Fusacandins A and B; Novel Antifungal Antibiotics of the Papulacandin Class from *Fusarium sambucinum*

II. Isolation and Structural Elucidation†

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Two novel antifungal compounds of the papulacandin class, named fusacandins A and B, have been isolated from *Fusarium sambucinum*. Each compound contains two units of galactose and one of glucose, the latter connected as a C-glycoside to an aromatic moiety. Fusacandin A is esterified at two sites with long-chain, unsaturated fatty acids and fusacandin B at only one site. The structures of the fusacandins were elucidated through analysis of mass spectral and 1-D and 2-D homonuclear and heteronuclear NMR data.

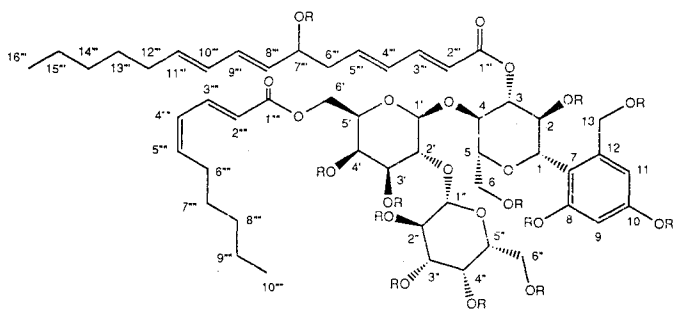
In the course of screening for new antifungal agents, two compounds, named fusacandins A and B, were isolated from *Fusarium sambucinum*. Each is a member of the papulacandin class[1,2] of antifungals but differs from all previous members of this class in containing an additional galactose unit. The fusacandins differ from one another in the number of unsaturated, long-chain, fatty acids attached to the trisaccharide backbone. This paper details the isolation and structural elucidation of the fusacandins. A companion paper[3] discusses the producing organism, fermentation and biological activity of the fusacandins.

Isolation of Fusacandin A

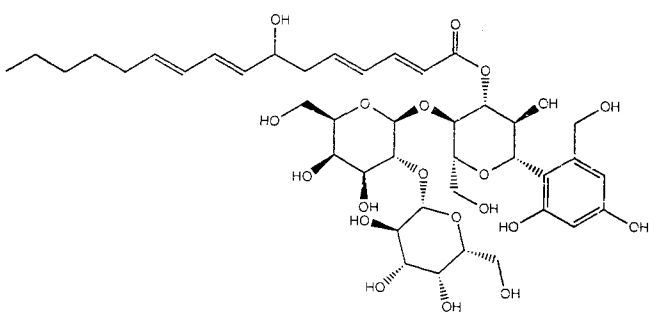
To 15 liters of whole culture broth was added 8 liters of acetone and the mixture agitated for 1 hour, after which 15 liters of EtOAc was added, the mixture was agitated for an additional 2 hours and upper layer was removed. An additional two, 8-liter extracts were made, combined with the first and concentrated to yield 15 g of brown oil. This oil was triturated sequentially with 2 liters each of; Hexane, EtOAc and MeOH. The MeOH soluble material was concentrated to yield 780 mg of brown oil which was subjected to silica gel chromatography on 500 g of Varian 40 μ silica gel eluted sequentially with 1 liter each of; EtOAc, 2, 5, 10, 20 and 50% MeOH in EtOAc, and finally with 100% MeOH. Material which eluted with 50% MeOH in EtOAc was subjected to size exclusion chromatography on a Sephadex LH-20 column developed in MeOH. Active fractions from this column were combined to yield 160 mg of pure fusacandin A (**1**).

Isolation of Fusacandin B

To 4,900 liters of whole broth was added 3,350 liters of acetone and 3,700 liters EtOAc and the mixture was agitated for approximately 12 hours after which the upper layer was removed and concentrated under vacuum and



1, Fusacandin A (R=H)
3, Fusacandin A peracetate (R=OAc)



2, Fusacandin B

† Dedicated to Professor SATOSHI ŌMURA on the happy occasion of his 60th birthday.

the concentrate was deposited onto 10 kg of silica gel. This was loaded on a 240 kg silica gel column developed sequentially with; 300 liter portions of EtOAc, 25, 50 and 75% MeOH in EtOAc and 100% MeOH. A portion (25 g) of the material which eluted with 50% MeOH in EtOAc was partitioned between EtOAc-EtOH-H_2O (3 : 1 : 2) and the upper layer was concentrated to an oily solid residue. The residue was subjected to size exclusion chromatography on a Sephadex LH-20 column developed in MeOH. Active fractions from this column were combined based upon their behavior on thin layer chromatography to yield fusacandin A (2.65 g) and fusacandin B (**2**) (62 mg).

