XYLOCANDIN: A NEW COMPLEX OF ANTIFUNGAL PEPTIDES

II. STRUCTURAL STUDIES AND CHEMICAL MODIFICATIONS

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Xylocandins A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_2 are new antifungal peptides isolated from *Pseudomonas cepacia* ATCC 39277. The molecular weights of the xylocandins were determined by fast atom bombardment mass spectrometry (A_1 m/z 1,215; A_2 1,199; B_1 1,229; B_2 1,213; C_1 1,097; C_2 1,081; D_1 1,083; D_2 1,067). Each xylocandin is a cyclic peptide containing glycine (1), serine (2), asparagine (1~3 residues), β-hydroxytyrosine (1), and an unusual amino acid with the formula $C_{18}H_{37}NO_5$ (1). Additionally A_1, A_2, D_1 and D_2 contain 2,4-diaminobutyric acid (1); A_1, B_1, C_1 and D_1 contain *erythro*-β-hydroxyasparagine (1); and A_1, A_2, B_1 and B_2 contain xylose (1). For each xylocandin pair, an *erythro*-β-hydroxyasparagine residue in the first component of the pair is thus replaced by an asparagine in the second component, accounting for the 16 dalton mass difference for each pair. Chemical modification of A_1 and A_2 at the diaminobutyric acid and β-hydroxytyrosine residues was used to probe structural requirements for activity.

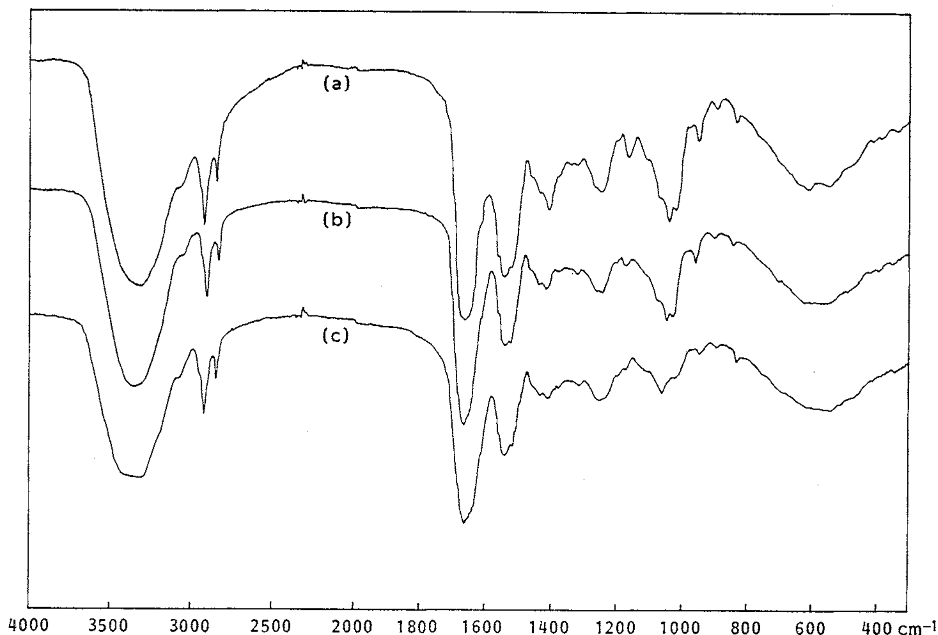
Xylocandin is a complex of novel peptide antibiotics produced by *Pseudomonas cepacia* exhibiting potent activity *in vitro* against dermatophytes and yeasts, including *Candida albicans*. Details of the discovery, isolation and biological activity of four pairs of xylocandin components (A_1 and A_2, B_1 and B_2, C_1 and C_2, and D_1 and D_2) are described in the preceding paper[1]. In this paper we describe the characterization of these eight components as well as chemical modifications of the A_1/A_2 pair designed to probe structural requirements for activity.

Efficient preparative-scale procedures for the separation of the two components of each xylocandin pair were not found, although small quantities of A_1 and A_2 could be isolated by preparative TLC as described below. Therefore, with the exception of some separate analyses for A_1 and A_2, all chemical and spectral analyses were carried out on the xylocandins as two-component mixtures, *i.e.* A_1/A_2, B_1/B_2, C_1/C_2 and D_1/D_2. Some analyses were not carried out on the D_1/D_2 pair due to insufficient quantities of this material.

Low resolution fast atom bombardment mass spectrometry (FAB-MS) of each xylocandin pair provided clean parent ion data for each component, indicating the following molecular weights: A_1 m/z 1,215; A_2 1,199; B_1 1,229; B_2 1,213; C_1 1,097; C_2 1,081; D_1 1,083; D_2 1,067. The IR and ^1H NMR spectra for the A_1/A_2, B_1/B_2 and C_1/C_2 pair are shown in Figs. 1 and 2, respectively. UV absorbance data is shown in Table 1.

