Structure of Furanocandin, a New Antifungal Antibiotic from *Tricothecium* sp.

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Papulacandins[1] are compounds active against *Candida albicans* and various other yeasts.

In the course of our screening for new antifungal antibiotics, a new compound, furanocandin, a papulacandin, was isolated from the fermentation broth of *Tricothecium* sp. It was found to have marked activity against *Candida albicans* as much as amphotericin B. MIC data are given in Table 1.

There are some structural differences in the diglycoside moiety between furanocandin and papulacandins, which prompted us to examine the structure of furanocandin.

The active compound was isolated from the cultured mycelia of *Trichothecium* sp. by extraction with 70% aqueous acetone. This extract was concentrated and was extracted with ethyl acetate, and reconcentrated to an oily residue. Further purification of the compound from the crude extract was carried out by chromatography on a silica gel column with chloroform and increasing amounts of methanol as eluent.

Table 1. Antimicrobial activity of furanocandin and amphotericin B.

	MIC (μg/ml)	
	Furanocandin	Amphotericin B
Candida albicans TIMM 1768	0.20	0.78
C. albicans C-a-24	0.39	0.39
C. glabrata IFO-0005	0.78	0.78
C. glabrata IFO-0622	1.56	0.78
C. tropicalis IFO-0589	0.20	0.78
C. guilliermondii IFO-1972	1.56	1.56
C. krusei IFO-0584	6.25	1.56
C. parapsilosis IFO-0585	0.20	3.13
C. neoformans Cr-1	>100	0.78
C. neoformans IMC F-10	>100	0.39
Saccharomyces cerevisiae X2180-1A	6.25	0.78
Aspergillus fumigatus saito	>100	12.5
A. fumigatus TIMM 1775	>100	3.13

Medium : Yeast morphology agar

Purified furanocandin (**1**) was obtained as a colorless powder (Scheme 1). Physico-chemical properties of **1** are as follows : amorphous powder. $C_{45}H_{64}O_{17}$; $[\alpha]_D$ +3.6 (*c* 1.0, MeOH); UV λ_{max} nm (ε) 206 (52500), 224 sh (41200), 231 (41500), 263 (53900) (MeOH), 205 (45700), 225 sh (39500), 231 (39800), 264 (55000) (HCl-MeOH), 214 (102400), 260 (53900), 300 sh (7900), 312 sh (4400) (NaOH-MeOH); IR (KBr) cm^{-1} 3416, 2928, 2859, 1698, 1636, 1460, 1414, 1379, 1333, 1310, 1269, 1202, 1150, 1090, 1069, 1044. FAB-MS *m/z* 899 $(M+Na)^+$ high resolution FAB-MS calcd for $(M+Na)^+$: 899.4042, found: 899.4011; elemental analysis calcd for $C_{45}H_{64}O_{17}$: C 61.63, H 7.36, O 31.01, found: C 59.47, H 7.88, O 28.88; Assignments of the ^1H NMR and ^{13}C NMR spectra are shown in Table 2.

Based on the above, antibiotic **1** was concluded to be a papulacandin. However, it was not identical with any papulacandins so far reported[2~8], based on spectral data. There appeared to be differences in the diglycoside moiety. For further structural elucidation, **1** (75 mg) was treated with alkaline [0.5 N - NaOH, MeOH - water (1 : 1), room temperature, 2 hours]. The aqueaus solution was neutralized with HCl and extracted with ethyl acetate. Two esters of fatty acids (**2**, 7.0 mg; **3**, 8.2 mg) and diglycoside compound possessing an aromatic ring (**4**, 7.9 mg) were isolated by preparative TLC ($CHCl_3$: MeOH = 3 : 1) of the residue (Scheme 2).

The molecular weight of **2** was 278 based on EI-MS data. ^1H NMR and ^{13}C NMR spectra showed 25 protons and 17 carbons. The carbons were two methyls, five methylenes, one oxy methine, eight sp^2 methines and one carbonyl carbon. The molecular formula was thus concluded to be $C_{17}H_{26}O_3$. Proton linkages were shown by the HH-COSY spectrum, from which **2** could be seen to be straight chained fatty acid with 16 carbons with one hydroxyl group and four unsaturated bonds. The ^1H NMR coupling constant (15 Hz) indicated the four unsaturated bonds each had a *trans* configuration. A

Scheme 1. Isolation of furanocandin.

