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Note

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Flavimycins A and B, Dimeric 1,3-Dihydroisobenzofurans with Peptide Deformylase Inhibitory Activity from *Aspergillus flavipes*

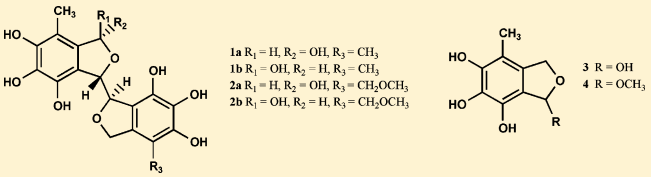
Yun-Ju Kwon,[†] Mi-Jin Sohn,[†] Chang-Jin Kim,[†] Hiroyuki Koshino,[‡] and Won-Gon Kim*[,†]

[†]Korea Research Institute of Bioscience and Biotechnology, Yusong, Daejeon 305-806, Republic of Korea

[‡]Molecular Characterization Team, RIKEN Advanced Science Institute, Hirosawa 2-1, Wako, Saitama 351-0198, Japan

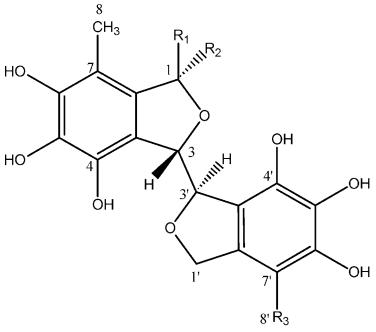
Supporting Information

ABSTRACT: Flavimycins A (**1**) and B (**2**), novel dimeric 1,3-dihydroisobenzofurans, were isolated as inhibitors of peptide deformylase from cultures of *Aspergillus flavipes*. Their chemical structures were established by NMR and MS data analysis. Compounds **1** and **2** exist as epimeric mixtures at C-1 through fast hemiacetal−aldehyde tautomerism. Compounds **1** and **2** inhibited *Staphylococcus aureus* peptide deformylase with IC_{50} values of 35.8 and 100.1 μM, respectively. Consistent with their PDF inhibition, **1** showed two times stronger antibacterial activity than **2** on *S. aureus* including MRSA, with MIC values of 32−64 μg/mL.

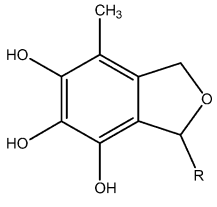


Emergence of resistance to all classes of antibiotics is a serious threat, and continued discovery of new antibiotics with novel modes of action is critical to overcoming antibiotic-resistant bacteria.[1] Bacterial genomics has revealed a plethora of previously unknown potential targets that could be used in the discovery of new antibacterial drugs.[2]

Among novel antibacterial targets, peptide deformylase (PDF) (EC 3.5.1.31) has received an increasing amount of attention.[3,4] 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 mammalian toxicity. Recent studies from several research groups have shown that PDF inhibitors act as broad-spectrum antibacterial agents,[5,6] and the PDF inhibitor GSK1322322 is currently in phase II clinical trial.[7] A number of PDF inhibitors have been reported, although most of them are peptidic.[8−11]



1a R_1 = H, R_2 = OH, R_3 = CH_3
1b R_1 = OH, R_2 = H, R_3 = CH_3
2a R_1 = H, R_2 = OH, R_3 = CH_2OCH_3
2b R_1 = OH, R_2 = H, R_3 = CH_2OCH_3



3 R = OH
4 R = OCH_3

In the course of screening for PDF inhibitors, we discovered the previously reported FR198248 (**3**) and FR202306 (**4**) from the fermentation broth of *Aspergillus flavipes* F543.[12] Compound **4**, a methyl acetal of compound **3**, was an artifact formed during the isolation procedure with methanol.

Further investigation of less polar fractions from this fermentation led to discovery of two novel dimeric, highly hydroxylated isobenzofurans, subsequently named flavimycins A (**1**) and B (**2**). Compound **2** is a C-8′ methoxymethyl analogue of **1**. Compounds **1** and **2** are present as epimeric mixtures, **1a** and **1b**, and **2a** and **2b**, respectively, at the hemiacetal carbon of C-1. Major (**1a** or **2a**) and minor (**1b** or **2b**) forms were inseparable epimers through hemiacetal−aldehyde tautomerism. The hemiacetal−aldehyde tautomerism is not surprising, as it has been reported that 30% of the monomeric compound **3** was converted to its methyl acetal **4** at 1 min in methanol.[12] Compounds **1** and **2** were also changed to methyl acetal mixtures when dissolved in methanol, and the ratio of methyl acetal **1** is 1:1.[12]

In this paper, we present the isolation, structure determination, and biological activities of **1** and **2**.

The molecular formula of **1** was determined to be $C_{18}H_{18}O_9$ on the basis of high-resolution ESIMS and ^{1}H and ^{13}C NMR data. The IR absorbption at 3397 cm^{-1} suggested the presence of hydroxyl moieties. The signals with different intensities in the ^{1}H and ^{13}C NMR spectra (Table 1) dissolved in DMSO-d_6 suggested that **1** is a mixture of two epimers, **1a** and **1b**, in the ratio of 3:1 to 5:1 depending on the concentration.