The total acid hydrolysate (6 N HCl, 18 hours, 105°C) of each xylocandin pair was analyzed and the results are summarized in Table 2. For each xylocandin pair, integral ratios of glycine, serine, and, where present, 2,4-diaminobutyric acid were found. Non-integral values were recorded for aspartic acid, however. These values fell between one and two residues (relative to glycine) for A_1/A_2 and D_1/D_2, and between two and three residues (relative to glycine) for B_1/B_2 and C_1/C_2. *Erythro*-

Fig. 1. IR spectra of xylocandins A_1/A_2 (a), B_1/B_2 (b) and C_1/C_2 (c) in KBr.



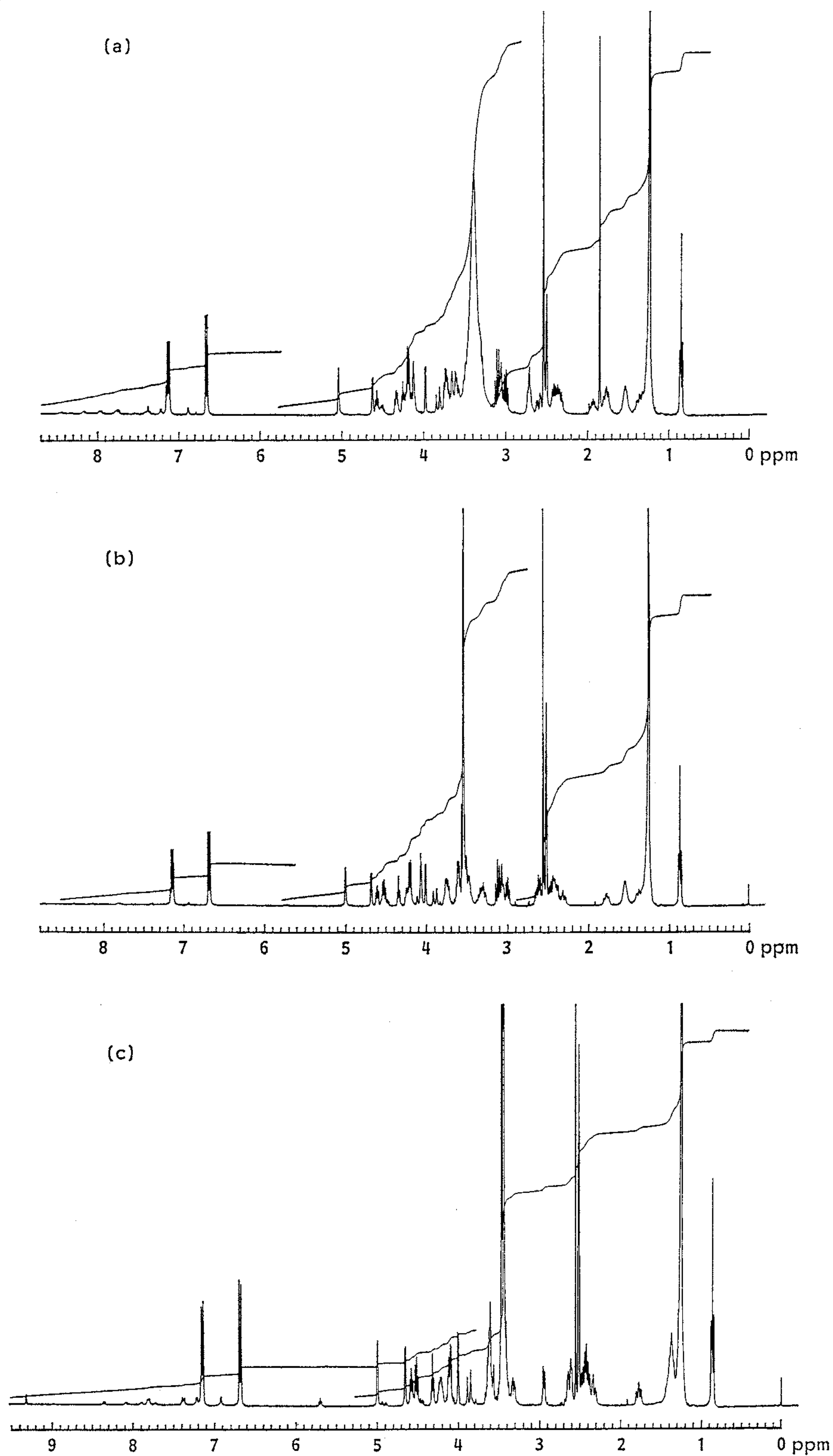
β-hydroxyaspartic acid was detected in the total hydrolysate of each xylocandin pair. The *erythro* configuration of this amino acid was established unambiguously by TLC and ^{1}H NMR comparison of the amino acid with authentic samples of both *erythro*- and *threo*-β-hydroxyaspartic acid.

