

The structure determination was mainly focused on arborcandin C. The ^{1}H and ^{13}C NMR spectral data of arborcandin C are summarized in Table 2. In the ^{1}H NMR spectrum of arborcandin C, signals from the multiple amide groups (7.03~8.29 ppm), hydroxyl groups (4.97~5.51 ppm) and α-methine of the amino acid residues (4~5 ppm) were observed. In the region of the alkyl group, complex overlapping methylene or methyl proton signals (1.2~1.3 ppm) and some other methyl proton signals (0.8~1.1 ppm) were observed. Although ten carbon signals overlapped in the ^{13}C NMR spectrum, all fifty-nine carbons could be classified by DEPT, HMQC and HMBC spectra into thirteen carbonyl, thirteen methine, twenty-eight methylene and five methyl carbons. According to these

* Corresponding author: inukai@shina.sankyo.co.jp

Table 1. Physico-chemical properties of arborcandins A, B, C, D, E and F.

	Arborcandin A	Arborcandin B	Arborcandin C
Appearance	Colorless powder	Colorless powder	Colorless powder
Molecular formula	$C_{57}H_{101}N_{13}O_{18}$	$C_{58}H_{103}N_{13}O_{18}$	$C_{59}H_{105}N_{13}O_{18}$
FAB-MS (*m/z*)	1278 $(M+Na)^+$	1270 $(M+H)^+$	1284 $(M+H)^+$
HRFAB-MS (*m/z*)	for $C_{57}H_{101}N_{13}O_{18}Na$	for $C_{58}H_{104}N_{13}O_{18}$	for $C_{59}H_{106}N_{13}O_{18}$
Found	1278.7295	1270.7628	1284.7784
Calcd.	1278.7285	1270.7622	1284.7778
IR ν_{max} (KBr) cm^{-1}	3310, 3069, 2928, 2856, 1664, 1536, 1454, 1379, 1249, 1104, 1024, 610	3308, 3068, 2928, 2856, 1663, 1538, 1436, 1407, 1380, 1297, 1252, 1105, 1023, 590	3317, 3068, 2928, 285 1662, 1536, 1455, 1409, 1378, 1294, 1258, 110 589
HPLC (Rt. Minutes)*	3.6	5.0	7.4
	Arborcandin D	**Arborcandin E**	**Arborcandin F**
Appearance	Colorless powder	Colorless powder	Colorless powder
Molecular formula	$C_{59}H_{103}N_{13}O_{18}$	$C_{60}H_{107}N_{13}O_{18}$	$C_{61}H_{109}N_{13}O_{18}$
FAB-MS (*m/z*)	1282 $(M+H)^+$	1298 $(M+H)^+$	1312 $(M+H)^+$
HRFAB-MS (*m/z*)	for $C_{59}H_{104}N_{13}O_{18}$	for $C_{60}H_{108}N_{13}O_{18}$	for $C_{61}H_{110}N_{13}O_{18}$
Found	1282.7609	1298.7928	1312.8105
Calcd.	1282.7623	1298.7935	1312.8091
IR ν_{max} (KBr) cm^{-1}	3310, 3062, 2928, 2856, 1664, 1538, 1454, 1409, 1380, 1297, 1253, 1104, 1024, 603	3313, 3069, 2927, 2855, 1663, 1536, 1455, 1407, 1384, 1252, 1105, 1024, 580	3315, 3065, 2926, 2855, 1664, 1537, 1456, 1409, 1379, 1252, 1102, 1024, 583
HPLC (Rt. Minutes)*	9.2	11.6	18.8

* Symmetry ODS 4.6 x 150 mm, Waters, CH_3CN-H_2O (50:50), 1.0 ml / minute, UV 210 nm

NMR data and the physico-chemical properties as well as the degree of unsaturation, it was suggested that arborcandin C belongs to the family of cyclic lipopeptide antibiotics. Amino acid analysis of the hydrolyzed products detected two moles of aspartic acid, two moles of threonine, one mole of alanine, one mole of glycine and one mole of β-alanine.

The structure of arborcandin C was elucidated by several NMR techniques such as DQFCOSY, HMQC, HMBC and ROESY, which accounted for the result of amino acid analysis. The DQFCOSY spectrum revealed the intra-residual spin system of each amino acid residue, and suggested the presence of ten amino acid residues including three uncommon amino acids. As shown in Fig. 1, HMBC spectral analysis revealed that two moles of aspartic acid detected in the amino acid analysis were derived from asparagines and the structure of residue (d) to be γ-hydroxyglutamine (γ-OH-Gln). Although the structures of the remaining two uncommon amino acid residues tentatively named Xaa-1 (g) and Xaa-2 (h) were not fully clarified by NMR spectral analysis, partial structure of positions 1 to 5 in Xaa-1 as shown in Fig. 1 was revealed by the DQFCOSY and HMBC spectral analysis. In the same way, partial structure of positions 1 to 4 in Xaa-2 was assigned. Further structural analysis of Xaa-1 and Xaa-2 by NMR was impossible because of the severe overlapping of the methylene proton region. The connection of the ten amino acid residues was determined by the ROESY spectrum, which showed the correlation between each amide proton and the α-methine proton of each neighboring amino acid residue. Inter-residual long-range correlation was observed in the HMBC spectrum (Fig. 1).

