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Fumimycin: A Peptide Deformylase Inhibitor with an Unusual Skeleton Produced by *Aspergillus fumisynnematus*

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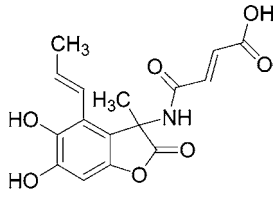
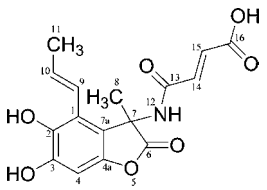
ABSTRACT



Fumimycin (**1**)

Fumimycin, an unusual metabolite incorporating an unusual alanine unit linked to a phenyl group at the α-carbon with both lactone and amide moieties, was isolated from cultures of *Aspergillus fumisynnematus*. Its structure was established by spectral analysis. Fumimycin was found to inhibit *Staphylococcus aureus* peptide deformylase with an IC_{50} value of 4.1 μM and also showed antibacterial activity against *S. aureus*.

Emergence of bacterial resistance to all known classes of antibiotics is a serious threat to humans, and continued discovery of new antibiotics with novel modes of action is critical to overcoming resistant bacteria. Bacterial genomics has revealed a plethora of previously unknown targets of potential use in the discovery of novel antibacterial drugs.[1] One novel antibacterial target that has received an increasing amount of attention lately is the bacterial peptide deformylase (PDF) (EC 3.5.1.31).[2] PDF, a member of a unique subclass of metalloenzymes, catalyzes the removal of the formyl group at the N-terminus of bacterial proteins. PDF is essential for bacterial growth, but not required by mammalian cells, which potentially makes it possible to identify a selective mechanism-based antibacterial agent without toxicity. Recent studies from several research groups have shown that PDF inhibitors act as broad-spectrum antibacterial agents.[2a,3] However, only few PDF inhibitors have been reported so far, and most of them are peptidic.[4]



In the course of screening for PDF inhibitors as potential leads to mechanism-based antibacterial agents, a novel metabolite named fumimycin (**1**) was isolated from the fermentation broth of *Aspergillus fumisynnematus* F746. It has an unprecedented skeleton and shows inhibition of *S. aureus* PDF with an IC_{50} of 4.1 μM. In this communication,

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we present the isolation, structure determination, and activities of fumimycin, an unusual metabolite incorporating an unusual alanine unit linked to a phenyl group at the α-carbon with both lactone and amide moieties.

Aspergillus fumisynnematus F746 was isolated from a soil sample collected in Buyeo-city, Chungcheongnam-do, Korea.[5] The culture broth (12 liters) was extracted with ethyl acetate, and the extract was concentrated and chromatographed on silica gel with $CHCl_3$−MeOH on Sephadex LH-20 with MeOH, and by reversed-phase C_{18} HPLC to afford **1**[6] (11 mg) as a white powder.