Additionally a novel, polyhydroxy amino acid of molecular weight 347 was found in the total hydrolysate of each xylocandin pair. High resolution FAB-MS established the molecular formula as $C_{18}H_{37}NO_5$ (found 348.2749, calcd 348.2750 for the $(M+H)^+$ ion). Detailed mass spectrometric studies of the amino acid and its corresponding methyl ester, produced by treatment of the amino acid with methanolic HCl, aided in assigning a structure (excluding stereochemistry) for this xylocandin component (Fig. 3). The fragmentation of the amino acid and its methyl ester observed in chemical ionization (CI) and FAB-MS/MS spectra along with the accurate mass measurements of several of these fragments are depicted in Fig. 3a. The MS/MS spectrum of the C-11 to C-18 (129 dalton) fragment illustrated in Fig. 3b indicated a linear hydrocarbon chain for the terminal section of the amino acid. The lack of hydrocarbon branching was supported by the ^{1}H NMR spectra of the parent xylocandins (Fig. 2) which in each case show a single terminal methyl group. The MS/MS spectra of the carboxy-containing 102 and 146 dalton fragments are shown in Fig. 3c.

Xylocandin pairs A_1/A_2, B_1/B_2 and C_1/C_2 were analyzed for carbohydrate content after mild acid hydrolysis (1 N HCl, 100°C, 3 hours). Xylose, identified by electrophoresis and HPLC, was present in the hydrolysates of A_1/A_2 and B_1/B_2 but no carbohydrate was detected in the C_1/C_2 hydrolysate.

The ^{1}H NMR data for A_1/A_2, B_1/B_2 and C_1/C_2 indicated the presence of an aromatic residue which had not been identified from the amino acid analyses of the acid hydrolysates. The coupled doublet resonances near 7.1 ppm (J=8.4 Hz) and 6.7 ppm (J=8.4 Hz) as well as coupled doublet resonances near 5.0 ppm (J=2.5 Hz) and 4.1 ppm (J=2.5 Hz) suggested a β-hydroxytyrosine residue. Confirmation of its presence was obtained by chemical means. Thus, treatment of each of A_1/A_2, B_1/B_2 and C_1/C_2 with sodium hydroxide in water or dimethyl sulfoxide liberated *p*-hydroxybenzalde-

Fig. 2. ^{1}H NMR spectra (400 MHz) of xylocandins A_1/A_2 (a), B_1/B_2 (b) and C_1/C_2 (c) in DMSO-d_6-D_2O, 19 : 1.



hyde by a retro-aldol cleavage[2]. Further, treatment of each of A_1/A_2, B_1/B_2 and C_1/C_2 with neat trifluoroacetic acid effected a smooth dehydration[3] ($T_{1/2}$ *ca.* 10 minutes at room temperature) of the β-hydroxytyrosine residue (Fig. 4) as evidenced by changes in the UV and mass spectra (Table 3). In trifluoroacetic acid solution, λ_{max} values underwent bathochromic shifts to 308~311 nm with large increases in the log ε values. This new UV maximum is at the same wavelength as that for methyl *p*-hydroxycinnamate, as expected for this unsaturated chromophore[4]. A comparison of the UV spectral data under neutral and basic conditions for methyl *p*-hydroxycinnamate and the anhydro derivative of xylocandin B_1/B_2, **1b**, is shown in Table 4. FAB-MS data (Table 3) confirmed the mono-dehydration of each component pair. More direct evidence for the presence of a β-hydroxytyrosine residue was obtained by treatment of xylocandin B_1/B_2 with triethylsilane and trifluoroacetic acid (1 : 9 mixture, 1 hour, 20°C) which gave a mixture of reduction[5] and dehydration products. Acid hydrolysis of this mixture produced tyrosine. β-Hydroxytyrosine has been identified previously in the glycoprotein cutinase[6], and in modified form in vancomycin-type antibiotics[2] and two monobactam antibiotics[7].

Table 1. UV absorbance data of xylocandins.

Xylocandin	λ_{max}^{DMSO} nm (log ε)
A_1/A_2	278 (3.16), 284 (sh, 3.09)
B_1/B_2	278 (3.25), 284 (sh, 3.19)
C_1/C_2	277.5 (3.21), 283 (sh, 3.14)

Treatment of the xylocandin pair A_1/A_2 with a β-xylosidase from *Charonia lampas* afforded a corresponding pair of products with molecular weights *m/z* 1,083 and 1,067 (FAB-MS) that were chromatographically identical to the xylocandin trace component pair D_1/D_2. Thus, D_1/D_2 is the aglycone of A_1/A_2.