Table 1. NMR assignments for fusacandins A and B (in CD_3OD).

C No.	A Carbon shift (δ)	B Carbon shift (δ)	A Proton shift (δ) and multiplicity	B Proton shift (δ) and multiplicity
1	78.5 (CH)	78.4 (CH)	4.78 (d,1H. J=9.5 Hz)	4.78 (d,1H. J=9.7 Hz)
2	72.0 (CH)	71.9 (CH)	3.97 (dd, 1H, J=9.5, 9.3 Hz)	4.00 (dd, 1H, J=9.7, 9.2 Hz)
3	79.2 (CH)	79.2 (CH)	5.16 (t, 1H, J=9.3 Hz)	5.15 (t, 1H, J=9.2 Hz)
4	78.3 (CH)	77.8 (CH)	3.86 (dd, 1H, J=9.5, 9.3 Hz)	3.90 (dd, 1H, J=9.7, 9.2 Hz)
5	82.0 (CH)	81.8 (CH)	3.70 (mult, 1H)	3.70 (mult, 1H)
6	61.7 (CH_2)	61.7 (CH_2)	4.06 (dd, 1H, J=12.2, 2.2 Hz), 4.02 (dd, 1H, J=12.2, 4.0 Hz)	4.07 (dd, 1H, J=12.6, 2.4 Hz), 4.01 (dd, 1H, J=12.6, 4.2 Hz)
7	114.4 (Q)	114.5 (Q)		
8	158.7 (Q)	158.6 (Q)		
9	104.5 (CH)	104.4 (CH)	6.26 (d, 1H, J=2.4 Hz)	6.27 (d, 1H, J=2.5 Hz)
10	159.5 (Q)	159.5 (Q)		
11	109.4 (CH)	109.4 (CH)	6.39 (d, 1H, J=2.4 Hz)	6.41 (d, 1H, J=2.5 Hz)
12	143.1 (Q)	143.1 (Q)		
13	63.7 (CH_2)	63.6 (CH_2)	4.64 (d, 1H, J=12.3 Hz), 4.52 (d, 1H, J=12.3 Hz)	4.64 (d, 1H, J=12.4 Hz), 4.53 (d, 1H, J=12.4 Hz)
1′	104.0 (CH)	103.9 (CH)	4.43 (d, 1H, J=7.1 Hz)	4.41 (d, 1H, J=7.3 Hz)
2′	81.8 (CH)	82.1 (CH)	3.71 (mult, 1H)	3.68 (mult, 1H)
3′	74.8 (CH)	75.0 (CH)	3.67 (mult, 1H)	3.65 (mult, 1H)
4′	70.2 (CH)	69.8(CH)	3.77 (mult, 1H)	3.80 (mult, 1H)
5′	73.8 (CH)	76.2 (CH)	3.68 (mult, 1H)	3.42 (mult, 1H)
6′	64.8 (CH_2)	62.1 (CH_2)	4.22 (dd, 1H, J=11.3, 5.9 Hz), 4.13 (mult, 1H)	3.59 (mult, 2H)
1″	107.0 (CH)	106.9 (CH)	4.50 (d, 1H, J=7.5 Hz)	4.51 (d, 1H, J=7.5 Hz)
2″	74.1 (CH)	74.1 (CH)	3.58 (dd, 1H, J=9.7, 7.5 Hz)	3.59 (mult, 1H)
3″	74.8 (CH)	74.7 (CH)	3.49 (dd, 1H, J=9.7, 3.3 Hz)	3.50 (dd, 1H, J=9.7, 3.3 Hz)
4″	70.6 (CH)	70.5 (CH)	3.81 (mult, 1H)	3.82 (mult, 1H)