Further structure elucidation of Xaa-1 and Xaa-2 was carried out by using mass spectral analysis combined with chemical methods. FAB-MS analysis of the acid hydrolysates of arborcandin C showed the protonated molecules $(M+H)^+$ from Xaa-1 and Xaa-2, with molecular mass of *m/z* 260 and 288, and the molecular formula of each amino acid was determined to be $C_{14}H_{29}NO_3$ and $C_{16}H_{33}NO_3$ by the HRFAB-MS.

The assignment of Xaa-1 and Xaa-2 was carried out by collisionally-activated dissociation (CAD) spectral analysis of the protonated molecule (*m/z* 1284.7), based on the amino acid sequence of arborcandin C determined by the

Table 2. NMR spectral data of arborcandin C.

	Position	δ_C	δ_H		Position	δ_C	δ_H
Asn-1 (a)	1	170.5		Thr-2 (f)	1	170.7	
	2	49.6	4.36 (1H, m)		2	58.2	4.28 (1H, m)
	3	36.2	2.51, 2.73 (2H, m)		3	66.7	4.06 (1H, m)
	4	172.3			4	19.6	1.10 (3H, d, 6.5)
	2-NH		8.25 (1H, m)		2-NH		7.83 (1H, m)
	4-NH_2		7.03, 7.58 (2H, br s)		3-OH		4.97 (1H, m)
Asn-2 (b)	1	170.7		Xaa-1 (g)	1	171.5	
	2	49.3	4.45 (1H, m)		2	54.2	3.86 (1H, m)
	3	36.2	2.49, 2.79 (2H, m)		3	26.2	1.70, 1.80 (2H, m)
	4	173.4			4	33.3	1.35 (2H, m)
	2-NH		8.03 (1H, d, 8.1)		5	69.1	3.36 (1H, *)
	4-NH_2		7.34, 7.56 (2H, br s)		6-13	*	*
Ala (c)	1	172.3			14	14.0	0.86 (3H, t, 6.5)
	2	48.5	4.23 (1H, m)		2-NH		8.19 (1H, d, 6.2)
	3	17.1	1.24 (3H, m)		5-OH		4.32 (1H, d, 5.2)
	2-NH		7.76 (1H, d, 8.1)	Gly (h)	1	169.0	
γ-OH-Gln (d)	1	171.9			2	42.2	3.78, 3.85 (2H, m)
	2	49.3	4.60 (1H, m)		2-NH		8.13 (1H, m)
	3	36.5	1.70, 2.30 (2H, m)	β-Ala (i)	1	170.8	
	4	67.6	3.67 (1H, m)		2	34.6	2.59, 2.29 (2H, m)
	5	177.5			3	35.3	3.15, 3.43 (2H, m)
	2-NH		7.63 (1H, d, 8.1)		2-NH		7.69 (1H, m)
	4-OH		5.51 (1H, d, 5.8)	Xaa-2 (j)	1	172.9	
	5-NH_2		7.30, 7.34 (2H, br s)		2	53.4	4.16 (1H, m)
Thr-1 (e)	1	170.5			3	31.5	1.65 (2H, m)
	2	58.8	4.35 (1H, m)		4	25.6	1.24 (2H, m)
	3	67.4	3.78 (1H, m)		5-9	*	*
	4	20.4	1.08 (3H, d, 6.5)		10	69.6	3.33 (1H, *)
	2-NH		8.29 (1H, m)		11-15	*	*
	3-OH		4.97 (1H, m)		16	14.0	0.86 (3H, t, 6.5)
					2-NH		8.29 (1H, m)
					10-OH		4.17 (1H, d, 5.2)

Spectra were recorded at 360 and 90 MHz for ^1H and ^{13}C, respectively, in DMSO-d_6.
Chemical shifts are given in ppm referenced to TMS at 0 ppm for ^1H and DMSO-d_6 solvent signal at 39.5 ppm for ^{13}C.
* : not assigned due to overlapping signals

NMR analysis. As shown in Fig. 3, the fragmentation pattern of the CAD spectrum revealed that the molecular formulae of Xaa-1 and Xaa-2 corresponded to $C_{14}H_{29}NO_3$ and $C_{16}H_{33}NO_3$, respectively, and the alkyl chain length of each amino acid residue and the presence of a hydroxyl group in Xaa-2 were clarified. Finally, the location of the hydroxyl group in Xaa-2 was determined by CAD spectral analysis with the acid hydrolysates of pyridinium dichromate oxidized arborcandin C, according to the method described by CHENG *et al.*[9,10]. The CAD spectrum of the lithiated molecule [$(M-H+2Li)^+$, *m/z* 298] derived from the oxidized Xaa-2 is shown in Fig. 4. In the spectrum, a typical charge-remote fragmentation pattern of alkyl chains and the characteristic two product ions *m/z* 240 and 170, which indicate the position of the saturated carbonyl[9,10] in the oxidized Xaa-2, were observed. Accordingly, the position of the hydroxyl group in Xaa-2 was determined to be position 10. The planar structure of arborcandin C was thus elucidated as shown in Fig. 2.