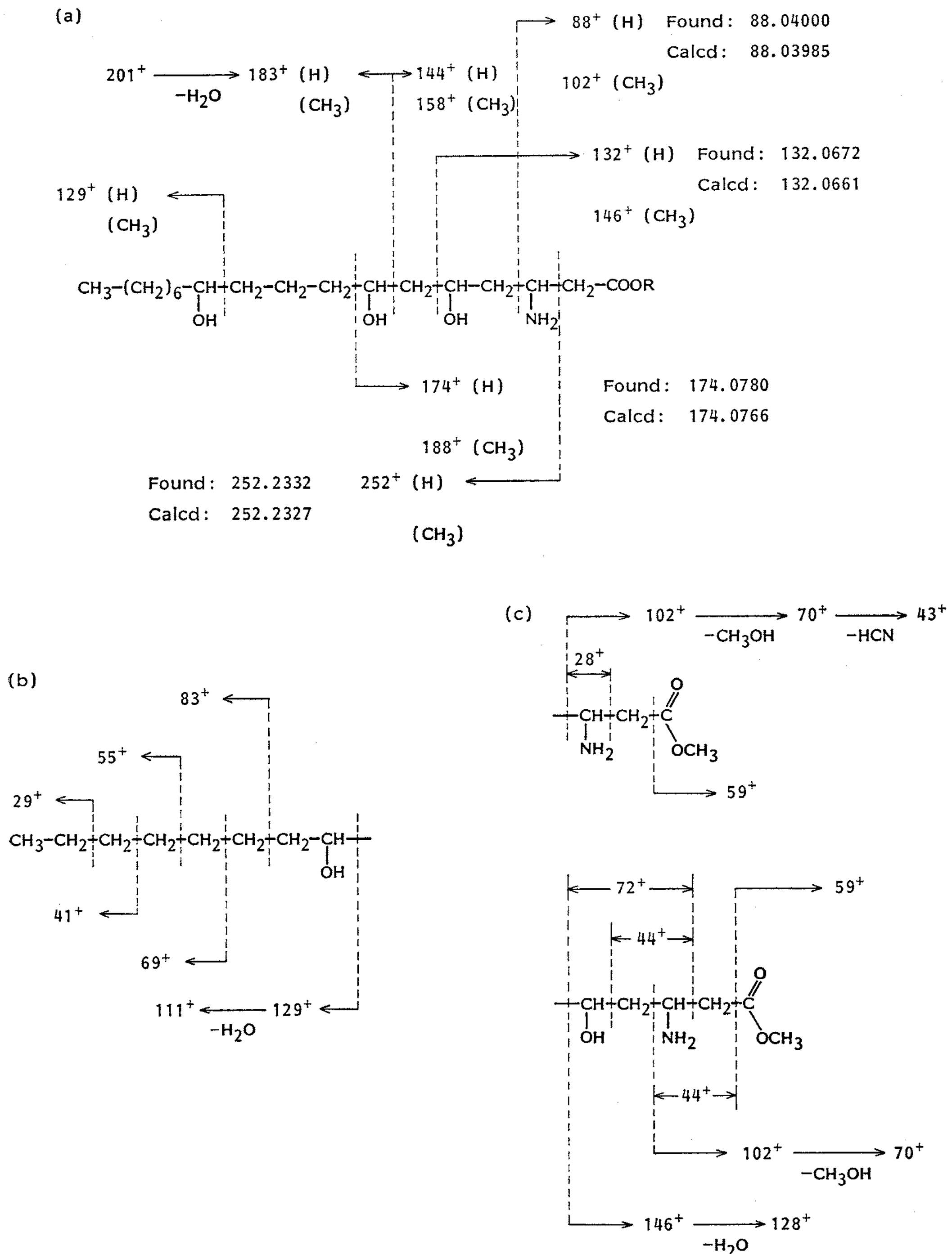
Isolation of small quantities of the separate A_1 and A_2 components was accomplished by preparative TLC on silica gel eluting with 2-propanol - concentrated ammonia - water, 4 : 2 : 1. The bands were visualized by spraying the plate with water to reveal white "anti-wetting" zones, and the compounds were eluted from the silica gel with dimethyl sufloxide. Bioautography on *Candida albicans* SC5314 demonstrated approximately equivalent activities for each of the two components. FAB-MS analysis of each component indicated a molecular weight of *m/z* 1,215 (*i.e.* xylocandin A_1) for the lower Rf component and a molecular weight of *m/z* 1,199 (*i.e.* xylocandin A_2) for the higher Rf component. Total acid hydrolysis of A_1 afforded glycine, serine, aspartic acid, 2,4-diaminobutyric acid, *erythro*-β-hydroxyaspartic acid and the $C_{18}H_{37}NO_5$ amino acid. Total hydrolysis of A_2 afforded glycine, serine, aspartic acid, 2,4-diaminobutyric acid and the $C_{18}H_{37}NO_5$ amino acid.