The molecular formula of **1** was determined to be $C_{16}H_{15}NO_7$ on the basis of high-resolution ESI-MS [$(M + Na)^+$, 356.07275 *m/z* (−1.32 mmu error)] in combination with ^1H and ^{13}C NMR data. The IR absorptions at 1797 and 3429 cm^{-1} suggested the presence of carbonyl and hydroxyl moieties, respectively. The ^1H and ^{13}C NMR (Table 1), ^1H-^1H COSY, DEPT, and HMQC data revealed the presence of isolated methyl, 1-propenyl, and 1, 2-disubstituted olefin units as well as an isolated aromatic methine (δ 6.23, s; δ 96.9), one exchangeable proton (δ 9.20, brs), an sp^3 quaternary carbon (δ 58.1), five nonprotonated sp^2 carbons, and three carbonyl carbons (δ 164.1, 168.5, and 177.5). The connectivities among these partial structures and atoms were determined by analysis of HMBC spectral data (Table 1). The olefinic proton at δ 6.07 (H-9) of the $^{11}CH_3$−^{10}CH═^{9}CH− group was long-range coupled to three nonprotonated sp^2 carbons (C-7a, C-1, and C-2) as well as the methyl carbon and the olefinic methine. The isolated aromatic methine (H-4) showed strong HMBC correlations with four nonprotonated sp^2 carbons (C-7a, C-2, C-3, and C-4a) and a weak HMBC correlation with C-1. These spectral data clearly indicated the presence of a pentasubstituted benzene ring in which the $^{11}CH_3$−^{10}CH═^{9}CH− group was attached to the C-1 position. On the other hand, long-range couplings were observed from the isolated methyl signal at δ 1.37 (H_3-8) to C-7a, C-7, and the carbonyl carbon at δ 177.5 (C-6). In addition, the exchangeable proton at δ 9.20 (NH-12) was long-range coupled to the carbons at C-6, C-7, and C-8. Together with the ^{13}C NMR chemical shift (δ 58.1) of C-7, these data suggested the presence of an alanine moiety of which the α-carbon (C-7) was linked to C-7a of the benzene ring. The presence of this moiety was supported by the four-bond correlation from H-4 of the benzene ring to C-7, assigned to W coupling, in HMBC optimized for 10 Hz. Both olefinic protons of the −CH═CH− group showed HMBC correlations with the carbonyl carbons at δ 164.1 (C-13) and 168.5 (C-16). The carbonyl carbon C-13 was in turn long-range coupled with the amide proton (NH-12) of the alanine moiety. These data indicated that the −CH═CH− group must be located between C-13 and C-16 and that C-13 is connected to the amino group of the alanine moiety via the amide bond. Considering the molecular formula of **1**, one of the remaining two carbonyl carbons at C-6 and C-16 could

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Spectral Data of Fumimycin (**1**) in $CDCl_3$ + CD_3OD

position	δ_H (*J*, Hz)	δ_C	HMBC
1		121.3	
2		140.3	
3		145.5[a]	
4	6.23 (1H, s)	96.9	C-1, C-2, C-3, C-4a, C-7, C-7a
4a		145.8[a]	
6		177.5	
7		58.1	
7a		115.8	
8	1.37 (3H, s)	22.4	C-6, C-7, C-7a
9	6.07 (1H, dq, 15.9, 1.2)	121.8	C-1, C-2, C-7a, C-10, C-11
10	6.36 (1H, dq, 15.9, 6.3)	133.4	C-1, C-9, C-11
11	1.61 (3H, dd, 6.3, 1.2)	19.1	C-9, C-10
12	9.20 (1H, brs)[b]		C-6, C-7, C-8, C-13
13		164.1	
14	6.59 (1H, d, 15.3)	133.1	C-13, C-15, C-16
15	6.35 (1H, d, 15.3)	133.3	C-13, C-14, C-16
16		168.5	

[a] Interchangeable. [b] The signal integrated to be 1H in the DMSO-d_6.

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(5) The solate was identified as *Aspergillus fumisynnematus* Horie et al. 1993 on the basis of standard biological and physiological tests and taxonomic determination. Fermentation was carried out in liquid culture using YPS medium (2% glucose, 0.2% yeast extract, 0.5% peptone, 0.05% $MgSO_4$, and 0.1% KH_2PO_4, pH 5.7 before sterilization). A piece of a colony from a mature agar plate culture was inoculated into a 500-mL Erlenmeyer flask containing 80 mL of the above sterile seed liquid medium and cultured on a rotary shaker (150 rpm) at 28 °C for 3 days. For the production of active compounds, 15 mL of the seed culture were transferred into 1000-mL Erlenmeyer flasks containing 300 mL of YPS medium and cultivated on a rotary shaker (150 rpm) for 7 days at 28 °C.