5″	77.2 (CH)	77.1 (CH)	3.55 (mult, 1H)	3.55 (mult, 1H)
6″	62.9 (CH_2)	62.9 (CH_2)	3.78 (mult, 1H), 3.73 (dd, 1H, J=11.6, 4.4 Hz)	3.79 (mult, 1H), 3.73 (mult, 1H)
1‴	168.9 (Q)	169.1 (Q)		
2‴	121.6 (CH)	121.7 (CH)	5.87 (d, 1H, J=15.4 Hz)	5.92 (d, 1H, J=15.4 Hz)
3‴	146.1 (CH)	146.1 (CH)	7.25 (dd, 1H, J=15.4, 11.0 Hz)	7.27 (dd, 1H, J=15.4, 10.8 Hz)
4‴	131.9 (CH)	131.9 (CH)	6.27 (br d, 1H, J=15.4, 11.0 Hz)	6.32 (br d, 1H, J=15.4, 10.8 Hz)
5‴	141.3 (CH)	141.3 (CH)	6.13 (mult, 1H)	6.17 (mult, 1H)
6‴	42.2 (CH_2)	42.2 (CH_2)	2.37 (mult, 2H)	2.39 (mult, 2H)
7‴	72.6 (CH)	72.6 (CH)	4.14 (mult, 1H)	4.16 (br q, 1H, J=6.6 Hz)
8‴	134.0 (CH)	134.0 (CH)	5.55 (dd, 1H, J=15.2, 6.8 Hz)	5.57 (dd, 1H, J=15.2, 6.6 Hz)
9‴	132.1 (CH)	132.1 (CH)	6.16 (dd, 1H, J=15.2, 10.8 Hz)	6.18 (mult, 1H)
10‴	131.0 (CH)	131.0 (CH)	6.01 (br dd, 1H, J=15.2, 10.8 Hz)	6.02 (br dd, 1H, J=15.0, 10.8 Hz)
11‴	136.1 (CH)	136.1 (CH)	5.67 (dt, 1H, J=15.2, 7.0 Hz)	5.68 (dt, 1H, J=15.0, 7.2 Hz)
12‴	33.6 (CH_2)	33.6 (CH_2)	2.06 (mult, 2H)	2.06 (br q, 2H, J=7.2 Hz)
13‴	30.1 (CH_2)	30.1 (CH_2)	1.38 (mult, 2H)	1.38 (mult, 2H)
14‴	32.5 (CH_2)	32.5 (CH_2)	1.32 (mult, 2H)	1.29 (mult, 2H)
15‴	23.6 (CH_2)	23.5 (CH_2)	1.32 (mult, 2H)	1.31 (mult, 2H)
16‴	14.6 (CH_3)	14.4 (CH_3)	0.87 (t, 3H, J=7.0 Hz)	0.89 (t, 3H, J=7.0 Hz)
1⁗	168.6 (Q)			
2⁗	121.7 (CH)		5.97 (d, 1H, J=15.2 Hz)	
3⁗	141.5 (CH)		7.69 (ddd, 1H, J=15.2, 11.7, 1.1 Hz)	
4⁗	127.5 (CH)		6.21 (br dd, 1H, J=11.7, 11.0 Hz)	
5⁗	143.4 (CH)		5.93 (mult, 1H)	
6⁗	29.2 (CH_2)		2.33 (mult, 2H)	
7⁗	30.1 (CH_2)		1.44 (mult, 2H)	
8⁗	32.6 (CH_2)		1.32 (mult, 2H)	
9⁗	23.6 (CH_2)		1.32 (mult, 2H)	
10⁗	14.4 (CH_3)		0.87 (t, 3H, J=7.0 Hz)	

