Discovery of Saricandin, a Novel Papulacandin, from a *Fusarium* Species

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We have identified a novel antifungal antibiotic of the papulacandin family,[1] saricandin (**1**), which was produced by the fungal culture, AB 2202W-161. PF-1042/BE-29602 (**2**), a previously described papulacandin,[2,3] was also present in the fermentation broth. These compounds were isolated by bioactivity guided fractionation using an assay[4] for agents which inhibit synthesis of the fungal cell wall. In this report we wish to describe the fermentation, isolation, characterization and biological properties of **1** and **2** (Fig. 1).

Saricandin is produced by *Fusarium* sp. AB 2202W-161. This fungus was isolated from a soil sample collected in a temperate forest in Nepal. A subculture of the microorganism was deposited in the permanent collection of the National Center for Agricultural Utilization Research, United States Department of Agriculture, 1815 North University Street, Peoria, IL, 61604, U.S.A. The accession number at this depository is NRRL 25158. The color names and numbers in parenthesis in the following strain description were taken from the ISCC-NBS Centroid Color Charts.[5]

Colonies of strain AB 2202W-161 grew slowly on potato-dextrose agar reaching 12 mm in diameter after 7 days at 20°C under continuous fluorescent light. Under these conditions the colonies were colored moderate yellowish brown (77) in the center with a dark orange yellow (72) outer edge. No soluble pigment was produced. There was dense growth of tangled aerial mycelia in the center of the colonies. No spores were seen after seven days, but macroconidia were produced abundantly as the culture aged. Macroconidia measured $25 \sim 38 \times 2.5 \sim 4\,\mu m$. They were three to four septate, fusiform, slightly curved with basal cells that were not distinctly foot shaped or notched. No microconidia were observed.

The active compounds were produced by solid state fermentation using Nabisco Shredded Wheat as the stationary support, a modification of the procedure described previously.[6] The producing strain was maintained as frozen mycelium at -70°C and was later used as the seed inoculum. The seed culture was prepared at 1% inoculum in 500-ml Erlenmeyer flasks containing 100 ml of a tomato paste-oat flour medium[7] and was incubated at 28°C for 72 hours on a rotary shaker operated at 225 rpm. Solid state fermentations were carried out in three 20-liter glass carboys, each containing 300 g of Nabisco Spoon Size Shredded Wheat. The fermentation medium consisted of 2.4% Dextrin (Staclipse JUB), 1.6% molasses, 1.6% Primatone CLT (Sheffield Product, Norwich, NY)), 0.4% Brewer's yeast (Wind Gap Farms, Baconton, GA), 0.16% $CaCO_3$, pH 7.0. Sixty ml of seed culture was added in a 500 ml flask containing 360 ml sterile fermentation medium. Subsequently, the inoculated medium was aseptically transferred to each carboy and mixed to achieve even distribution of the inoculum The carboy was incubated at 20°C for 21 days.

At harvest, 300 ml of acetone was added to each fermentation vessel. After six hours, 375 ml of ethyl acetate : toluene (1 : 1) was added to the vessel, which was then stored at 4°C for eighteen hours. The lysed fungal mat was steeped with two 6 liter volumes of ethyl acetate, followed by an 8 liter volume of methanol. After removal of the solvent, the oily residue was triturated in a sequence of solvents with increasing polarity, beginning with hexane, followed by ethyl acetate, methylene chloride, methanol and ending with water. The hexane and ethyl acetate triturates were pooled together (pool 1), as were the methanol triturates (pool 2). Pool 1 was chromatographed using preparative droplet countercurrent with a 2 : 4 : 3 : 2 system of H_2O - MeOH - EtOAc - hexane in an ascending mode. The active fractions were concentrated and the residue was chromatographed on an LH-20 column (2 cm × 120 cm) in 50% aqueous methanol and fractions of *ca.* 10 ml volume collected. Active fractions (52 ~ 61) were pooled and concentrated. The residue was purified by planetary coil centrifuge countercurrent using 2 : 4 : 5 : 1 system of H_2O - MeOH - $CHCl_3$ - hexane in the descending mode and yielded two

Fig. 1. Structures of saricandin (**1**) and PF-1042 (**2**).