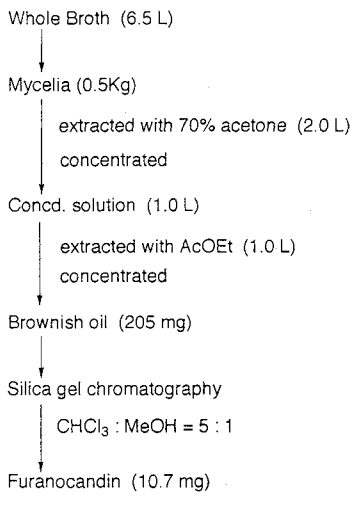
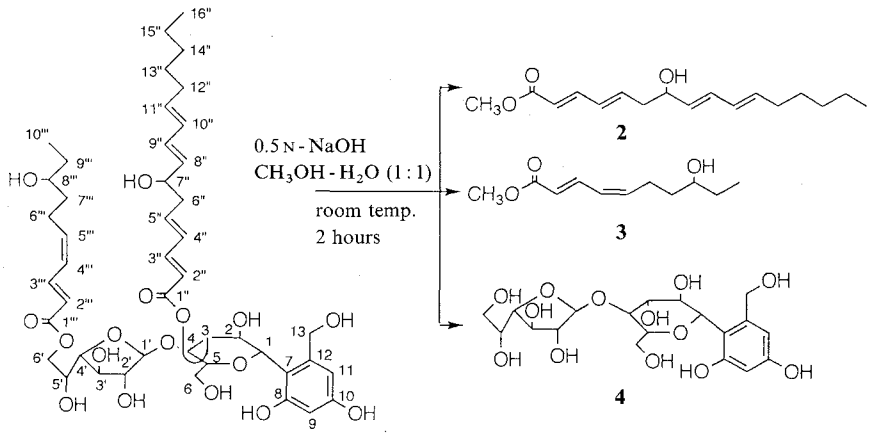


Table 2. ^{1}H NMR and ^{13}C NMR spectra of furanocandin.

Carbon	^{1}H-NMR	^{13}C-NMR	Carbon	^{1}H-NMR	^{13}C-NMR
1	4.87 (d, J = 10.0Hz)	78.6	6"	2.43 (dt, J = 7.2, 6.6Hz)	42.2
2	4.07 (m)	72.0	7"	4.20 (dt, J = 6.6, 6.5Hz)	72.6
3	5.26 (dd, J = 9.4, 9.2Hz)	78.9	8"	5.61 (dd, J = 15.1, 6.5Hz)	134.0
4	4.00 (dd, J = 10.0, 9.7Hz)	76.4	9"	6.23 (dd, J = 15.0, 10.5Hz)	132.1
5	3.65 (dt, J = 10.0, 2.5 Hz)	81.5	10"	6.07 (dd, J = 15.0, 10.5Hz)	131.0
6	3.90-3.96 (m)	61.2	11"	5.75 (dt, J = 15.0, 7.1Hz)	136.1
7		114.4	12"	2.14 (dt , J = 7.1, 7.1Hz)	33.7
8		158.7	13"		30.2
9	6.35 (d, J = 2.5Hz)	104.4	14"	1.36-1.50 (m)	32.6
10		159.4	15"		23.6
11	6.48 (d, J = 2.5Hz)	109.3	16"	0.97 (t, J = 6.9Hz)	14.4
12		143.2			
13	4.62 (d, J = 12.5Hz)	63.7	1"'		168.8
	4.70 (d, J = 12.2Hz)		2"'	6.03 (d, J = 15.5Hz)	122.0
			3"'	7.81 (dd, J = 15.1, 11.8Hz)	141.3
1'	5.14 (d, J = 2.5Hz)	110.2	4"'	6.28 (dd, J = 11.5, 11.5Hz)	127.8
2'	3.97 (dd, J = 4.4, 2.5Hz)	82.7	5"'	6.01 (m)	142.8
3'	4.08 (dd, J = 6.1, 4.7Hz)	77.7	6"'	2.52 (dt)	25.7
4'	3.90 (dd, J = 6.1, 2.5Hz)	84.9	7"'	1.50-1.70 (m)	37.5
5'	3.90-3.96 (m)	69.5	8"'	3.55 (m)	73.2
6'	4.21 (dd, J = 28.0, 11.7Hz)	67.8	9"'	1.43-1.64 (m)	31.2
	4.23 (dd, J = 32.5, 11.4Hz)		10"'	1.02 (t, J = 7.5Hz)	10.4
1"		168.8			
2"	6.05 (d, J = 15.5Hz)	121.4			
3"	7.39 (dd, J = 15.6, 10.8Hz)	146.7			
4"	6.40 (dd, J = 15.3, 11.1Hz)	131.9			
5"	6.23 (dt, J = 15.0, 7.2Hz)	141.8			

δ in ppm downfield from internal TMS. CD_3OD was used as solvent.

Scheme 2.

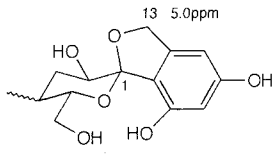


Furanocandin **1**

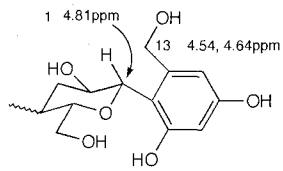
methyl singlet seen at 3.78 ppm was confirmed to be a methyl ester.

EI-MS data indicated the molecular weight of **3** as 198. ^{1}H NMR and ^{13}C NMR spectra disclosed 17 protons and 11 carbons. The carbons were two methyls, three methylenes, one oxy methine, four sp^2 methines and one carbonyl carbon. The molecular formula was thus considered to be $C_{11}H_{18}O_3$. Proton linkages were evident in the HH-COSY spectrum. From the spectrum, 3 appeared to be a C_{10}-normal fatty acid possessing one hydroxyl group and two unsaturated bonds. The two double bonds had the *trans* (H2"'-H3"', 15 Hz) and *cis* (H4"'-H5"', 11 Hz) configuration, respectively, based on the ^{1}H NMR coupling constant. A methyl singlet of 3.80 ppm indicated this fatty acid to be a methyl ester.