Structural Elucidation of Fusacandin A

^{13}C NMR and DEPT spectra of fusacandin A contained fifty one unique carbon signals and indicated sixty two attached protons (see Table 1). Three sugar moieties were evident in the COSY spectrum of fusacandin A, two as *O*-glycosides and one as a *C*-glycoside.

One O-linked sugar was galactose as defined by NMR signals for an anomeric with proton signal at δ 4.50 (d, 1H, J=7.5 Hz) correlating in an HMQC spectrum to a carbon signal at δ 107.0 (CH). The coupling system from this proton signal included, sequentially, signals at δ 3.58 (dd, 1H, J=9.7, 7.5 Hz), 3.49 (dd, 1H, J=9.7, 3.3 Hz), 3.81 (mult, 1H), 3.55 (mult, 1H) and ultimately methylene proton signals at δ 3.73 (dd, 1H, J=116, 4.4 Hz) and 3.78 (mult, 1H). Further, NOEs were observed between the proton signals at δ 4.50 (C-1″), 3.49 (C-3″) and 3.55 (C-5″). These data indicate that the protons at positions 1, 3 and 5 of this sugar must be axial and the coupling constants require that the proton at 4″ be equatorial.

The stereochemistry of the second O-glycosidic sugar could not be determined through examination of the spectral data of fusacandin A itself as significant signal overlap was observed. The sequence of proton signals, however, was assigned by COSY as in Table 1. The stereochemistry of this sugar was elucidated from an analysis of the spectral data of the preacetate derivative.

Peracetate of Fusacandin

The ^1H NMR of the peracetate of fusacandin A (**3**) had an anomeric proton signal at δ 4.61 (d, 1H, J= 7.5 Hz). The spin system for the protons of this sugar was analysed tracing a coupling of this anomeric proton to the 2′ proton signal at δ 3.71 (dd, 1H, J=9.9, 7.5 Hz), to the 3′ proton signal at δ 5.04 (dd, 1H, J=9.9, 3.7 Hz), the 4′ proton signal at δ 5.27 (dd, 1H, J=3.7, 0.9 Hz), the 5′ proton signal at δ 4.03 (ddd, 1H, J=7.7, 6.6, 0.9 Hz) and finally the coupled 6′ methylene proton signals at δ 4.08 (mult, 1H) and 3.91 (mult, 1H). That this was the central sugar was established by an HMBC correlation from the 2′ proton signal at δ 3.71 to the 1″ carbon signal at δ 102.3, as well as by a strong NOE between the 2′ and 1″ proton signals. The stereochemistry of the central sugar was established as follows; NOEs are observed from each of the protons at δ 4.61 (C-1′), 5.04 (C-3′) and 4.03 (C-5″) to the other two. Thus each must be in an axial position. The coupling constants of the proton on C-2′ (9.9 and 7.5 Hz) indicate that it must also be axial. The coupling constants of the proton on C-4′ (3.7 and 0.9 Hz) indicate that it must be equatorial. Thus the central sugar in fusacandin must be β-galactose.

The *C*-glycosidic sugar moiety was shown to have the glucose stereochemistry†. The proton spin system starting with the pseudo-anomeric proton signal at δ 4.78 (d, 1H, J=9.5 Hz) with an attached carbon signal at δ 78.5 (CH) proceeded in order to the proton signal at δ 3.97 (dd, 1H, J=9.5, 9.3 Hz), the signal at δ 5.16 (t, 1H, J=9.3 Hz), a signal at δ 3.86 (dd, 1H, J=9.5, 9.3 Hz), one at δ 3.70 (mult, 1H), and finally two methylene proton signals at δ 4.06 (dd, 1H, J=12.2, 2.2 Hz) and 4.02 (dd, 1H, J=12.4, 4.0 Hz). The contiguous large coupling constants require that this sugar contain all axial protons (*cf.* β-glucose) and the requisite NOEs were observed between proton signals at δ 4.78, 5.16 and 3.70.

The order of attachment of sugars within the trisaccharide was deduced through long-range proton-carbon coupling *via* an HMBC experiment in which the anomeric proton signal of the galactose at δ 4.50 showed long range coupling to the carbon signal at δ 81.8 for the 2 carbon of the sugar whose stereochemistry required derivatization to define. The anomeric proton signal (at δ 4.43) of this same sugar showed long range coupling to the carbon signal of the 4 position in glucose at δ 78.3.

