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PREPARATION AND ANTIFUNGAL ACTIVITY OF FUSACANDIN ANALOGS: C-6' SIDECHAIN ESTERS

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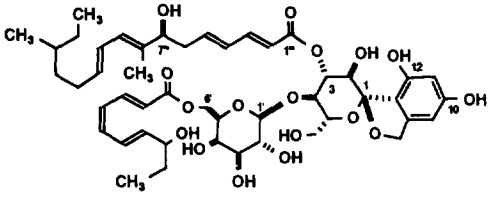
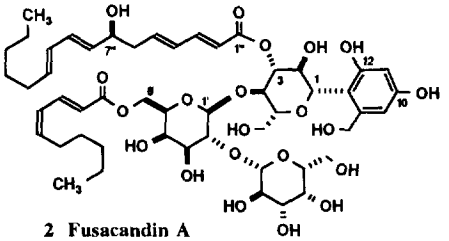
Abstract: The preparation of C-6' ester analogs of fusacandin (**2**) is described leading to derivatives exhibiting improved *in vitro* activity in the presence of mouse serum.

The papulacandins are a series of naturally occuring antifungal agents whose isolation and characterization were initially reported in 1977[1,2] and whose structures are typified by **1**. Related isolates have also been reported such as chaetiacandin which was isolated in 1985 and has a reduced anomeric center.[3] These compounds have been shown to inhibit the production of β-1,3-glucan, an essential fungal cell wall component which is not found in mammals and is, therefore, a target of significance and selectivity.[4]

Previously, several reports describing the synthesis of analogs of papulacandin have appeared,[5] though little work has been performed on the sidechains and none of these analogs exhibited significantly better activity than the parent compound. Although a number of these derivatives showed excellent inhibition of β-1,3-glucan synthase and whole cell activity, these compounds, as for **1**, had little or no efficacy in animal models of fungal infection. Whether a covalent (metabolic) inactivation or a non-covalent (binding) phenomenon was occuring was not described. In our hands, fermentations of papulacandin were complicated by the existence of various olefin congeners whose placement and geometry made the separation and purification of the individual components difficult. Furthermore, the instability of the electron-rich anomeric spiro-ketal at C-1 limits reaction conditions which can be used for modification of the molecule.



1 Papulacandin B

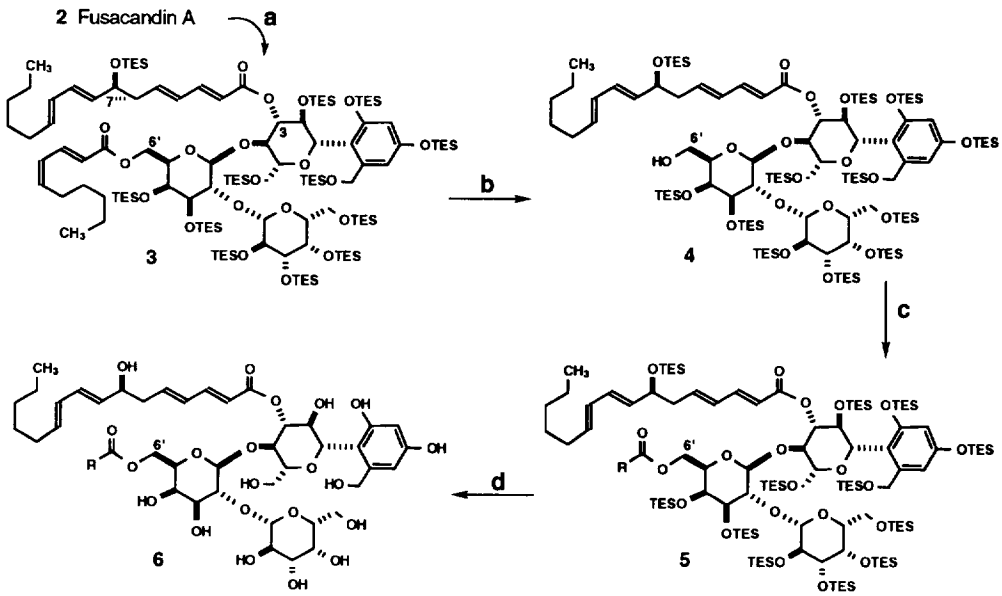


2 Fusacandin A

Recently, a trisaccharide analog named fusacandin A (**2**) has been isolated and characterized by workers at Abbott.[6] The structure of this molecule differs from that of papulacandin in three respects: (1) the C-1 position bears the more stable ether functionality as found in chaetiacandin; (2) an additional galactose ring is connected β-1,2 to the existing galactose thereby forming a novel trisaccharide array; and (3) the sidechains at C-3 and C-6' are less substituted.[7] While this new compound shared similar in vitro antifungal activity with papulacandin, it also shared the inactivity in animal models. Studies carried out at Abbott regarding this inactivation have determined that fusacandin was highly protein bound such that in the presence of serum its minimum inhibitory concentration (MIC) increased from 0.78 μg/mL to >100 μg/mL.[8] This effect most probably accounted for its poor in vivo activity.

