VOL. 55 NO. 8, AUG. 2002 THE JOURNAL OF ANTIBIOTICS pp. 758 — 763

NOTES

F-10748 A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_2, Novel Papulacandins

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

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

(Received for publication February 21, 2002)

In the course of our screening program for 1,3-β-glucan synthase inhibitors, we discovered novel antifungal antibiotics of a papulacandin family[1,2], F-10748 A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C_1 (**5**), C_2 (**6**), D_1 (**7**) and D_2 (**8**). These compounds were isolated from the culture broth of fungus, SANK 18496. This fungus was isolated from a leaf of Japanese red pine (*Pinus densiflora*), collected in Mt. Tsukuba, Japan. Based on the cultural and morphological characteristics, strain SANK 18496 was determined to belong to the genus *Lophodermium*. The strain SANK 18496 was deposited at the National Institute of Advanced Industrial Science and Technology, Patent and Bio-Resource Center, Japan (accession No. FERM BP-5620). In this paper, we describe the fermentation, isolation, structure elucidation (mainly compounds **5** and **6**), and biological properties of compounds **3** to **8**. GPMY medium (glycerol 5%, potato 5%, malt extract (Difco) 0.5% and yeast extract (Difco) 0.5%) was used as a seed medium and the production medium. A slant culture of the strain SANK 18496 grown on potato dextrose agar (Difco) for 7 days at 26°C were inoculated into each of five 500-ml Erlenmeyer flasks containing 100 ml of GPMY medium. The flasks were incubated on a rotary shaker at 23°C, 210 rpm for 7 days. Four hundred fifty ml of the seed culture was transferred into each of two 30-liter jar fermentors containing 15 liters of GPMY medium. The fermentation was carried out at 26°C for 72 hours with aeration of 7.5 liters/minute and agitation of 210 rpm.

The 31 liters of the 72-hour old whole broth was added to an equal volume of acetone, and the mixture was stirred for 1 hour. The filtrate of the mixture was adjusted to pH 3 with HCl and extracted with 30 liters of ethyl acetate twice. The ethyl acetate extract was concentrated *in vacuo* to yield 31.7 g of oily material. The oily material was subjected to a 3-liter Cosmosil column (140C18OPN, Nakarai Tesque) with a stepwise elution using CH_3CN-H_2O (20 : 80, 6.5 liters), (40 : 60, 6.6 liters), (50 : 50, 7.1 liters), (60 : 40, 12 liters), and (80 : 20, 7.7 liters). Active fractions were eluted with CH_3CN-H_2O (50 : 50), and (60 : 40). The active fractions were combined, and concentrated *in vacuo* to 4 liters. The concentrate was extracted with 4 liters of ethyl acetate, and the solvent layer was concentrated *in vacuo* to yield 1.3 g of crude powder. The crude powder was subjected to a 400-ml Cosmosil column with a stepwise elution using CH_3CN-H_2O (20 : 80, 600 ml), (40 : 60, 1.2 liters), (50 : 50, 1 liter), (60 : 40, 800 ml) and (80 : 20, 800 ml). Active fractions were eluted separately with CH_3CN-H_2O (40 : 60, fraction A), (50 : 50, fraction B), (60 : 40, fraction C) and (80 : 20, fraction D). Each active fraction was concentrated *in vacuo* to yield 244 mg (fraction A), 131 mg (fraction B), 336 mg (fraction C) and 67 mg (fraction D) of crude powder. Active fractions containing F-10748 A_1 (**1**), F-10748 A_2 (**2**) (from fraction A), F-10748 B_1 (**3**), F-10748 B_2 (**4**) (from fraction B), F-10748 C_1 (**5**), F-10748 C_2 (**6**) (from fraction C), F-10748 D_1 (**7**) and F-10748 D_2 (**8**) (from fraction D) were obtained by preparative HPLC (PEGASIL ODS, 20×150 mm, Senshu; CH_3CN-MeOH-H_2O (54 : 10 : 36); 10 ml/minute, UV at 210 nm). The active fractions were concentrated *in vacuo* to yield colorless amorphous powder of **3** (48 mg), **4** (6 mg), **5** (210 mg), **6** (20 mg), **7** (35 mg), **8** (7 mg) and mixture of **1** and **2** (150 mg).