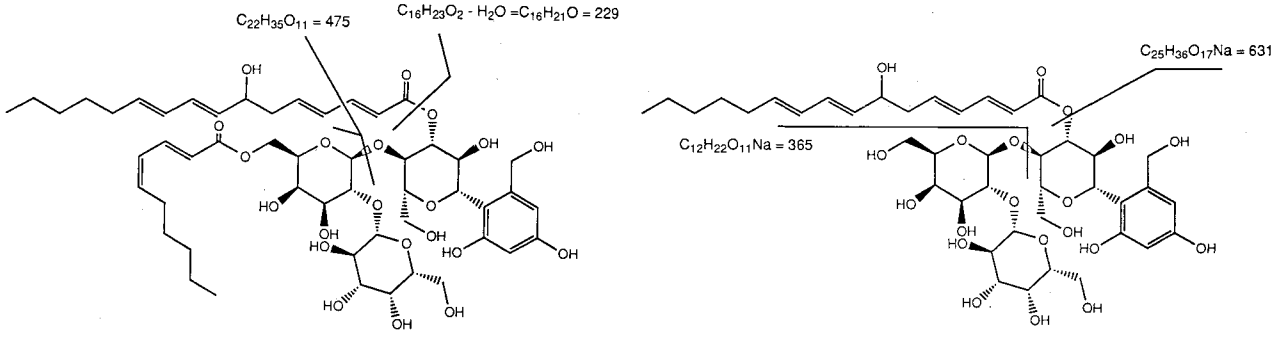
The *C*-glycosidic linkage of glucose attaches to a 3,5-dihydroxybenzylic alcohol aromatic as evidenced by a strong NOE between the *C*-glycosidic proton signal at δ 4.78 (C-1) and the methylene proton signals of the hydroxymethylene at δ 4.64 and 4.52 (C-13). This aromatic moiety is defined by analysis of HMBC and HMQC data as follows; the benzylic hydroxymethylene proton signals at δ 4.64 and 4.52 each show long range coupling to two quaternary carbon signals at δ 114.5 (C-7) and 143.1 (C-12) as well as an aromatic methine carbon at δ 109.4 (C-11). This C-11 carbon signal correlates to a proton signal at δ 6.39 (d, 1H, J=2.4 Hz) which shows meta-coupling to a proton signal at δ 6.26 (d, 1H, J=2.4 Hz) as well as long range proton-carbon coupling to carbon signals at δ 159.5 (C-10), 104.5 (C-9), 143.1 (C-12) and 114.5 (C-7). The aromatic methine at 6.26 shows long range proton-carbon coupling to carbon signals at δ 159.5 (C-10), 109.4 (C-11), 158.7 (C-8) and 114.5 (C-7).

Two unsaturated, long-chain fatty acids were evident

† The authors are aware that the numbering system used here is not the approved one for C-glycosides but it is the same as that used in reference 2 for the chaetiacandins. The relative stereochemistry has been deduced from NMR considerations only and although the structures are presented as D-sugars, no determination of absolute stereochemistry has been made.

Fig. 1. Mass spectral fragmentation of fusacandins A and B.

(Fusacandin A = $C_{51}H_{74}O_{21}$ = 1,022, Fusacandin B = $C_{41}H_{60}O_{20}$ = 872).



from the COSY of fusacandin A in which 1-bond couplings allowed assignment of the sequence from C-2‴ through C-14‴ and C-2⁗ through C-8⁗. The exact length of each fatty acid was deduced from mass spectral fragmentation data in which peaks were observed at *m/z* 1,045 and *m/z* 1,005 for the parent ion sodium adduct and parent ion with loss of H_2O. Further fragmentation of the 1,005 peak gave a strong fragment at *m/z* 229 for a loss of H_2O from the sixteen carbon fatty acid. The sodium ion adduct at *m/z* 1,045 fragmented to give a strong ion at *m/z* 475 for loss of a disaccharide with a ten carbon fatty acid (see Fig. 1).

Structural Elucidation of Fusacandin B

A ^{13}C NMR spectrum of fusacandin B contained forty one carbon signals, or ten less than that of fusacandin A. Immediately obvious from the ^{1}H NMR spectrum of fusacandin B was the absence of signals for the *cis-trans* diene moiety of the 2,4-dienedecanoic acid (C-1⁗ through C-10⁗) seen in the spectrum of fusacandin A. Signals for the remainder of the structure of fusacandin B equivalent to that in fusacandin A could be assigned by a series of COSY, HMBC and HMQC experiments (see Table 1). An electrospray mass spectrum and collision induced dissociation of fusacandin B confirmed the structure of fusacandin B as the 6′-desacyl derivative of fusacandin A. The highest mass ion in this mass spectrum was *m/z* 895 consistent with a sodium salt adduct of $C_{41}H_{60}O_{20}$. Further, a strong fragment of *m/z* 365, consistent with loss of a disaccharide as the sodium salt with no fatty acid attached was observed as well as a fragment of *m/z* 631 as the sodium salt for loss of the 7-hydroxy-2,4,8,10-tetrahexadecanoic acid from the parent ion of fusacandin B (see Fig. 1).