In order to determine whether this non-selective binding could be circumvented through the modification of **2**, we embarked on a program to produce compounds which retain in vitro activity in the presence of serum. The C-3 and C-6' lipophilic sidechains appear necessary for activity (see below), and since it was assumed that the serum protein binding was probably related to these chains, we felt that these moieties were a key site for modification. Unfortunately, initial analogs whose C-6' sidechains carried amino, anilino, or carboxylic acid groups exhibited little or no in vitro antifungal activity;[9] therefore, we describe here the preparation and antifungal activity of several series of aliphatic and aromatic sidechain analogs of fusacandin bearing oxygen substituents.

Chemistry. Toward this end we attempted to acylate, alkylate, silylate, sulfonate, and ketalize fusacandin in a selective manner but could not attain useful chemical selectivity. Based on these unsatisfactory procedures, we decided to utilize a comprehensive protection-deprotection scheme employing triethylsilyl (TES) groups for fusacandin. This persilylation reaction proceeded smoothly giving persilylfusacandin **3**; however, alkaline hydrolysis of the C-6' sidechain of **3** failed, and forcing conditions effected loss of the phenolic silyl groups. Ultimately, we utilized a reductive deacylation (diisobutylaluminum hydride) to remove the 2,4-decadienoate chain and obtained **4** in good yield.[10] Compound **4** was actually the dodecasilyl derivative of the previously reported fusacandin B.[6] Various aromatic acids were prepared via standard Suzuki chemistry,[11] and acylation of persilylalcohol **4** with these residues was carried out using either acid/DCC coupling procedures or standard acid



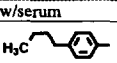
Scheme 1. (a) 25 eq. TESOTf, collidine, -15 °C, 2 h, 80%; (b) 2 eq. DiBAl, hexane, 10 min, -78 °C, 70%; (c) 3 eq. RCOCl, 20 eq. DMAP, $CHCl_3$, 25 °C, 2 h or RCO_2H, 0.5 eq. DMAP, 3 eq. DCC, toluene, 80 °C, 3 h, 20 - 90%; (d) 5% HF in CH_3CN, THF, 25 °C, 1.5 h, 50 - 80%.

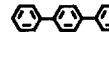
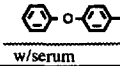
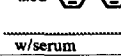
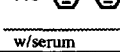
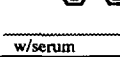
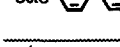
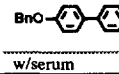
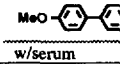
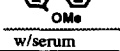
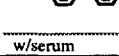
chloride/amine conditions and produced the new esters **5** at the C-6' position. Deprotection using either HF/pyridine or tetrabutylammonium fluoride succeeded in producing the desired C-6' analogs **6** which were isolated by silica gel chromatography and characterized as to their structure by ^1H NMR and high resolution mass spectral data and as to

their purity by reverse phase HPLC.[12]

Biological Activity. Our goal was not only to retain or improve the whole cell antifungal activity in these analogs but also to determine whether any structural variation of this chain would allow for activity in the presence of mouse serum. Minimum inhibitory concentrations (MICs) were determined in the presence or absence of serum (50% v/v) using microtiter broth dilution in Yeast Nitrogen Base (YNB) containing 1% glucose.[13] As shown for fusacandin B (**2b**) in Table 1, the absence of the C-6' sidechain caused a great loss in activity; however simple acyl derivatives such as the acetyl analog **6b** were also devoid of activity. As the chain was lengthened e.g. decanoyl compound **6c**, activity improved. The double bond stereochemistry was shown not to be important as *E,E*-isomer **6a** is equally active as its *E,Z*-isomer. Unfortunately, these analogs were inactive in the presence of serum.

Table 1. Minimum Inhibitory Concentrations of fusacandin analogs vs. *Candida albicans* strains

No.	R	MIC (mcg/mL) *Candida albicans* ATCC 10231	579a	CCH 442	ATCC 38247	ATCC 62376
1	Papulacandin B	0.2	0.78	0.78	0.39	0.39
	w/serum	>100	>100	>100	>100	>100
2 a	Fusacandin A	1.56	0.78	0.78	0.78	1.56
	w/serum	>100	>100	>100	>100	>100
2 b	Fusacandin B	50	25	25	>100	50
	w/serum	>100	>100	>100	>100	>100
6 a		1.56	0.78	1.56	1.56	3.12
	w/serum	>100	>100	>100	>100	>100
6 b	CH_3	>100	>100	>100	>100	>100
	w/serum	>100	>100	>100	>100	>100
6 c		12.5	12.5	6.25	12.5	12.5
	w/serum	>100	>100	>100	>100	>100
6 d		12.5	6.25	12.5	6.25	12.5
	w/serum	>100	>100	>100	>100	>100
6 e		3.12	0.78	3.12	1.56	1.56
	w/serum	>100	>100	>100	>100	>100
6 f		3.12	0.78	1.56	0.78	3.12
	w/serum	50	50	50	>100	25
6 g		3.12	3.12	3.12	1.56	6.25
	w/serum	50	50	50	50	100
6 h		1.56	0.39	0.39	0.78	1.56
	w/serum	25	25	25	25	25
6 i		3.12	3.12	3.12	6.25	3.12
	w/serum	50	100	50	100	100