Structures of Arborcandin C Related Compounds

The structural differences between arborcandin C and its related compounds were identified from the structures of the Xaa-1 and Xaa-2 residues by amino acid analysis and NMR spectral analyses. To elucidate their structures, mass spectral analysis of arborcandins A, B, D, E and F was carried out in the same way as described above. Consequently, the planar structures of arborcandins A, B, D, E and F were elucidated as shown in Fig. 2.

Fig. 1. DQFCOSY, HMBC and ROESY correlations of arborcandin C.

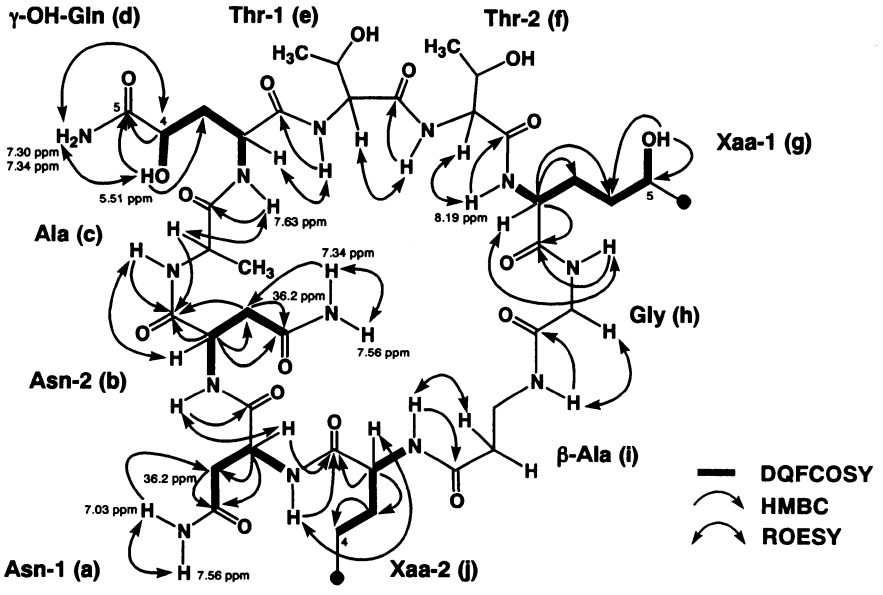
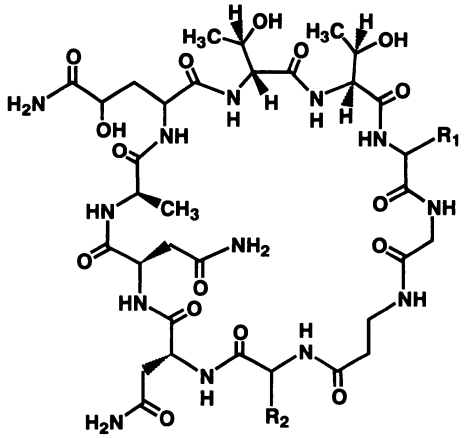
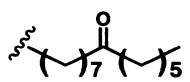


Fig. 2. Structures of arborcandins.



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

Stereochemistry of Arborcandin C

The stereochemistry of each amino acid residue of arborcandin C was determined by the advanced Marfey's method[11]. Acid-hydrolyzed products of arborcandin C were L-FDLA-derived, and analyzed by LC/MS. By comparing each authentic amino acid standard, the chiralities of two Asn, two Thr and Ala were determined to be D and L configuration, D-*allo* and L configuration and D configuration, respectively.

The configurations of the two Asn residues, Asn-1 and Asn-2, were determined by selective reduction of Asn residues in arborcandin C. Each of the two Asn residues of arborcandin C was selectively converted to 2,4-diaminobutyric acid (DAB), and the derivatives were named arborcandin C–NH_2-1 and –NH_2-2. The converted sites of arborcandin C–NH_2-1 and –NH_2-2 were determined by CAD spectral analysis of the *N*-acetylated products, as shown in Fig. 5. The converted sites of each derivative were distinguished by the different *m/z* values of the product ions in the spectra. The NH_2-1 derivative gave product ions at *m/z* 773 and *m/z* 431, whereas the NH_2-2 derivative generated the product ions at *m/z* 801 and *m/z* 358. These results revealed that Asn-1 and Asn-2 were selectively converted to DAB in NH_2-1 and NH_2-2, respectively. Finally, the configurations of each remaining Asn residue were determined by the advanced Marfey's method, and

Fig. 3. High-energy CAD spectrum obtained from the protonated molecule (*m/z* 1284) of arborcandin C.