Table 2. Analyses of total acid hydrolysates of xylocandins.

Amino acid	Number of residues			
	A_1/A_2	B_1/B_2	C_1/C_2	D_1/D_2
Glycine	1	1	1	1
Serine	2	2	2	2
2,4-Diaminobutyric acid	1	0	0	1
Aspartic acid	(a)	(b)	(b)	(a)
erythro-β-Hydroxyaspartic acid	(c)	(c)	(c)	(c)
$C_{18}H_{37}NO_5$ Amino acid	(c)	(c)	(c)	(c)

(a): Non-integral, between 1 and 2 residues.
(b): Non-integral, between 2 and 3 residues.
(c): Amino acid identified but not quantitated.

Fig. 3. MS/MS spectra of the $C_{18}H_{37}NO_5$ amino acid and its methyl ester (a) and of several fragments (b and c).



The explanation for the 16 dalton molecular weight difference between the two A components is therefore the presence of an *erythro*-β-hydroxy-Asx residue in A_1 which corresponds to an Asx residue in A_2. The non-integral aspartic acid content in the total acid hydrolysate of A_1/A_2 (*vide*

Table 3. Treatment of xylocandins with TFA.

Xylocandin	Anhydro product **1**		
	Compound	UV λ_{max}^{TFA} nm (log ε)	MW[a] (*m/z*)
A_1/A_2	**a**	311 (4.10)	1,197/1,181
B_1/B_2	**b**	308 (4.17)	1,211/1,195
C_1/C_2	**c**	308 (4.12)	1,079/1,063

[a] Determined by FAB-MS.

Fig. 4.

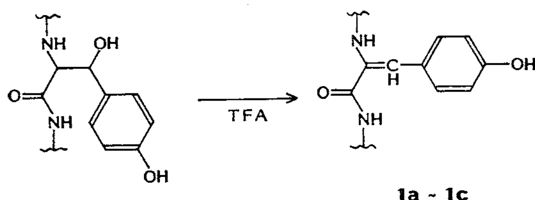


Table 4. UV comparison of xylocandin B_1/B_2 derivative **1b** with methyl *p*-hydroxycinnamate.

Compound	Solvent	λ_{max} nm (log ε)
Methyl *p*-hydroxy-cinnamate	MeOH	311 (4.33)
	0.01 M NaOH in MeOH	358 (4.45)
1b	DMSO - H_2O (7 : 3)	311 (4.28)
	0.01 M NaOH in DMSO - H_2O (7 : 3)	357 (4.35)

Table 5. Composition of xylocandins.

Component	Xylocandin							
	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
Glycine	1	1	1	1	1	1	1	1
Serine	2	2	2	2	2	2	2	2
Asparagine	1	2	2	3	2	3	1	2
erythro-β-Hydroxyasparagine	1	0	1	0	1	0	1	0
2,4-Diaminobutyric acid	1	1	0	0	0	0	1	1
β-Hydroxytyrosine	1	1	1	1	1	1	1	1
$C_{18}H_{37}NO_5$ Amino acid	1	1	1	1	1	1	1	1
Xylose	1	1	1	1	0	0	0	0

supra) is consistent with an Asx con tent in A_2 of two residues and in A_1 of one residue along with one residue of *erythro*-β-hydroxy-Asx. As in the A_1/A_2 pair, the two components of each of the B_1/B_2 and C_1/C_2 pairs differ in molecular weight by 16 daltons and the total hydrolysis of each pair afforded *erythro*-β-hydroxyaspartic acid along with non-integral values for aspartic acid (*vide supra*). Consistent with the above data is an Asx content in B_2 and C_2 of 3 residues each and in B_1 and C_1 of 2 residues each along with one residue of *erythro*-β-hydroxy-Asx.

A complete description of the compositional content of all eight xylocandins consistent with all analytical data obtained to date is shown in Table 5. Correlation of composition with the observed molecular weights for the various xylocandins requires for each component a cyclic structure and the assignment of asparagine and *erythro*-β-hydroxyasparagine for the Asx and *erythro*-β-hydroxy-Asx content. The lack of fragment ions in the FAB-MS of the xylocandins also supports cyclic structures[8]. High resolution FAB-MS measurements (Table 6) for each xylocandin are in good agreement with the calculated values for the molecular formulae derived from the above compositions. β-Hydroxyasparagine has previously been identified in vitamin K-dependent protein S[9].

The γ-amino function of the 2,4-diaminobutyric acid residue in A_1/A_2 was determined to be a free

Table 6. Accurate mass measurements (FAB-MS) of xylocandins.