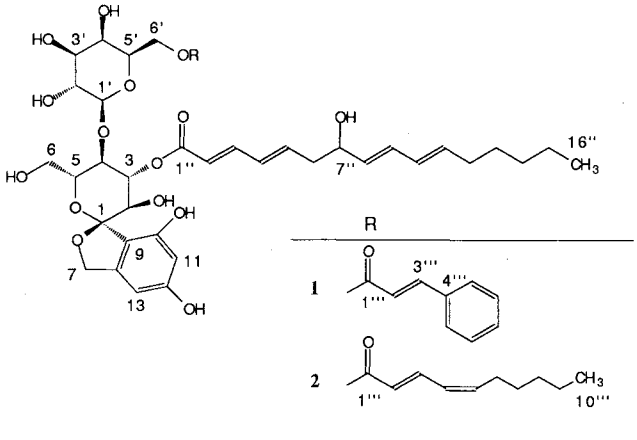


Table 1. ^{1}H NMR and ^{13}C NMR spectra of saricandin (**1**) and PF-1042 (**2**).

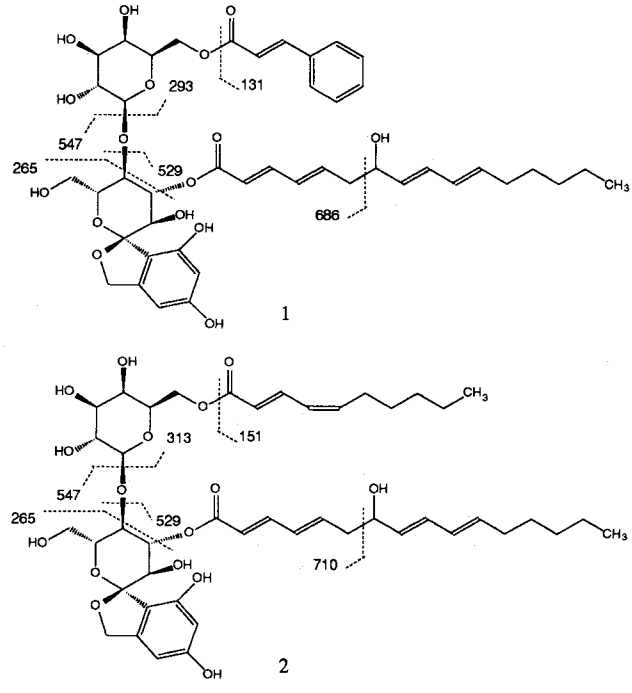
Position	1		2	
	C shift	H shift	C shift	H shift
1	111.9	—	111.9	—
2	71.9	4.40	71.9	4.36
3	76.4	5.44	76.4	5.41
4	77.8	4.02	77.8	3.95
5	74.7	4.02	74.7	4.00
6	61.6	3.80, 4.02	61.6	3.78, 3.78
7	73.9	5.02	73.9	5.02, 5.05
8	145.4	—	145.5	—
9	116.5	—	116.5	—
10	161.8	—	161.6	—
11	103.2	6.21	103.0	6.22
12	154.9	—	154.6	—
13	100.0	6.18	100.0	6.17
1′	105.4	4.38	105.4	4.34
2′	74.8	3.48	74.8	3.46
3′	72.6	3.48	72.5	3.46
4′	70.3	3.80	70.3	3.75
5′	74.0	3.73	73.9	3.67
6′	64.9	4.21, 4.30	64.7	4.24, 4.15
1″	169.0	—	169.0	—
2″	121.7	5.92	121.7	5.90
3″	146.0	7.28	146.0	7.27
4″	131.9	6.29	131.9	6.28
5″	141.2	6.13	141.2	6.15
6″	42.2	2.37	42.3	2.37
7″	72.6	4.13	72.6	4.15
8″	134.0	5.53	134.0	5.56
9″	132.1	6.14	132.1	6.17
10″	131.0	5.98	131.0	6.01
11″	136.1	5.69	136.0	5.67
12″	33.6	2.04	33.6	2.06
13″	30.1	1.37	30.1	1.39
14″	32.6	1.29	32.5	1.31
15″	23.5	1.31	23.5	1.31
16″	14.4	0.89	14.4	0.89
1‴	168.3	—	168.6	—
2‴	118.9	6.58	121.7	5.97
3‴	146.7	7.76	141.5	7.70
4‴	135.8	—	127.5	6.22
5‴	129.4	7.63	143.4	5.94
6‴	130.1	7.42	29.2	2.34
7‴	131.6	7.42	30.2	1.45
8‴	130.1	7.42	32.6	1.32
9‴	129.4	7.63	23.5	1.31
10‴			19.4	1.31

d_4-methanol was used as solvent. δ in ppm downfield form TMS.