The physico-chemical properties of compounds **5** and **6** are summarized in Table 1. The molecular formula of **5** was determined to be $C_{42}H_{62}O_{16}$ from the HRFAB-MS (found *m/z* 823.4129 $(M+H)^+$, calcd. *m/z* 823.4116 for $C_{42}H_{63}O_{16}$) and NMR spectral analysis (Table 2). IR spectrum of **5** showed the absorption bands corresponding to the hydroxyl group at 3387 cm^{-1}, ester group at 1713 cm^{-1}, and conju-

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

Table 1. Physico-chemical properties of F-10748 C_1 (**5**) and F-10748 C_2 (**6**).

	5	6
Appearance	Colorless amorphous powder	Colorless amorphous powder
Specific rotation		
$[\alpha]_D$ (MeOH)	+ 7.34° (*c* 1.49)	+ 4.86° (*c* 0.350)
Molecular formula	$C_{42}H_{62}O_{16}$	$C_{42}H_{64}O_{16}$
FAB-MS (*m/z*)	823 $(M+H)^+$, 845 $(M+Na)^+$	825 $(M+H)^+$, 847 $(M+Na)^+$
HRFAB-MS (*m/z*)		
Found	823.4129	825.4240
Calcd	823.4116 ($C_{42}H_{63}O_{16}$)	825.4273 ($C_{42}H_{65}O_{16}$)
Found		847.4089
Calcd		847.4092 ($C_{42}H_{64}O_{16}Na$)
UV (MeOH) λ_{max} nm (ε)	263 (29,200), 223 (sh 13,700), 204 (49,400)	263 (21,300), 205 (32,900)
IR ν_{max} (KBr) cm^{-1}	3387, 2961, 2931, 2874, 1713, 1640, 1612, 1464, 1346, 1263, 1149, 1073, 1036, 1007	3374, 2961, 2930, 2874, 1714, 1641, 1622, 1459, 1338, 1265, 1147, 1078, 1035, 971
HPLC (Rt. minute)*	9.0	9.6

* Symmetry C_{18} 4.6φ x 150 mm, CH_3CN : MeOH : H_2O = 54 : 10 : 36, 1.0 ml/minute, UV 210 nm

gated dienes at 1640 and 1612 cm^{-1}. The UV spectrum of **5** in methanol showed absorption maxima at 204, 223 and 263 nm. The molecular formula of **6** was determined as $C_{42}H_{64}O_{16}$ (HRFAB-MS found *m/z* 825.4240 $(M+H)^+$, calcd. *m/z* 825.4273 for $C_{42}H_{65}O_{16}$). In the IR spectrum of **6**, characteristic absorption bands for the same functional group as **5** (3374, 1714, 1641 and 1622 cm^{-1}) was observed. The molecular formulae of the other related compounds, **1**, **2**, **3**, **4**, **7** and **8** were determined to be $C_{37}H_{54}O_{15}$ (HRFAB-MS found *m/z* 761.3370 $(M+Na)^+$, calcd. *m/z* 761.3361 for $C_{37}H_{54}O_{15}Na$), $C_{37}H_{56}O_{15}$ (HRFAB-MS found *m/z* 763.3463 $(M+Na)^+$, calcd. *m/z* 763.3517 for $C_{37}H_{56}O_{15}Na$), $C_{41}H_{60}O_{16}$ (HRFAB-MS found *m/z* 809.3962 $(M+H)^+$, calcd. *m/z* 809.3959 for $C_{41}H_{61}O_{16}$), $C_{41}H_{62}O_{16}$ (HRFAB-MS found *m/z* 811.4119 $(M+H)^+$, calcd. *m/z* 811.4116 for $C_{41}H_{63}O_{16}$), $C_{43}H_{64}O_{16}$ (HRFAB-MS found *m/z* 837.4291 $(M+H)^+$, calcd. *m/z* 837.4272 for $C_{43}H_{65}O_{16}$) and $C_{43}H_{66}O_{16}$ (HRFAB-MS found *m/z* 861.4242 $(M+Na)^+$, calcd. *m/z* 861.4249 for $C_{43}H_{66}O_{16}Na$), respectively. Compounds, **1**, **2**, **3**, **4**, **5**, **6**, **7** and **8** were eluted with retention times of 3.5, 3.6, 6.9, 7.2, 9.0, 9.6, 12.7 and 13.7 minutes, respectively by HPLC (Waters Symmetry ODS, 4.6×150 mm, Waters; CH_3CN - MeOH - H_2O (54 : 10 : 36), 1.0 ml/minute; UV at 210 nm).