Xylocandin	Formula	m/z (M+H)$^+$	
		Calcd	Expt (Δ ppm)
A_1	$C_{52}H_{86}N_{11}O_{22}$	1,216.5949	1,216.6015 (5.4)
A_2	$C_{52}H_{86}N_{11}O_{21}$	1,200.5999	1,200.6033 (2.8)
B_1	$C_{52}H_{84}N_{11}O_{23}$	1,230.5741	1,230.5773 (2.6)
B_2	$C_{52}H_{84}N_{11}O_{22}$	1,214.5791	1,214.5867 (6.3)
C_1	$C_{47}H_{76}N_{11}O_{19}$	1,098.5320	1,098.5259 (−5.6)
C_2	$C_{47}H_{76}N_{11}O_{18}$	1,082.5370	1,082.5347 (−2.0)
D_1	$C_{47}H_{78}N_{11}O_{18}$	1,084.5525	1,084.5531 (0.5)
D_2	$C_{47}H_{78}N_{11}O_{17}$	1,068.5576	1,068.5570 (−0.5)

Table 7. Anticandidal activity *in vitro* of xylocandin A_1/A_2 derivatives compared to xylocandin A_1/A_2.

Organism	SC No.[a]	MIC (μg/ml)				
		A_1/A_2	Derivative			
			1a	**2a**	**2b**	**2c**
Candida albicans	5314	0.4	6.3	>100	50	0.8
C. albicans	9177	0.2	12.5	>100	100	0.8
C. albicans	11422	0.4	12.5	>100	>100	0.8
C. albicans	10580	0.2	6.3	>100	100	0.8
C. albicans (bacilysin^R)[b]	12734	0.2	6.3	>100	50	0.8
C. tropicalis (amphotericin B^R)[b]	9861	0.2	6.3	>100	>100	0.8
C. tropicalis	10597	0.05	6.3	>100	50	0.2
C. krusei	2968	0.4	12.5	>100	>100	1.6
C. parakrusei	2621	0.4	12.5	>100	25	0.2
C. parakrusei	2966	0.4	25	>100	>100	0.4
C. pseudotropicalis	11241	0.1	12.5	>100	100	0.4
C. guilliermondii	2210	0.4	12.5	>100	>100	0.8
C. stellatoidea	2211	0.4	12.5	>100	>100	0.2
C. glabrata	9342	0.1	12.5	>100	100	0.4
C. glabrata	11267	0.2	25	>100	100	1.6

[a] Squibb culture.
[b] Bacilysin^R; bacilysin-resistant, amphotericin B^R; amphotericin B-resistant.

basic site by the following method. A_1/A_2 was treated with 2,4-dinitrofluorobenzene (*N,N*-dimethylformamide, triethylamine) to afford a yellow solid which was subjected to total acid hydrolysis. By TLC, 2,4-diaminobutyric acid was absent and a single new yellow component was detected that corresponded in Rf to authentic γ-dinitrophenylated 2,4-diaminobutyric acid and that differed in Rf from the authentic α-dinitrophenylated amino acid (silica gel impregnated with copper(II) acetate, eluting with ethyl acetate - pyridine - acetic acid - water, 60 : 20 : 6 : 11).

Three derivatives at this γ-amino site of xylocandin A_1/A_2 were prepared (Fig. 5) to determine the effect on anticandidal activity. *N*-Acetylation was effected with *p*-nitrophenylacetate to provide **2a**. The *N*-succinylated derivative **2b** was prepared using succinic anhydride in *N,N*-dimethylformamide. *N*-Formimidination to provide **2c** was carried out using benzylformimidate hydrochloride in *N,N*-dimethylformamide.

The anticandidal activities of these three derivatives along with that of the anhydro derivative of xylocandin A_1/A_2 (**1a**, Fig. 4) are shown in Table 7. The basic *N*-formimidoyl derivative **2c** displays activity *in vitro* similar to the parent compounds A_1/A_2, while the neutral *N*-acetyl (**2a**) and acidic *N*-succinyl (**2b**) derivatives display little or no activity. Preservation of the basic site of A_1/A_2 is thus

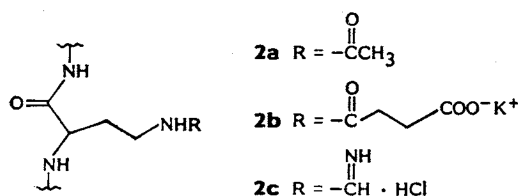


Fig. 5. Xylocandin A_1/A_2 derivatives.

Table 8. Effect of serum on anticandidal activity of xylocandin A_1/A_2 derivatives.

Derivative	MFC (μg/ml)[a]	
	Standard assay medium	Standard assay medium+human serum (1 : 1)
1a	8.0	50
2c	0.4	25

[a] Against *Candida albicans* SC5314.
MFC: Minimum fungicidal concentration.

seen as critical for maintaining potent activity. The anhydro derivative **1a**, resulting from dehydration of the β-hydroxytyrosine moiety of A_1/A_2, was much less active than the parent compound. Unfortunately, both **1a** and **2c**, like the parent A_1/A_2[1)], suffered substantial loss of activity in serum (Table 8).