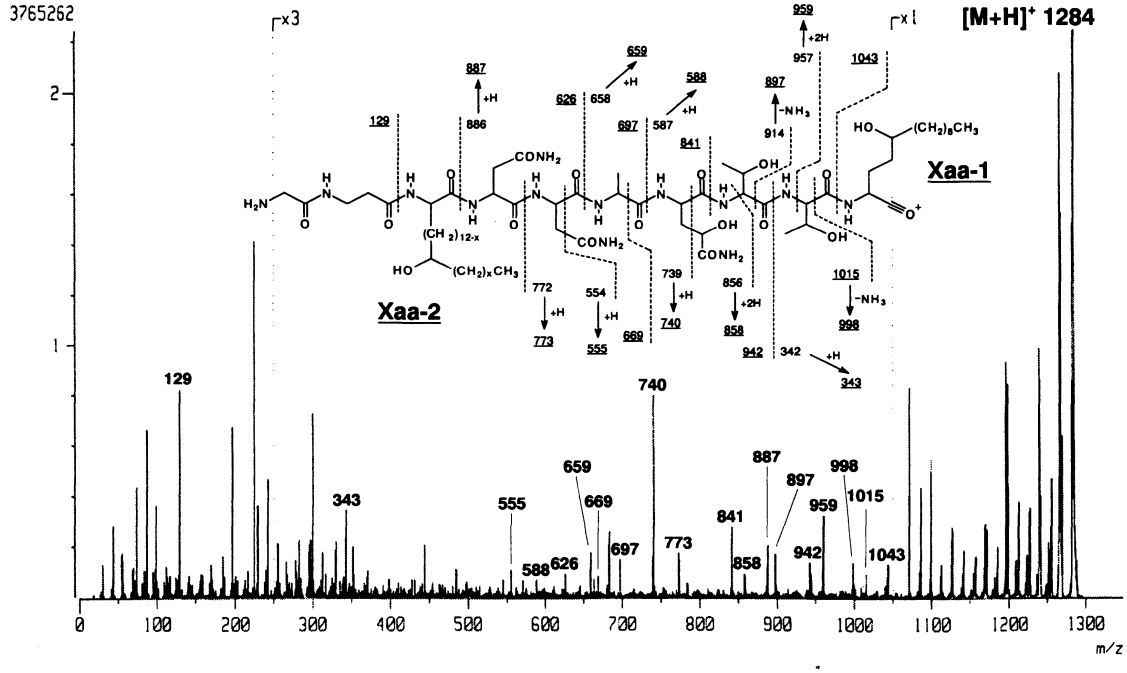
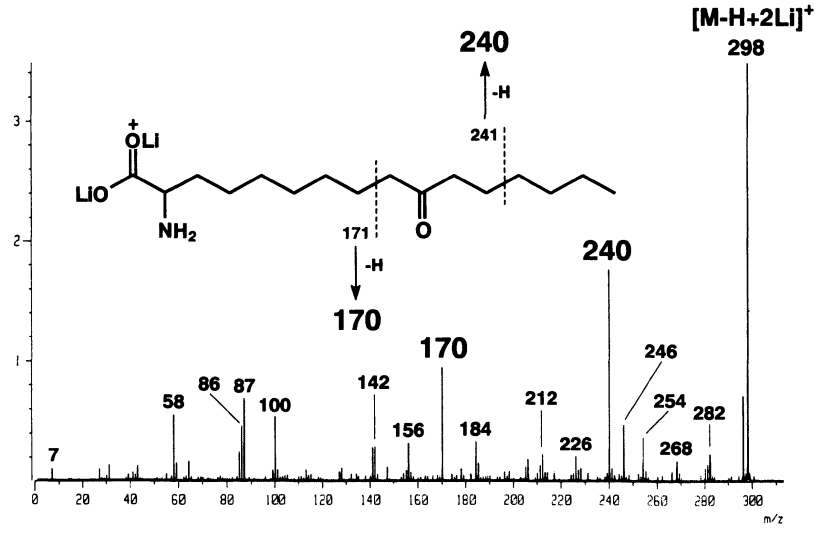


Fig. 4. MS/MS analysis of the lithiated molecule (*m/z* 298) from the acid hydrolysate of pyridinium dichromate oxidized arborcandin C.



only L-Asp from NH_2-1 and D-Asp from NH_2-2 were detected. These results revealed that the configurations of Asn-1 and Asn-2 were D and L, respectively.