active compounds, **1** (1.4 mg) and **2** (30 mg). Purification of pool 2 by similar partition and LH-20 chromatography resulted in an additional 22 mg of compound **2**. The structure determinations of compounds **1** and **2** (Fig. 1) were based on spectral interpretation of the ^{1}H and ^{13}C NMR experiments (Table 1) and mass spectral information (Fig. 2).

The sodium adduct of **1**, $[M+Na]^+ = m/z$ 861, was the predominant ion in the FAB-MS over the protonated molecular ion, $[M+H]^+ = m/z$ 839. The FAB high resolution peak match of the protonated molecular ion was measured at 839.3499 amu and corresponds to a formula of $C_{44}H_{55}O_{16}$ (calculated: 839.3490). The sodium adduct of **2**, $[M+Na]^+ = m/z$ 881, was the predominant ion in the FAB-MS spectrum. The FAB high resolution peak match of the protonated molecular ion measured the ion at 859.4109 amu and corresponds to a formula of $C_{45}H_{63}O_{16}$ (calculated: 859.4116).

Fig. 2. MS/MS daughter ions.



The ^{1}H and ^{13}C NMR data of the glycoside core of **1** are characteristic of papulacandin type compounds,[1] which are differentiated from one another by features occuring in the acyl lipid side chains. The 16-carbon unbranched ester at C-3 was characterized by COSY and HMBC/HMQC correlations. The COSY analysis of the spin system places the olefins at C-2″, C-4″, C-8″ and C-10″ which by the magnitude of their coupling constants (J=14.7 to 15.5 Hz) are assigned as *trans*. The hydroxyl is located on C-7″. Only the assignment of 14″-H and 15″-H required HMBC/HMQC correlations to eliminate ambiguity, due to signal overlap. The *trans*-cinnamyl ester at C-6′ was identified by its NMR resonances. Additional support for the structural assignments comes from the FAB MS/MS data. Daughter ions indicative of the side chains and their locations are observed in the spectra (B/E linked-scans) CAD $[M+H]^+$ (Fig. 2).

The ^{1}H and ^{13}C NMR spectral data of **2** are identical to those given for PF-1042 and BE-29602. The UV and IR data (not shown) are also identical. The 6′ acylating lipid is 2*E*,4*Z*-decadienoic acid. Daughter ions in the MS/MS fragmentation spectrum (Fig. 2) also support this structural assignment.

Table 2. Response of **1** and **2**, and related compounds, in the microtiter broth dilution in the presence and absence of 0.8 M sorbitol assay using *Candida albicans* CCH 442 and in the β-(1,3)-glucan synthase assay.

Compound	Broth dilution assay MIC at 2 day growth (μg/ml)		Glucan synthase assay IC_{50} (μg/ml)
	No sorbitol	With sorbitol	
PF-1042 (**2**)	0.49	>250	6.5
Saricandin (**1**)	7.80	>250	242.0
Fusacandin A	0.49	>250	15.0
Papulacandin B	0.98	>250	2.3
Amphotericin B	0.48	0.48	ND

The broth dilution assay was performed as described previously,[4] except that the final concentration of cells was 2×10^5 organisms/ml. The antifungal activity of PF-1042 was comparable to fusacandin A and papulacandin B while the activity of saricandin was weaker (Table 2). Sorbitol could rescue the growth of cells when treated with the papulacandin-related compounds indicating that these agents act on the fungal cell wall. The higher MIC of saricandin compared to the other papulacandin-related compounds was paralleled with a higher IC_{50} in the *in vitro* β-(1,3)-glucan synthase assay.[8]

Saricandin is a novel papulacandin by virtue of its unique combination of acylating lipids. A cinnamyl ester at the C-6′ has been previously reported in one instance.[9]

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