Since their structures were classified into two groups based on the similarity of the core structure, the structure determination was mainly focused on compounds **5** and **6**. The ^1H and ^{13}C NMR spectral data of **5** are summarized in Table 2. In the ^1H NMR spectrum of **5**, eight olefinic and aromatic proton signals were observed in the range between 5.25 and 7.29 ppm, and the presence of two sugar moieties was suggested from the signals between 3.45 and 5.41 ppm. In the range of alkyl group, methine or methylene proton signals (1.29~2.39 ppm) and four methyl protons (0.8~1.0 ppm) were observed. The ^{13}C NMR and DEPT spectrum displayed 42 signals classified into two carbonyl, five quaternary, twenty methine, elven methylene, and four methyl carbons. These NMR data and the physico-chemical properties suggested that **5** belongs to papulacandin family antibiotics and further structural analysis suggested the presence of the spirocyclic glycoside structure identical to those of papulacandins. It was also supported by the analysis of the hydrolyzed product of **5** (**9**). Compound **9** and the hydrolyzed of papulacandin B showed identical ^1H and ^{13}C NMR spectrum data, and the specific rotation ($[\alpha]_D$ +31° (*c* 0.43, MeOH))[2]. From the data above, the absolute configuration of the core skeleton of **5** was assigned to be identical to that of papulacandin B[1,2].

Major difference between **5** and previously reported papulacandins was found in the acyl side chains. By analyzing the DQFCOSY and the HMBC spectrum, one side chain of **5** was determined to be valeryl moiety represented as R_1, and the other was determined to be 7-hydroxy-8,14-dimethyl-2,4,12-hexadecatrienoyl moiety (Fig. 1). All of the geometries of the double bond at 2″, 4″ and 12″ position (Fig. 1) were confirmed to be *E* by the

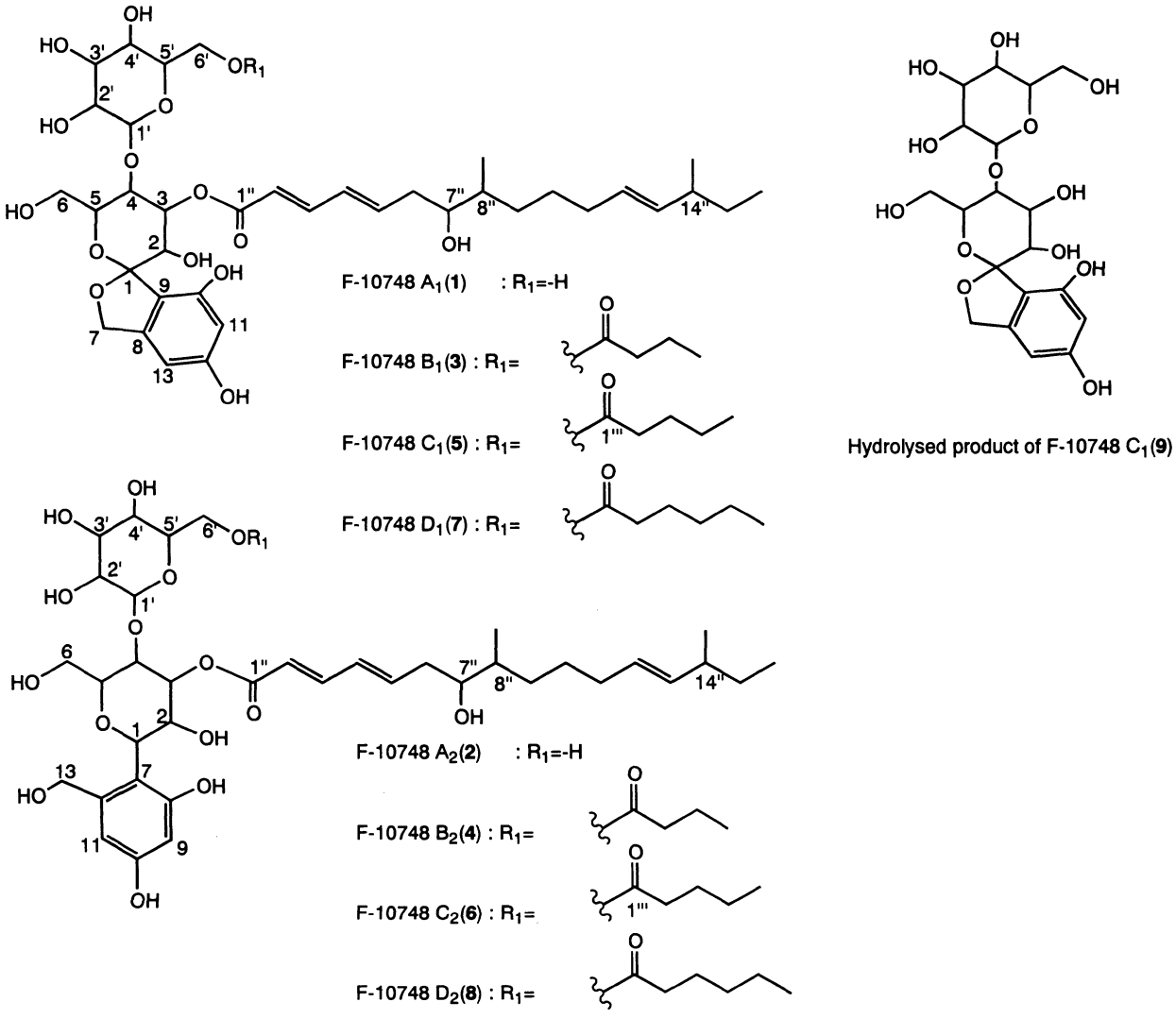
Table 2. ^{1}H NMR and ^{13}C NMR data of F-10748 C_1 (**5**) and C_2 (**6**).