(6) The culture broth (12 liters) was extracted with 50% aqueous acetone and evaporated to remove acetone. The resultant water phase was partitioned with an equal volume of EtOAc three times and the EtOAc layer was concentrated in vacuo. The resultant residue (4.39 g) was subjected to SiO_2 (Merck Art No. 7734.9025) column chromatography using stepwise elution with $CHCl_3$−MeOH (10:1, 5:1, 2:1, 1:1). The active fractions eluted with $CHCl_3$−MeOH (1:1) were pooled and concentrated in vacuo to give an oily residue. The residue was applied to a Sephadex LH-20 column and then eluted with MeOH. The active fraction dissolved in MeOH was further purified by reversed-phase HPLC (20 × 250 mm, YMC C_{18}) with a photodiode array detector. The column was eluted with MeOH−H_2O (50:50) containing 0.01% TFA at a flow rate of 3.5 mL/minute to afford **1** (11 mg) with a retention time of 28 minutes as a white powder. Compound **1**: a white powder; λ_{max} nm (log ε) in MeOH: 212 (4.38), 239 (4.26), 257 (4.13), 266 (3.98), 316 (3.46). IR (KBr): 3429, 2927, 1797, 1637, 1315, 1151 cm^{-1}. $[\alpha]_D = -11.9$ (*c* 0.26, MeOH). ESI-MS: *m/z* 356.07275 $(M + Na)^+$, $C_{16}H_{15}NO_7Na$ requires 356.07407

(7) A mixture of 2 mL MeOH, 0.04 mL H_2SO_4, and 5 mg fumimycin was stirred for 6 h at room temperature, poured into 50 mL water, and extracted twice with 50 mL EtOAc. The solvent of the organic layer was evaporated. The residue was subjected to preparative ODS TLC developed with 50% aqueous MeOH containing 0.01% TFA to yield a methylated product (2 mg). Fumimycin methyl ester: a white powder. ESI-MS: *m/z* 346.3 $(M - H)^-$, 348.3 $(M + H)^+$, 370.3 $(M + Na)^+$. ^1H NMR (300 MHz, CD_3Cl + CD_3OD): δ 6.95 (1H, d, 15.6, H-14), 6.65 (1H, d, 15.6, H-15), 6.62 (1H, dq, 16.2, 6.6, H-10), 6.51 (1H, s, H-4), 6.33 (1H, dq, 16.2, 1.2, H-9), 3.75 (3H, s, $COOCH_3$), 1.89 (3H, dd, 6.6, 1.2, H-11), 1.65 (3H, s, H-8). ^{13}C NMR (800 MHz, CD_3Cl + CD_3OD) δ 177.0 (C-6), 166.2 (C-16), 163.1 (C-13), 145.6 (C-3, C-4a), 140.0 (C-2), 135.0 (C-14), 133.6 (C-10), 130.1 (C-15), 121.6 (C-9), 120.7 (C-1), 115.8 (C-7a), 96.9 (C-4), 58.0 (C-7), 51.9 ($COOCH_3$), 22.4 (C-8), 19.3 (C-11).

be connected to C-4a of the benzene ring via ester bond. To determine which carbonyl carbon participates in the lactone ring, **1** was methylated by teatment with $MeOH/H_2SO_4$ to yield a methyl ester.[1] The methoxy group in the methyl ester of **1** was determined to be correlated with C-16 by HMBC anaylsis, indicating that the lactone ring in **1** must be between C-4a and C-6. Thus, the planar structure of **1** was determined as shown.

The geometric configurations of the carbon−carbon double bonds were assigned on the basis of their ^1H coupling constants. The coupling constants between H-7 and H-8 and between H-14 and H-15 were 15.3 and 15.9 Hz, respectively, indicating that the double bonds both have *E* configurations.

Fumimycin (**1**) has an unusual skeleton incorporating an unusual alanine moiety linked to a phenyl group at the α-carbon, of which the amino and carboxylic groups participate in amide and lactone moieties, respectively. Structurally, fumimycin is related to sorbicillactones A and B, which were isolated from a sponge-derived *Penicillium chrysogenum* strain.[8] Sorbicillactones have a similar five-membered lactone and amide side chain, with the lactone fused to a six-membered ring. However, the six-membered ring of sorbicillactones is not aromatic, but highly oxidized. In addition, they have a longer and even-numbered side chain on the six-membered ring as well as two additional methyl substituents. Biosynthetically, the six-membered ring and the nitrogen of the amide unit in sorbicillactones were reported to originate from polyketide and alanine, respectively. Thus, it seems likely that the aromatic portion in fumimycin has a polyketide origin, but the odd number of carbon atoms in the chain requires loss of a carbon somewhere. Altogether, fumimycin has a structurally and biogenetically unusual skeleton. Also, this study is the first report of chemistry from *Aspergillus fumisynnematus.*