Experimental

Most low and high resolution FAB-MS were determined on a VG-ZAB-2F mass spectrometer. Chemical ionization mass and MS/MS spectra were recorded using a Finnigan TSQ-4600 mass spectrometer. Some FAB-MS/MS spectra were also obtained on this instrument. IR spectra were recorded on a Perkin-Elmer Model 983 IR spectrophotometer. UV spectra were measured on a Perkin-Elmer Model 202 spectrophotometer. ^1H NMR spectra were recorded using a Jeol GNM-GX400 spectrometer. TLC's were run on Merck Silica gel 60 F_{254} plates eluting with 2-PrOH - concd NH_4OH - H_2O, 4 : 2 : 1 (System I) and with the upper phase of BuOH - AcOH - H_2O, 4 : 1 : 5 (System II).

Amino Acid Analysis

Glycine, serine, aspartic acid and 2,4-diaminobutyric acid were analyzed by conventional Stein-Moore analysis (Sequemat, Inc., Watertown, MA). For identification of *erythro*-β-hydroxyaspartic acid, the aqueous solution of the total hydrolysate was separated from some insoluble material and was concentrated *in vacuo*, redissolved in water and lyophilized. ^1H NMR analysis of the lyophilate in D_2O using dioxane as internal standard (3.75 ppm) revealed a pair of doublet resonances at 4.72 and 4.56 ppm (J=2.6 Hz for each), identical to the spectrum of the HCl salt of authentic *erythro*-β-hydroxyaspartic acid. TLC analysis (System I) revealed a ninhydrin-positive spot at Rf 0.32, identical in color and Rf to authentic *erythro*-β-hydroxyaspartic acid. (The Rf of authentic *threo*-β-hydroxyaspartic acid in System I was 0.43). The insoluble material from the total hydrolysate was found to contain the novel $C_{18}H_{37}NO_5$ amino acid (Rf 0.70, System I; 0.47, System II).

Identification of Xylose

The mild acid (1 N HCl, 100°C, 3 hours) hydrolysate solution was passed successively through short columns of Bio-Rad AG-1 (acetate form) and AG MP-50 (H^+ form) ion exchange resins. The final eluent was analyzed by HPLC using a Waters μBondapak-NH_2 column, eluting with CH_3CN - H_2O, 85 : 15, at 1.5 ml/minute and detecting at 192 nm. For xylocandins A_1/A_2 and C_1/C_2, the peak eluting with a retention time of 4.61 minutes corresponded to either xylose or lyxose based on coinjection with the authentic pentoses. Electrophoretic analysis on Whatman CHR-20 paper using 0.05 M sodium borate at 2,000 V clearly distinguished the two pentoses and established xylose as the carbohydrate component.

Xylocandin A_1/A_2: Anhydro Derivative (1a)

A solution of 195 mg of xylocandin A_1/A_2 in 20 ml of TFA was stirred for 2 hours at room temp. Toluene (80 ml) was then added and the solution was concentrated to dryness *in vacuo*. The residue was dissolved in 0.5 ml of DMSO and precipitated by the rapid addition of 7 ml of EtOAc. The solid

was washed with EtOAc and dried *in vacuo*. The product was chromatographed on Diaion CHP20P reverse phase resin (Mitsubishi Chemical Industries Limited) eluting with a water to DMSO gradient containing 5% AcOH. The appropriate fractions were combined and concentrated to a small volume. The product was precipitated with EtOAc and dried, and then dissolved in water and lyophilized to afford 58 mg of derivative **1a**: FAB-MS *m/z* $(M+H)^+$ 1,198, 1,182; UV λ_{max} nm (log ε) 312 (4.32) in DMSO - H_2O (7:3) and 357 (4.36) in 0.01 N NaOH in DMSO - H_2O (7:3); ^1H NMR (DMSO-d_6 - D_2O, 19:1) δ 7.18 (1H, s, Ar-C*H*=C), absence of doublet resonances at 5.04 and 4.11.