The configurations of the two Thr residues, Thr-1 and Thr-2, were determined by the actual coupling constant between the α-methine proton and β-methine proton of each Thr residue and the NOE data. As shown in Fig. 6, the observed coupling constant between the α-proton and β-proton in Thr-1 and Thr-2 were 8.1 Hz and 3.2 Hz, respectively. By applying the Karplus equation to these

Fig. 5. Assignment of CAD-MS/MS fragments of *N*-acetylated arborcandin C–NH_2-1 and –NH_2-2.

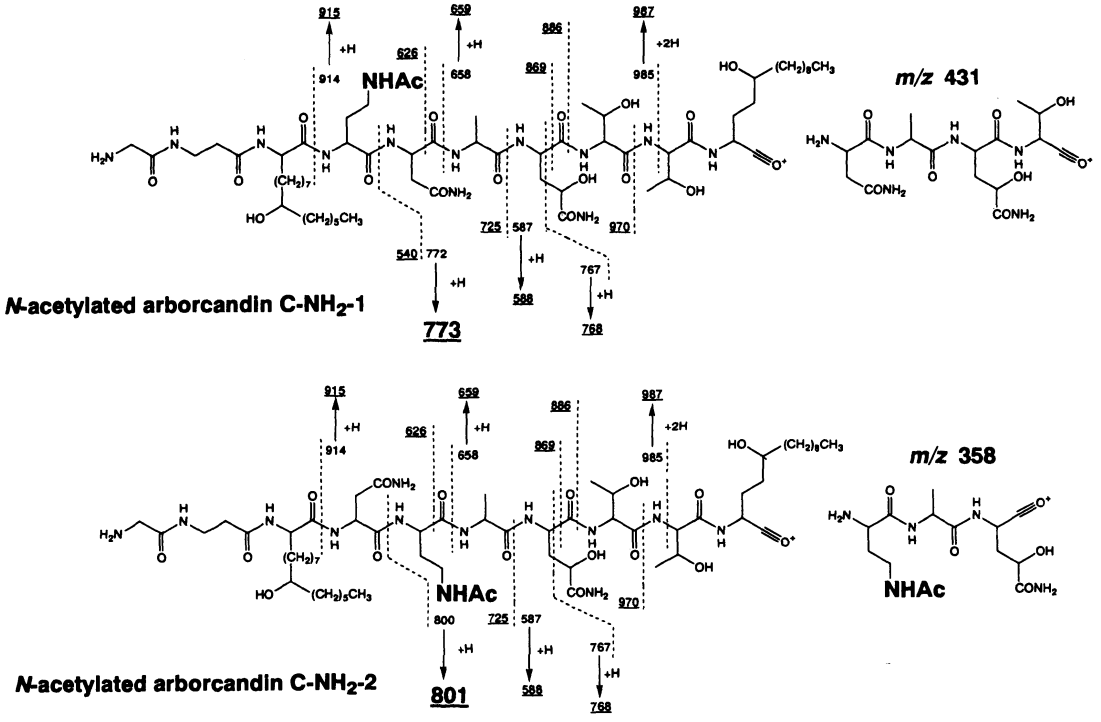
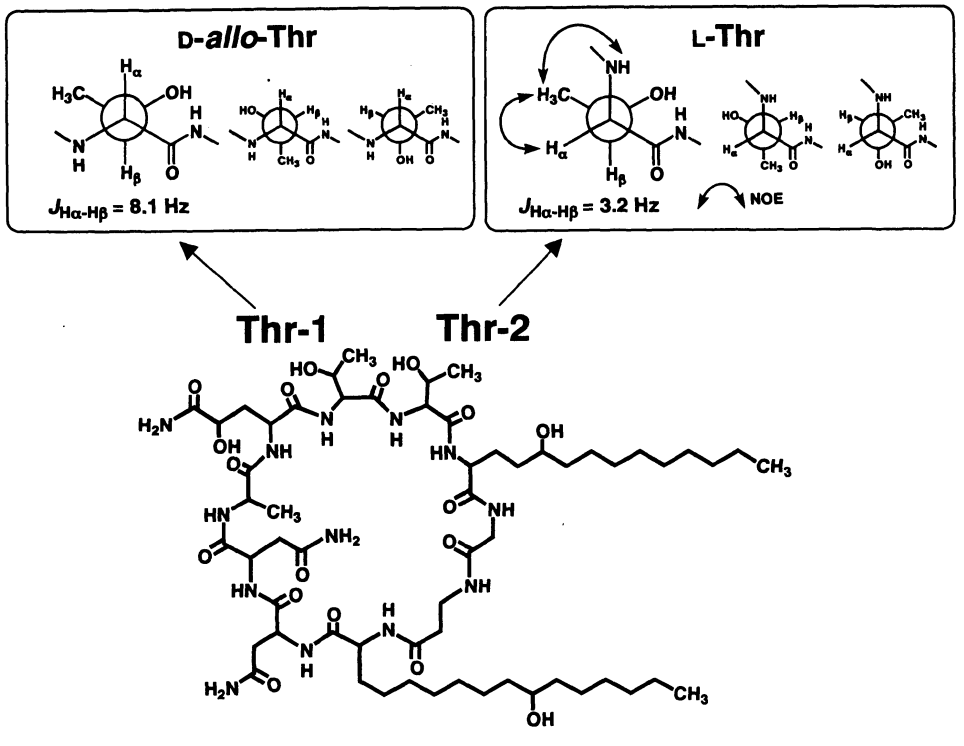