	5		**6**	
Position	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	112.1 (s)		78.8 (d)	4.81 (1H, d, J = 10.8 Hz)
2	72.0 (d)	4.38 (1H, d, J = 10.0 Hz)	72.1 (d)	4.01 (1H, t, J = 9.7 Hz)
3	76.4 (d)	5.41 (1H, t, J = 9.7 Hz)	79.1 (d)	5.17 (1H, t, J = 9.4 Hz)
4	77.6 (d)	3.96 (1H, m)	77.4 (d)	3.97 (1H, t, J = 9.4 Hz)
5	75.0 (d)	3.98 (1H, m)	81.6 (d)	3.61 (1H, dt, J = 9.4, 2.5 Hz)
6	61.6 (t)	3.99 (1H, m)	61.2 (t)	4.03 (1H, dd, J = 12.2, 3.2 Hz)
		3.76 (1H, m)		3.88 (1H, dd, J = 12.2, 2.2 Hz)
7	74.0 (d)	5.05, 5.00 (2H, ABq, J = 12.6 Hz)	114.6 (s)	
8	145.6 (s)		158.8 (s)	
9	116.6 (s)		104.6 (d)	6.28 (1H, d, J = 2.5 Hz)
10	154.8 (s)		159.5 (s)	
11	103.2 (d)	6.21 (1H, d, J = 0.9 Hz)	109.5 (d)	6.41 (1H, d, J = 2.5 Hz)
12	161.8 (s)		143.3 (s)	
13	100.1 (d)	6.20 (1H, d, J = 0.9 Hz)	63.9 (t)	4.64, 4.55 (2H, ABq, J = 12.2 Hz)
1'	105.4 (d)	4.32 (1H, d, J = 7.6 Hz)	105.4 (d)	4.34 (1H, d, J = 7.6 Hz)
2'	74.8 (d)*	3.45 (1H, m)	74.8 (d)*	3.46 (1H, m)
3'	72.7 (d)*	3.45 (1H, m)	72.7 (d)*	3.46 (1H, m)
4'	70.4 (d)	3.64 (1H, br s)	70.3 (d)	3.73 (1H, br s)
5'	74.0 (t)	3.65 (1H, t, J = 6.5 Hz)	74.0 (d)	3.64 (1H, t, J = 6.1 Hz)
6'	64.8 (t)	4.14 (2H, d, J = 6.3 Hz)	64.7 (t)	4.13 (2H, d, J = 6.1 Hz)
1"	169.3 (s)		169.2 (s)	
2"	121.7 (d)	5.91 (1H, d, J = 15.4 Hz)	121.6 (d)	5.89 (1H, d, J = 15.5 Hz)
3"	146.2 (d)	7.29 (1H, dd, J = 15.4, 10.3 Hz)	146.4 (d)	7.28 (1H, dd, J = 15.5, 10.4 Hz)
4"	75.4 (d)	3.55 (1H, m)	75.4 (d)	3.55 (1H, m)
8"	39.5 (d)	1.49 (1H, m)	39.5 (d)	1.48 (1H, m)
8"-CH_3	14.4 (q)	0.90 (3H, d, J = 6.6 Hz))	14.4 (q)	0.89 (3H, d, J = 6.5 Hz)
9"	34.1 (t)	1.46 (1H, m), 1.19 (1H, m)	34.1 (t)	1.46 (1H, m), 1.18 (1H, m)
10"	28.7 (t)	1.38 (2H, m)	28.7 (t)	1.39 (2H, m)
11"	34.1 (t)	1.99 (2H, m)	34.1 (t)	1.98 (2H, m)
12"	130.0 (d)	5.38 (1H, dt, J = 15.5, 6.5 Hz)	130.0 (d)	5.38 (1H, dt, J = 15.5, 6.5 Hz)
13"	137.7 (d)	5.25 (1H, dd, J = 15.3, 7.6 Hz)	137.7 (d)	5.25 (1H, dd, J = 15.3, 7.6 Hz)
14"	40.0 (d)	1.95 (1H, m)	40.0 (d)	1.95 (1H, m)
14"-CH_3	21.2 (q)	0.95 (3H, d, J = 6.7 Hz)	21.2 (q)	0.95 (3H, d, J = 6.8 Hz)
15"	31.1 (t)	1.29 (2H, m)	31.1 (t)	1.28 (2H, m)
16"	12.4 (q)	0.86 (3H, t, J = 7.4 Hz)	12.3 (q)	0.86 (3H, t, J = 7.4 Hz)
1'"	175.4 (s)		175.4 (s)	
2'"	35.0 (t)	2.39 (2H, t, J = 7.4 Hz)	35.0 (t)	2.37 (2H, t, J = 7.6 Hz)
3'"	28.4 (t)	1.66 (2H, m)	28.4 (t)	1.65 (2H, m)
4'"	23.5 (t)	1.42 (2H, m)	23.5 (t)	1.41 (2H, m)
5'"	14.3 (q)	0.97 (3H, t, J = 7.3 Hz)	14.3 (q)	0.96 (3H, t, J = 7.4 Hz)