Xylocandin A_1/A_2 Aglycone: Enzymatic Removal of Xylose

A suspension of 53 mg of xylocandin A_1/A_2 in 20 ml of H_2O containing 2 ml of 0.1 M sodium acetate buffer, (pH 4) 348 mg of NaCl, 5 μl of toluene, and 42 mg of a crude β-xylosidase preparation (mixed glycosylases from *C. lampas*, Miles Scientific) was stirred at 37°C. Both starting material and product were largely insoluble in the aqueous reaction medium. The reaction was monitored by TLC after dissolving aliquots of the reaction mixture solids in DMF. After 48 hours the reaction mixture was charged with additional crude β-xylosidase (22 mg). After a further 24 hours, the solids were collected and then resubmitted to fresh reaction conditions, as described above. After a further five days, TLC revealed only a trace of starting material remaining. The solids were separated and chromatographed on a column of Diaion CHP20P reverse phase resin, eluting with a linear gradient starting with DMSO - AcOH - H_2O, 13:1:6, and ending with DMSO - AcOH, 19:1. The appropriate fractions were combined and concentrated to a small volume and the product was precipitated by the rapid addition of EtOAc. The solid was separated, washed with EtOAc and dried *in vacuo* to afford 12.5 mg of xylocandin A_1/A_2 aglycone: Rf 0.64, 0.67 (System I); FAB-MS *m/z* $(M+H)^+$ 1,068, 1,084.

Xylocandin A_1/A_2: *N*-Acetyl Derivative (**2a**)

p-Nitrophenylacetate (7.5 mg, 41 μmol) in 100 μl of EtOAc was added to xylocandin A_1/A_2 (51 mg) in 5 ml of H_2O. The pH was adjusted to 7.5 with 1 M $NaHCO_3$ and the mixture was stirred for 8.5 hours at room temp. The pH was then lowered to 4.0 with 1 N HCl and the aqueous mixture was washed three times with EtOAc. The aqueous mixture was lyophilized and the resulting solid was triturated with water. To remove residual starting material, the product was dissolved in a small volume of DMSO and passed through a column of Bio-Rad AG MP-50 (H^+ form) ion exchange resin eluting with a stepwise water to DMSO gradient. The appropriate fractions were combined and concentrated to a small volume and the product was precipitated by the rapid addition of EtOAc. The solid was washed with EtOAc and dried *in vacuo* to afford 27.7 mg of the desired *N*-acetyl derivative: FAB-MS *m/z* $(M+H)^+$ 1,242, 1,258; ^1H NMR (DMSO-d_6 - D_2O, 19:1) δ 1.75 (3H, s, $NCOCH_3$).

Xylocandin A_1/A_2: *N*-Succinyl Derivative (**2b**)

A solution of 81.5 mg of xylocandin A_1/A_2, diisopropylethylamine (4.6 μl, 26.4 μmol), succinic anhydride (88 μl of a freshly made 0.101 M solution in DMF, 8.9 μmol) and a catalytic amount of *N*,*N*-dimethylaminopyridine in 8 ml of DMF was stirred at room temp for 2 hours. The reaction mixture was concentrated *in vacuo* to a small volume and the product was precipitated by the rapid addition of diethyl ether. The precipitate was washed with ether and dried *in vacuo*. The solid was dissolved in a small volume of DMF - H_2O, 1:1, and passed through a column of Bio-Rod AG MP-50 (K^+ form) ion exchange resin eluting with DMF - H_2O, 3:7. The appropriate fractions were combined and concentrated to a solid that was dissolved in water and lyophilized to afford 40 mg of the *N*-succinyl xylocandin A_1/A_2 derivative as the potassium salt: Rf 0.59, 0.63 (System I); FAB-MS *m/z* $(M+H)^+$ 1,338, 1,354; ^1H NMR (DMSO-d_6 - D_2O, 19:1) δ 2.2~2.4 (4H, m, $COCH_2CH_2CO$).

Xylocandin A_1/A_2: *N*-Formimidoyl Derivative (**2c**)

A solution of 106 mg of xylocandin A_1/A_2, benzylformimidate hydrochloride (299 mg, 1.74 mmol), and diisopropylethylamine (0.61 ml, 3.18 mmol) in 30 ml of DMF was stirred at room temp for 30 minutes and then at 5°C overnight. The reaction mixture was concentrated *in vacuo* to a small volume and CH_3CN was added in a fast stream. The resultant precipitate was washed with CH_3CN and dried

in vacuo to afford 76 mg of solid. The solid was dissolved in a small volume of DMSO and chromatographed on a column of Sephadex G-10 (pre-swollen in DMSO), eluting with DMSO. The relevant fractions were combined and concentrated to a small volume. EtOAc was added in a fast stream and the resultant precipitate was washed with EtOAc and dried *in vacuo* to afford 66 mg of the *N*-formimidoyl xylocandin A_1/A_2 derivative (HCl salt): Rf 0.53, 0.58 (System I); FAB-MS *m/z* (free base+H^+) 1,227, 1,243; ^{1}H NMR (DMSO-d_6 - D_2O, 19 : 1) δ 7.86 (1H, s, *H*C(NH)N).

Conclusions

The eight xylocandins have been characterized as novel lipopeptide and glycolipopeptide antifungal antibiotics. Chemical modification of the most active xylocandin pair, A_1/A_2, demonstrated that maintenance of a basic site at the 2,4-diaminobutyric acid residue was crucial to its potent anticandidal activity.

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