Fig. 6. Conformational analysis of two threonine residues of arborcandin C by NMR spectral data.



coupling constants, it was considered that the conformation of the α- and β-methine protons in Thr-1 and Thr-2 were *anti* and *gauche*, respectively. The NOE observed between the amide proton and methyl proton, and between the α-methine proton and methyl proton in Thr-2 well supported this conformation. The isomer, which satisfied such relationships in Thr-2 was only L-Thr but not D-*allo*-Thr. Since the existence of one mole each of L-Thr and D-*allo*-Thr was previously confirmed, the configuration of Thr-1 was inductively determined to be D-*allo*-Thr. The stereochemistry of the residual asymmetric centers in γ-OH-Gln, Xaa-1 and Xaa-2 remains to be determined.

Discussion

Several types of 1,3-β-glucan synthase inhibitors have been identified, such as echinocandins, papulacandins, enfumafungin, and FR901469/aerothricin3[12~14]. Echinocandins are cyclic hexapeptides with a lipophilic side chain. Papulacandins are liposaccharides. Enfumafungin is an acidic terpenoid. FR901469/aerothricin3 is a lipopeptidolactone.

In this study, the structures of arborcandins A, B, C, D, E and F were elucidated, and established to be novel cyclic peptides containing uncommon amino acid residues. Although arborcandins are structurally different from these known 1,3-β-glucan synthase inhibitors, they have similar structural features to known peptidic 1,3-β-glucan synthase inhibitors from the point of view of a cyclic peptide with lipophilic side chains. Furthermore, the differences among the arborcandins are in the structure of the alkyl side chains, which affect the biological activity of the arborcandins[8]. It is reported that 1,3-β-glucan synthase is composed of at least two membrane-localizing proteins, a putative catalytic subunit and a regulatory subunit[6,15,16], and that targets of echinocandins and FR901469/aerothricin3 are the putative catalytic subunit[17,18]. Therefore, it is possible that the arborcandins may also interact with the catalytic subunit of 1,3-β-glucan synthase complex, and that the alkyl side chains are essential for their interaction to the membrane localizing protein. Further study to determine the target molecule of the arborcandins might be helpful for understanding the inhibition-targeting site of 1,3-β-glucan synthase.

Experimental

General Experimental Procedures

IR spectra were obtained on a JASCO FT/IR-8900 spectrometer. UV spectra were recorded on a Shimazu UV-265FW spectrometer. NMR spectra were recorded on a Bruker AMX360 spectrometer in DMSO-d_6 solution. Chemical shifts are given in ppm referenced to TMS at 0 ppm for ^{1}H and DMSO-d_6 solvent signal at 39.5 ppm for ^{13}C. The analytical HPLC of the arborcandins was performed using an ODS column (Symmetry C_{18}, 4.6×150 mm, Waters; CH_3CN-H_2O (50:50); 1.0 ml/minute; UV at 210 nm).

Amino Acid Analysis

The compounds were completely hydrolyzed with 6 N HCl at 105°C for 15 hours. The hydrolysates were evaporated to dryness and dissolved in 0.5 ml of 0.02 N HCl, and then 20 μl of each was separately applied to a HITACHI L-8500 Amino Acid Analyzer.

The absolute configurations of the amino acids were determined by the advanced Marfey's method[11]. The hydrolysates of arborcandin C, arborcandin C–NH_2-1 or –NH_2-2 as described above were dissolved in water at the final concentration of about 10 mM. Then 10 μl of 1 M sodium bicarbonate and 20 μl of 1% L-FDLA (1-fluoro-2,4-dinitrophenyl-5-L-leucinamide) in acetone were added to the 10 μl aqueous solution, and incubated at 37°C. After one-hour incubation, the reaction was terminated by the addition of 10 μl of 1 N HCl. The reactant diluted with 50 μl of acetone was analyzed by LC/MS (Finnigan LCQ spectrometer, in ESI positive ion mode) with an HPLC column of Symmetry C_{18} (4.6×150 mm, Waters; 20%-15 minutes-60% CH_3CN/0.01% TFA aq., 0.5 ml/minute; UV at 340 nm). The chiralities of the hydrolyzed products were determined by comparing with each authentic amino acid standard.