Experimental

General Procedures

Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in a 10 cm cell. Rf values reported were determined on Merck Kiesel gel 60 F_{254} TLC plates and were visualized using ceric sulfate spray reagent[4]. Fast atom bombardment mass spectra were measured on a Kratos MS-50 mass spectrometer and electrospray mass spectra on a Finnigan-MAT TSQ 700 triple quadrupole mass spectrometer equipped with a Finnigan-MAT electrospray source. ESIMS-MS analysis was done on a Finnigan-MAT TSQ 700 using argon as the collision gas and 0.1% TFA - 5% acetonitrile as the mobile phase for electrospray. Ultraviolet spectra were recorded on a Hitachi U-2000 UV-visible spectrophotometer and infrared spectra on a Nicolet model 60SX FT-IR. Nuclear magnetic resonance spectra were acquired on a General Electric GN500 spectrometer.

Physiochemical Properties of Fusacandin A

Fusacandin A, of MW 1,022, ($C_{51}H_{74}O_{21}$), is a white solid, mp 114~119°C (dec.), $[\alpha]_D$ +58° (*c* 0.67, MeOH), with an Rf 0.00 in EtOAc, Rf 0.71 in MeOH - EtOAc (1 : 1), Rf 0.52 in acetone and Rf 0.40 in $CHCl_3$ - MeOH (3 : 2) on Merck silica gel TLC plates. An ultraviolet spectrum of fusacandin A acquired in MeOH or MeOH - 0.01 M HCl contained a band at λ_{max} 264 nm (ε 33,000), 232 (25,000), and end absorption. An ultraviolet spectrum of fusacandin A acquired MeOH - 0.01 M NaOH contained a band at λ_{max} 261 nm (ε 35,000) and end absorption. An infrared spectrum of fusacandin acquired in microscope mode contained bands at: 3372, 2955, 2927, 2858, 1702, 1634, 1459, 1411, 1375, 1335, 1396, 1267, 1146, 1076, 1049 and 1003 cm^{-1}.

Physiochemical Properties of Fusacandin B

Fusacandin B, MW 872, ($C_{41}H_{60}O_{20}$), is a white solid, mp 42~45°C, $[\alpha]_D$ +1° (*c* 0.4, MeOH), with an Rf 0.00 in EtOAc, Rf 0.60 in MeOH - EtOAc (1 : 1), Rf 0.46 in

acetone and Rf 0.19 in $CHCl_3$ - MeOH (3 : 2) on Merck silica gel TLC plates. An ultraviolet spectrum of fusacandin B acquired in MeOH or MeOH - 0.01 M HCl contained a band at λ_{max} 263 nm (ε 18,000), 231 (21,000) and end absorption. An ultraviolet spectrum of fusacandin B acquired in MeOH - 0.01 M NaOH contained a band at λ_{max} 256 nm (ε 22,000), and end absorption. An infrared spectrum of fusacandin B acquired in microscope mode contained bands at: 3305, 3040, 3005, 2880, 2850, 1708, 1645, 1625, 1570, 1465, 1410, 1380, 1315, 1265, 1155, 1080 and 1055 cm^{-1}.

Peracetylation of Fusacandin A

Fusacandin (15 mg) was dissolved in 2 ml of pyridine and 1 ml of acetic anhydride and the mixture stirred for 16 hours at room temperature. Liquid was removed under reduced pressure to leave pure fusacandin A peracetate.

References

1) TRAXLER, P.; H. FRITZ, H. FUHRER & W. J. RICHTER: Papulacandins, a new family of antibiotics with antifungal activity: structures of papulacandins A, B, C, and D. J. Antibiotics 33: 967～978, 1980
2) KOMORI, T & Y. ITOH: Chaetiacandin, a novel papulacandin II. Structure determination. J. Antibiotics 38: 544～546, 1985
3) JACKSON, M.; D. J. FROST, J. P. KARWOWSKI, P. E. HUMPHREY, S. K. DAHOD, W. S. CHOI, K. BRANDT, L.-H. MALMBERG, R. R. RASMUSSEN, M. H. SCHERR, R. K. FLAMM, S. KADAM & J. B. MCALPINE. Fusacandins A and B; Novel antifungal antibiotics of the papulacandin class from *Fusarium sambucinum*. I. Identity of the producing organism, fermentation and biological activity. J. Antibiotics 48: 608～613, 1995
4) KREBS, K. G.; D. HEUSSER & H. WIMMER: Spray reagents in thin layer chromatography. New York: Springer-Verlag, pp. 861, 1969 (STAHL E., *Ed.*)