Spectra were recorded at 360 and 90 MHz for ^{1}H and ^{13}C, respectively, in CD_3OD.
Chemical shifts are given in ppm referenced to the solvent signals at 3.31 ppm for ^{1}H and at 49.15 ppm for ^{13}C as internal standard.
* : Assignments are interchangeable.

coupling constants (J=15.4, 15.1 and 15.5 Hz, respectively) of the olefinic proton signals. The structures of the two side chains were also supported by the high-energy collision-induced dissociation (CID) spectrum (Fig. 2). The connectivity of the acyl side chains to the sugar moieties was determined by the presence of C-H long-range coupling between H-3 and C-1", H-6' and C-1'" in the HMBC spectrum. Therefore, the structure of **5** was

Fig. 1. Structures of F-10748 A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), C_1 (**5**), C_2 (**6**), D_1 (**7**), D_2 (**8**) and **9**.



determined as shown in Fig. 1. In comparison with the structures of previously reported papulacandins, F-10748 series compounds possess no conjugated double bonds at position 8″ and 10″, and possess only one double bond at position 12″.

The ^1H and ^{13}C NMR spectral data of **6** are summarized in Table 2. In the ^1H NMR spectrum, the proton signal (4.81 ppm, d, J=10 Hz) was assigned to the proton at H-1, and high field shift of the benzylic protons (4.55 and 4.64 ppm) was characteristic to **6**. These data suggested that the glycoside core structure of **6** is similar to that of chaetiacandin[3,4], which belongs to papulacandin family. This structure was elucidated mainly by the DQFCOSY, HMQC and HMBC spectral analysis supported by the ^1H and ^{13}C NMR signal assignment of chaetiacandin. Finally, the structure of **6** was determined as shown in Fig. 1.

Compounds **3** and **7** differed from **5** in their R_1 side chain, *i.e.* the valeryl group of **5** was replaced with butyryl and hexanoyl group in **3** and **7**, respectively. These structures were supported by high-energy CID analysis (data not shown). Compounds **4** and **8** also differed from **6** in their R_1 side chain. By comparison of ^1H NMR and FAB-MS of **4** and **8** with that of **6**, the structures of **4** and **8** were determined as shown in Fig. 1.

Although **1** and **2** were obtained as a mixture, the ^1H NMR spectrum and FAB-MS data clearly indicated the loss of the R_1 side chain. Furthermore, the proton signals derived from spirocyclic moiety (H-7) and benzyl alchol

Fig. 2. Assignment of CID-MS/MS fragments of F-10748 C_1 (**5**).

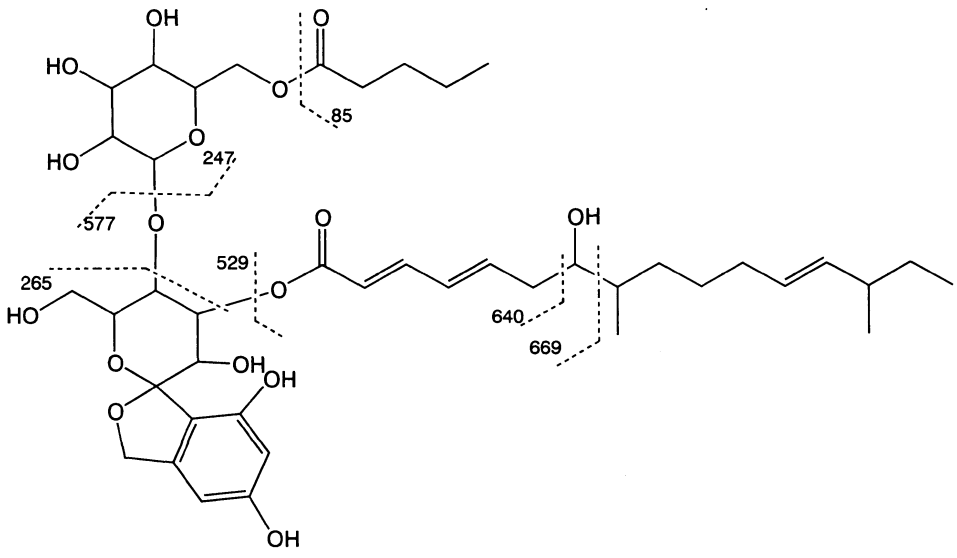


Table 3. Inhibition of 1,3-β-glucan synthase[a] by F-10748 C_1 and its related compounds.

Sample	Glucan synthase inhibitory activity IC_{50} (μg/ml)
F-10748 B_1 (3)	0.2
F-10748 B_2 (4)	2
F-10748 C_1 (5)	0.1
F-10748 C_2 (6)	1
F-10748 D_1 (7)	0.2
F-10748 D_2 (8)	2

a) Solubilized membrane fraction of *Aspergillus fumigatus* was used.

group (H-13) were observed in the spectrum. These results revealed the structures of **1** and **2** as shown in Fig. 1.

Inhibitory activity against fungal 1,3-β-glucan synthase was determined according to the method described previously[5]. As shown in Table 3, **3** to **8** inhibited 1,3-β-glucan synthase. The inhibitory activities ranged from 0.1 to 2 μg/ml. The inhibitory activities of papulacandin type compounds (**3**, **5** and **7**) were much stronger than that of chaetiacandin type compounds (**4**, **6** and **8**). This suggests that the spirocyclic glycoside core skeleton is related to the glucan synthase inhibitory activity of papulacandins.