Pyridinium Dichromate Oxidization of Arborcandin C

Four-hundred milligrams of arborcandin C were dissolved in 40 ml of DMF and incubated with 7.5 equivalents (880 mg) of pyridinium dichromate at room temperature. After overnight incubation, the reaction was terminated by the addition of 80 ml of H_2O, and the reactant was desalted using an HP-20 column, and purified by preparative HPLC (Pegasil ODS 20×150 mm, Senshu Scientific; CH_3CN-H_2O (55:45), 10 ml/minute; UV at 210 nm). The peak eluted at a retention time of 14.4 minutes was collected and concentrated *in vacuo* to yield the powder of pyridinium dichromate oxidized arborcandin

C (70.6 mg).

Selective Reduction of Asn Residue

Two-hundred milligrams of arborcandin C was dissolved in 20 ml of DMF and incubated with five equivalents (140 mg) of cyanuric chloride at room temperature. After the 15-minute incubation, the reaction was terminated by the addition of 1.5 ml of 2 M sodium acetate aq., and the reactant was desalted using an HP-20 column, and purified by a preparative HPLC (Pegasil ODS 20×150 mm, Senshu Scientific; CH_3CN-H_2O (55:45), 10 ml/minute; UV at 210 nm). The peaks eluted at retention times of 12.5 minutes and 11.7 minutes were collected and concentrated *in vacuo* to yield the powder of arborcandin C–CN-1 (7.6 mg) and –CN-2 (6.9 mg). Arborcandin C–CN-1 and –CN-2 were determined to be the isomers, in which the carbamoyl group in one of the two Asn residues is differently converted to a cyano group [arborcandin C–CN-1: *m/z* 1288.7489 $(M+Na)^+$; arborcandin C–CN-2: *m/z* 1288. 7499 $(M+Na)^+$]. Four milligrams each of arborcandin C–CN-1 and –CN-2 were dissolved in 2 ml of MeOH and reduced with eight equivalents (6.0 mg) of $CoCl_2$ $6H_2O$ and sixty equivalents (7.0 mg) of $NaBH_4$ at room temperature. After 30-minute incubation, the reaction was terminated by the addition of 0.1 N HCl, and the reactant was desalted using a Sep-Pak PS-2 column, and purified by preparative HPLC (Pegasil ODS 20×150 mm; CH_3CN-0.5% triethylamine-phosphate buffer, pH 3.2 (45:55), 10 ml/minute; UV 210 nm). The peaks eluted at retention times of 12.6 minutes and 17.0 minutes were collected, diluted with H_2O, desalted using Sep-Pak PS-2, and concentrated *in vacuo* to yield arborcandin C–NH_2-1 (1.5 mg) and –NH_2-2 (2.3 mg). Arborcandin C–NH_2-1 and –NH_2-2 were determined to be derivatives in which one of the two Asn residues is converted to 2,4-diaminobutyric acid (DAB) [arborcandin C–NH_2-1: *m/z* 1270.7985 $(M+H)^+$; arborcandin C–NH_2-2: *m/z* 1270.7976 $(M+H)^+$]. Decrease of one mole of Asp in each acid hydrolysate of arborcandin C–NH_2-1 and –NH_2-2, compared to arborcandin C, was observed by amino acid analysis.

FAB-MS and FAB-MS/MS (Collisionally-Activated Dissociation (CAD)) Experiments

FAB-MS and FAB-MS/MS (collisionally-activated dissociation (CAD)) spectra were obtained using a JEOL JMS-SX/SX 102A four-sector tandem mass spectrometer. Xenon (6 keV) was used for the FAB ionization. The mass spectrometer was operated at an accelerating voltage of 10 kV in the positive ion mode. CAD was accomplished *via* the introduction of argon into the collision cell, which was floated at 5 kV, at a pressure sufficient to give approximately 80% attenuation of the precursor ion beam.

Acknowledgements

We wish to thank Dr. TOSHIO TAKATSU, Exploratory Chemistry Research Laboratories, Sankyo Co., Ltd., for reading the manuscript.