The inhibitory activity of **1** against *S. aureus* PDF was evaluated according to our previously reported method.[9] The antibacterial activity of **1** against various strains of *S. aureus* including two methicillin-resistant *S. aureus* (MRSA) strains (CCARM3167, CCARM3506) and two quinolone-resistant *S. aureus* (QRSA) strains (CCARM 3505, CCARM 3519) was examined using the microdilution broth method.[10] The minimum inhibitory concentration (MIC) was the lowest antibiotic concentration that completely prevented visible growth after incubation for 18 h; the minimum restrictive concentration (MRC) was defined as the lowest antibiotic concentration that caused at least 50% retraction of growth at 37 °C for 18 h.[11]

Compound **1** inhibited *S. aureus* PDF in a dose-dependent manner with an IC_{50} value of 4.1 μM. The inhibition pattern of PDF by **1** with respect to the substrate, f-MAS, was examined with a Lineweaver−Burk plot analysis. As shown in Figure 1, **1** exhibited mixed-type inhibition with f-MAS and its K_i and K_m values for PDF were 3.1×10^{-6} and 4.9×10^{-4} M, respectively. Also, **1** exhibited antibacterial activity against MRSA with an MIC (μg/mL) of 100 and against both *S. aureus* and QRSA with an MRC (μg/mL) of 100.

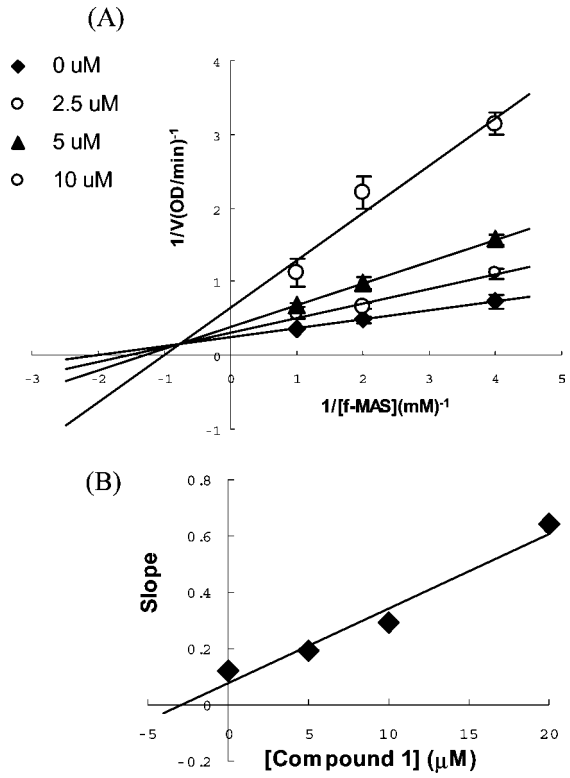

Figure 1. The inhibition of *S. aureus* PDF by **1** with respect to the substrate (f-MAS) (A), and K_i determination of **1** (B). The values were represented as the means ± SD in triplicates.

In summary, fumimycin is a unique antibiotic isolated from a fermentation culture of *Aspergillus fumisynnematus* F746. Fumimycin has an unusual metabolite incorporating an aromatic skeleton fused to an alanine unit of which the amino and carboxylic groups participate in amide and lactone moieties, respectively. Fumimycin exhibited potent inhibition on *S. aureus* PDF and also showed antibacterial activity against *S. aureus*, MRSA, and QRSA. Fumimycin may represent a new class of PDF inhibitor for development of novel antibacterials.

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Supporting Information Available: 1D and 2D NMR and MS data of **1**; HMBC data of its methyl ester. This material is available free of charge via the Internet at http://pubs.acs.org.

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