No.	R	MIC (mcg/mL) *Candida albicans* ATCC 10231	579a	CCH 442	ATCC 38247	ATCC 62376
6 j		0.78	0.78	0.78	1.56	0.78
	w/serum	12.5	12.5	12.5	25	12.5
6 k		1.56	0.78	0.78	3.12	0.78
	w/serum	12.5	12.5	12.5	25	12.5
6 l		3.12	1.56	3.12	3.12	3.12
	w/serum	50	50	100	50	>100
6 m	Et 	0.78	0.2	0.78	0.78	0.39
	w/serum	25	25	12.5	25	25
6 n	MeO 	0.78	1.56	0.78	3.12	0.78
	w/serum	12.5	12.5	6.25	25	12.5
6 o	PrO 	0.78	0.78	0.78	0.78	0.78
	w/serum	12.5	25	6.25	25	12.5
6 p	*i*-PrO 	1.56	0.78	0.78	0.78	0.78
	w/serum	12.5	12.5	6.25	25	12.5
6 q	OctO 	25	25	25	50	12.5
	w/serum	100	100	100	>100	100
6 r	BnO 	1.56	1.56	3.12	3.12	1.56
	w/serum	25	25	12.5	25	25
6 s	MeO 	1.56	0.78	1.56	1.56	3.12
	w/serum	50	50	100	100	25
6 t	 OMe	6.25	1.56	6.25	6.25	1.56
	w/serum	50	50	50	100	50
6 u	HO 	12.5	6.25	6.25	6.25	12.5
	w/serum	>100	>100	>100	>100	>100

Replacing the diene chain with more rigid aromatic isosteres produced some promising results, thus several derivatives containing aromatic moieties in this sidechain were prepared. While the benzoyl analog **6d** showed poor activity, its corresponding *p*-butyl congener **6e** was almost as active as **2a**. Expanding the aromatic nucleus led to compounds **6f-i** which, while exhibiting similar activity as the *p*-butyl analog, were the first derivatives to present activity (<100 μg/mL) in presence of mouse serum assay. Tricyclic analog **6h** showed relatively good MIC's both with and without serum and pointed to biphenyl structures as a series worth studying.

Since the parent biphenyl compound **6j** exhibited good activity in serum, many analogs in this series were prepared. While the biphenyl and terphenyl compounds showed similar activity, the easier access of the former system led to its use as the template upon which optimization studies were performed. The ethylbiphenyl analog **6m** showed improved activity in this series and gave measurable MIC's in the serum assay.[14] The similarity in activity of the ethyl and methoxy substituents, **6m** and **6n**, respectively, indicated compatibility of the ether oxygen. Furthermore, since the hydroxy analog **6u** was quite accessible and, by virtue of its phenolic function, more easily modified than the corresponding alkyl-substituted analogs, we studied the ethers to a greater extent.

Lengthening of the alkoxy chain as for **6n - q** was compatible with retention of activity until the length approached that of the octyloxy analog **6q**. This effect was also seen in the terphenyl series.[9] Changing the position of the acyl or the methoxy substituent of **6n** led to ethers **6s** and **6t**, neither of which were more active than **6n**. The methoxybiphenyl analog **6n** was seen as the most active compound in this series and further study of this molecule in terms of its pharmacokinetics and in vivo activity is ongoing. Modification of the C-3 sidechain and the sugar moieties is also in progress.

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References and Notes

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7. The *(S)* absolute stereochemistry at C-7''' was established via degradation studies: methanolysis of **2**, followed by *(R)*-naphthylethylisocyanate, ozonolysis, and reduction to diol carbamate. This diol was compared to both *(R)*- and *(S)*-butanetriol carbamates prepared from commercially available materials . We appreciate HPLC help from G. Brill and Dr. R. Chen.
8. Dr. K. Marsh and Dr. H. W. Hua, unpublished results.
9. Klein, L. L.; Yeung, C. M. Unpublished results.
10. No removal of the C-3 sidechain occurs under these conditions probably due to the hindrance from proximal glycoside and TES groups. Under forcing conditions only 1,4-addition to this chain was observed.
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12. All compounds have been fully characterized by ^{1}H NMR, high resolution mass spectra, and HPLC purity >90%.
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14. During the deprotection of **6m** with TBAF, the C-4' ethylbiphenyl analog was produced presumably due to an acyl migration. This analog showed poor activity thereby establishing the importance of the positioning of this sidechain.

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