Fig. 1. H-1, H-13 chemical shifts of papulacandin B and chaetiacandin.



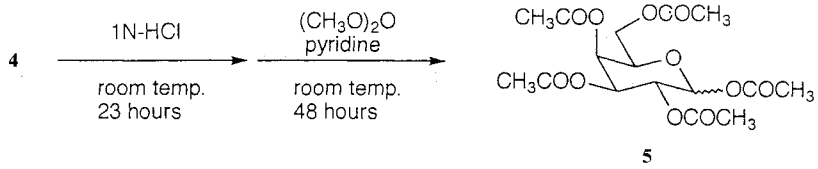
Papulacandin B



Chaetiacandin

The structure of compound **4** was investigated as follows. ^{1}H NMR and ^{13}C NMR spectra showed 18 protons and 19 carbons. The carbons were three oxy-

Scheme 3.



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methylenes, ten oxymethines, two sp^2 methine and four sp^2 quaternary carbons. Proton linkages were due to the HH-COSY and the selective proton decoupling spectra, showed compound **4** to possess two sugar units. The HMBC spectrum indicated compound **4** to have a dihydroxy benzyl alcohol. These components are common to all papulacandins, but the NMR spectra of **4** differed from those of known papulacandins. It is known that there are two connectivity types of the sugar unit to the benzyl alcohol. One type has a spirocyclic moiety such as papulacandin B[2], and the other, a benzyl alcohol group such as chaetiacandin[3]. The ^1H NMR spectrum is useful for distinguishing between them. The former type of compounds, H-13 methylene are observed around 5.0 ppm, and H-1 is not observed because of a spirocyclic bond. On the other hand, in later type of compounds, H-13 methylene are observed around 4.7 ppm, and H-1 is observed (Fig. 1).

In the ^1H NMR spectra of furanocandin and **4**, an AB-quartet assigned to H-13 methylene was observed at 4.59 and 4.70 ppm, and doublet assigned to H-1 was seen at 4.78 ppm. This result revealed that furanocandin is of the chaetiacandin type with no spirocyclic moiety.

The first sugar unit joined to the aromatic group was studied. Proton linkages were could be seen in the HH-COSY spectrum and the selective proton decoupling spectra, all coupling constants of H-1 through H-5 (*ca.* 10 Hz.) indicated the sugar unit to have the hexopyranosyl form. The configuration of each proton was *trans-diaxial* in all cases. This unit was thus considered to be 1-β-glucopyranose.

The second sugar unit was shown to have six carbons by the HH-COSY spectrum and the selective proton decoupling spectra. These proton and carbon chemical shifts differed from those of known papulacandins. ^{13}C NMR chemical shifts of this unit were compared with those of known methylglycosides. The chemical shifts of C-1′ to C-6′ agreed essentially with those of methyl β-galactofuranoside[9]. In the HMBC spectrum of **1**, the correlation from H-1 to C-4′ was observed. Furanocandin is thus shown to have a galactofuranosyl moiety differing from those of other papulacandins. To determine the absolute configuration of the galactofuranosyl moiety, compound **4** was acid hydrolyzed [1 N-HCl, room temperature, 23 hours] and acetylated [Ac_2O, pyridine, room temperature, 48 hours] to give penta-*O*-acetyl galactose (**5**) (Scheme 3).

Compound **5** was obtained as an α-, β- mixture (1 : 2). The ratio was appeared from the ^1H NMR data. The specific rotation of authentic penta-*O*-acetyl-α-D-galactopyranose is $+106.7°$ and β-form is $+25°$[10]. A specific rotation value ($+53°$) suggested the configuration of 5 as D-form.

The connection of three units which were revealed above, were established from the HMBC spectrum of furanocandin. The connection of benzyl alcohol to 1-β-glucopyranose was indicated by correlations between H-1 of glucose to C-7, C-8, C-12 of aromatic group. The long range correlations between H-1′ of galactose to C-4 of glucose showed the connection of 1-β-glucopyranose to β galactofuranose [$\beta(1\sim4)$].

The positions of two acyl-substituted hydroxy groups on each sugar were determined. In the HMBC spectrum of furanocandin, correlations between H-6′ of galactose to C-1‴ of short fatty acid, and H-3 of glucose to C-1″ of long fatty acid were observed. The long fatty acid **2** is thus shown to couple with the 3-hydroxyl group of glucose, and the short fatty acid **3** with the C-6′ hydroxyl group of galactose. The structure of furanocandin was thus indicated to be **1**.

Thus furanocandin is a new papulacandin. Its chemical structure differs in part from those of others. Furanocandin is the compound shown to possess a galactofuranosyl unit. The relationship between chemical structure and antifungal activity is a point of interest.

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