In vitro growth inhibitory activity against human pathogenic yeasts and a filamentous fungus were evaluated by the microbroth dilution method according to the procedure proposed by the Japanese Society for Medical Mycology[6]. As shown in Table 4, **3** to **8** have potent antifungal activities against *Candida* spp., although they were inactive against *Aspergillus fumigatus* and *Cryptococcus neoformans*, as observed for other papulacandins[7,8]. The antifungal activity of **3** and **5** against *Candida* spp. was almost the same as those of **4** and **6**, although the inhibitory activity of **3** and **5** against fungal glucan synthase was much

Table 4. Antifungal activity of F-10748 C_1 and its related compounds.

Strain	MIC (μg/ml)					
	F-10748 B_1 (3)	F-10748 B_2 (4)	F-10748 C_1 (5)	F-10748 C_2 (6)	F-10748 D_1 (7)	F-10748 D_2 (8)
Candida albicans ATCC 90028	2	2	2	2	0.5	0.5
C. albicans ATCC 90029	2	2	1	2	0.5	4
C. parapsilosis ATCC90018	2	2	1	1	0.5	2
C. tropicalis SANK59263	8	64	>128	>128	>128	>128
Cryptococcus neoformans SANK59863	>128	>128	>128	>128	>128	>128
Aspergillus fumigatus SANK 10662	>128	>128	>128	>128	>128	>128

MICs were determined using RPMI 1640 meduim (ICN Biomedicals Inc.) buffered with 0.165 M MOPS (pH 7.0).

stronger. This discrepancy may be explained by the difference in penetration of these compounds through the membrane or the difference between *Candida* spp. and *A. fumigatus* in sensitivity of glucan synthase for these compounds. A similar difference between the antifungal activity and the enzyme inhibitory activity was reported for other glucan synthase inhibitors[5,9].

F-10748 C_1 and its related compounds were novel papulacandin type antibiotics with a unique acyl side chain. This is the first report that papulacandin and chaetiacandin type antibiotics were co-produced in the same fermentation broth.

References

1) TRAXLER, P.; J. GRUNER & J. A. L. AUDEN: Papulacandins, a new family of antibiotics with antifungal activity. I. Fermentation, isolation, chemical and biological characterization of papulacandins A, B, C, D and E. J. Antibiotics 30: 289~296, 1977
2) TRAXLER, P.; J. GRUNER & J. A. L. AUDEN: Papulacandins, a new family of antibiotics with antifungal activity. Structures of papulacandins A, B, C and D. J. Antibiotics 33: 967~978, 1980
3) KOMORI, T.; M. YAMASHITA, Y. TSURUMI & M. KOHSAKA: Chaetiacandin, a novel papulacandin. I. Fermentation, isolation and characterization. J. Antibiotics 38: 455~459, 1985
4) KOMORI, T. & Y. ITO: Chaetiacandin, a novel papulacandin. II. Structure determination. J. Antibiotics 38: 544~546, 1985
5) 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
6) Japanese Journal of Medical Mycology 36: 62~64, 1995
7) VANMIDDLESWORTH, F.; M. N. OMSTEAD, D. SCHMATZ, K. BARTIZAL, R. FROMTLING, G. BILLS, K. NOLLSTADT, S. HONEYCUTT, M. ZWEERINK & G. GARRITY: L-687,781, a new member of the papulacandin family of β-1,3-D-glucan synthesis inhibitors. I. Fermentation, isolation, and biological activity. J. Antibiotics 44: 45~51, 1991
8) AOKI, M.; T. ANDOH, T. UEKI, S. MASUYOSHI, K. SUGAWARA & T. OKI: BU-4794F, a new β-1,3-glucan synthase inhibitor. J. Antibiotics 46: 952~960, 1993
9) BARTIZAL, K.; G. ABRUZZO, C. TRAINOR, D. KRUPA, K. NOLLSTADT, D. SCHMATZ, R. SCHWARTZ, M. HAMMOND, J. BALKOVEC & F. VANMIDDLESWORTH: *In vitro* antifungal activities and *in vivo* efficacies of 1,3-β-D-glucan synthesis inhibitors L-671,329, L-646,991, tetrahydroechinocandin B, and L-687,781, a papulacandin. Antimicrob. Agents Chemother. 36: 1648~1657, 1992