References

1) GEORGOPAPADAKOU, N. H. & T. J. WALSH: Human mycoses: drugs and targets for emerging pathogens. Science 264: 371~373, 1994
2) ANDRIOLE, V. T.: The 1998 Garrod lecture. Current and future antifungal therapy: new targets for antifungal agents. J. Antimicrob. Chemother. 44: 151~162, 1999
3) WHITE, C. C.; K. A. MARR & R. A. BOWDEN: Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin. Microbiol. Rev. 11: 382~402, 1998
4) FARKAS, V.: Fungal cell walls: their structure, biosynthesis and biotechnological aspects. Acta Biotechnol. 10: 225~238, 1990
5) DEBONO, M. & R. S. GORDEE: Antibiotics that inhibit fungal cell wall development. Annu. Rev. Microbiol. 48: 471~497, 1994
6) INOUE, S. B.; N. TAKEWAKI, T. TAKASUKA, T. MIO, M. ADACHI, Y. FUJII, C. MIYAMOTO, M. ARISAWA, Y. FURUICHI & T. WATANABE: Characterization and gene cloning of 1,3-β-glucan synthase from *Saccharomyces cerevisiae*. Eur. J. Biochem. 231: 845~854, 1995
7) MIO, T.; M. ADACHI-SHIMIZU, Y. TACHIBANA, H. TABUCHI, S. B. INOUE, T. YABE, T. YAMADA-OKABE, M. ARISAWA, T. WATANABE & H. YAMADA-OKABE: Cloning of the *Candida albicans* homologue of *Saccharomyces cerevisiae GSC1/FKS1* and its involvement in β-1,3-glucan synthase. J. Bacteriol. 179: 4096~4105, 1997
8) OHYAMA, T.; Y. KURIHARA, Y. ONO, T. ISHIKAWA, S. MIYAKOSHI, K. HAMANO, M. ARAI, T. SUZUKI, H. IGARI, Y. SUZUKI & M. INUKAI: Arborcandin A, B, C, D, E and F, novel 1,3-β-glucan synthase inhibitors: production and biological activity. J. Antibiotics 53: 1108~1116, 2000
9) CHENG, C.; D. GIBLIN & M. L. GROSS: Structural determination of oxofatty acids by charge-remote fragmentation. J. Am. Soc. Mass Spectrom. 9: 216~224, 1998
10) CHENG, C. & M. L. GROSS: Fragmentation mechanisms of oxofatty acids *via* high-energy collisional activation. J. Am. Soc. Mass Spectrom. 9: 620~627, 1998
11) FUJII, K.; T. SHIMOYA, Y. IKAI, H. OKA & K. HARADA: Further application of advanced Marfey's method for determination of absolute configuration of primary amino compound. Tetrahedron Lett. 39: 2579~2582, 1998
12) KURTZ, M. B. & C. M. DOUGLAS: Lipopeptide inhibitors of fungal glucan synthase. J. Med. Vet. Mycol. 35: 79~86, 1997
13) FUJIE, A.; T. IWAMOTO, H. MURAMATSU, T. OKUDAIRA, I. SATO, T. FURUTA, Y. TSURUMI, Y. HORI, M. HINO, S. HASHIMOTO & M. OKUHARA: FR901469, a novel

antifungal antibiotic from an unidentified fungus No. 11243. II. *In vitro* and *in vivo* activities. J. Antibiotics 53: 920~927, 2000

14) ONISHI, J.; M. MEINZ, J. THOMPSON, J. CUROTTO, S. DREIKORN, M. ROSENBACH, C. DOUGLAS, G. ABRUZZO, A. FLATTERY, L. KONG, A. CABELLO, F. VICENTE, F. PELAEZ, M. T. DIEZ, I. MARTIN, G. BILLS, R. GIACOBBE, A. DOMBROWSKI, R. SCHWARTZ, S. MORRIS, G. HARRIS, A. TSIPOURAS, K. WILSON & M. B. KURTZ: Discovery of novel antifungal (1,3)-β-D-glucan synthase inhibitors. Antimicrob. Agents Chemother. 44: 368~377, 2000

15) DRGONOVA, J.; T. DRGON, K. TANAKA, R. KOLLAR, G. C. CHEN, R. A. FORD, C. S. CHAN, Y. TAKAI & E. CABIB: Rho1p, a yeast protein at the interface between cell polarization and morphogenesis. Science 272: 277~279, 1996

16) QADOTA, H.; C. P. PYTHON, S. B. INOUE, M. ARISAWA, Y. ANRAKU, Y. ZHENG, T. WATANABE, D. E. LEVIN & Y. OHYA: Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-β-glucan synthase. Science 272: 279~281, 1996

17) DOUGLAS, C. M.; F. FOOR, J. A. MARRINAN, N. MORIN, J. B. NIELSEN, A. M. DAHL, P. MAZUR, W. BAGINSKY, W. LI, M. EL-SHERBEINI, J. A. CLEMAS, S. M. MANDALA, B. R. FROMMER & M. B. KURTZ: The *Saccharomyces cerevisiae FKS1* (*ETG1*) gene encodes an integral membrane protein which is a subunit of 1,3-β-D-glucan synthase. Proc. Natl. Acad. Sci. USA 91: 12907~12911, 1994

18) KONDOH, O.; T. TAKASUKA, M. ARISAWA, Y. AOKI & T. WATANABE: Differential sensitivity between Fks1p and Fks2p against a novel β-1,3-glucan synthase inhibitor, aerothricin3 [corrected]. J. Biol. Chem. 277: 41744~41749, 2002, Erratum in: J. Biol. Chem. 278: 17580, 2003