The planar structure of the major epimer (**1a**) of **1** was determined by ^{1}H−^{1}H COSY, HMQC, and HMBC analysis (Figure 1). In the ^{1}H−^{1}H COSY, the methine at δ 6.30 (H-1) was coupled with the exchangeable proton at δ 7.19 (1-OH),

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data[a] of Flavimycin A (1a and 1b) and Flavimycin B (2a and 2b)

	1a		1b		2a		2b	
position	δ_H, multi. (J in Hz)	δ_C, multi.	δ_H, multi. (J in Hz)	δ_C, multi.	δ_H, multi. (J in Hz)	δ_C, multi.	δ_H, multi. (J in Hz)	δ_C, multi.
1	6.30, d (9.6)	101.4, CH	6.39, dd (8.4, 1.6)	101.0, CH	6.31, d (9.9)	101.4, CH	6.40, dd (8.6, 1.8)	101.0, CH
1-OH	7.19, d (9.6)		6.74, d (8.4)		7.23, d (9.9)		6.75, d (8.6)	
3	4.98, d (9.6)	83.9, CH	5.25, dd (8.4, 1.6)	83.9, CH	5.00, d (9.0)	83.7, CH	5.26, dd (8.1, 1.8)	83.7, CH
		116.7, C		116.3, C		116.7, C		116.2, C
		137.4, C		137.4, C		137.4, C		137.4, C
		134.0, C		133.8, C		134.0, C		133.7, C
		145.1, C		145.0, C		145.0, C		144.9, C
		110.7, C		110.6, C		110.6, C		110.6, C
		128.3, C		129.4, C		128.2, C		129.5, C
8	2.05, s	11.1, CH_3	2.03, s	11.1, CH_3	2.05, s	11.1, CH_3	2.03, s	11.1, CH_3
1′	5.01, d (10.4)	73.9, CH_2	4.96, d (11.1)	73.0, CH_2	5.04, d (11.3)	73.0, CH_2	5.01, d (11.3)	72.8, CH_2
	5.26, dd (10.4, 1.2)		5.17, dd (11.1, 2.4)		5.29, dd (11.3, 2.7)		5.19, dd (11.3, 2.7)	
3′	5.24, dd (9.6, 1.2)	87.8, CH	5.12, dd (8.4, 2.4)	86.6, CH	5.23, dd (9.0, 2.7)	87.2, CH	5.11, dd (8.1, 2.7)	86.1, CH
		114.7, C		114.7, C		115.4, C		115.4, C
		138.1, C		138.3, C		139.7, C		139.7, C
		132.6, C		132.5, C		132.4, C		132.3, C
		145.0, C		144.9, C		145.5, C		145.3, C
		108.3, C		108.2, C		109.6, C		109.5, C
		127.7, C		128.2, C		128.8, C		129.2, C
8′	1.94, s	12.1, CH_3	1.93, s	12.1, CH_3	4.29, d (10.8)	66.2, CH_2	4.28, d (11.7)	66.3, CH_2
					4.32, d (10.8)		4.30, d (11.7)	
8′-OMe					3.20, s	57.2, CH_3	3.20, s	57.1, CH_3

[a]Recorded in DMSO-d_6.

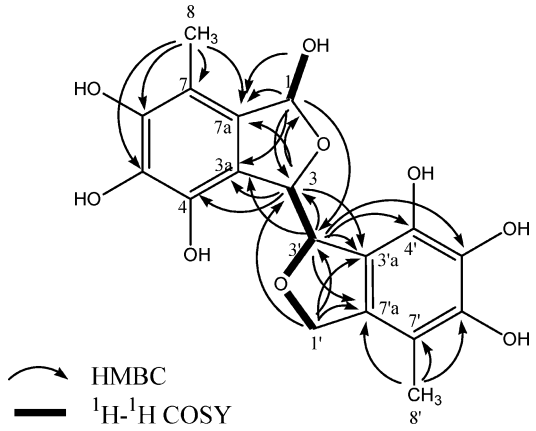

Figure 1. HMBC and ^{1}H−^{1}H COSY correlations of compound **1a**.

suggesting, together with the ^{13}C NMR chemical shift of C-1 at δ 101.4, that H-1 could be a hemiacetal methine proton. In the HMBC spectrum, the hemiacetal methine proton of H-1 was long-range coupled to two sp^2 quaternary carbons at δ 116.7 (C-3a) and 128.3 (C-7a) and an oxygenated methine carbon at δ 83.9 (C-3). Long-range couplings were observed from the proton of H-3 to the carbons of C-1, C-3a, C-7a, and C-4 (δ 137.4). The aromatic methyl signal at δ 2.05 (H_3-8) showed strong HMBC correlations with three sp^2 quaternary carbons at C-6, C-7, and C-7a and a weak HMBC correlation with C-5. These spectral data indicated the presence of a 1,4,5,6-tetrahydroxy-7-methyl-1,3-dihydroisobenzofuran moiety. On the other hand, the other aromatic methyl signals at δ 1.94 (H_3-8′) showed HMBC correlations with three sp^2 quaternary carbons at δ 145.0 (C-6′), 108.3 (C-7′), and 127.7 (C-7′a). The methylene protons at δ 5.01 (H-1′a) and 5.26 (H-1′b) were long-range coupled with the other oxygenated methine carbon at δ 87.8 (C-3′) and three sp^2 quaternary carbons at C-3′a, C-7′a, and C-7′. These spectral data indicated the presence of a 4,5,6-trihydroxy-7-methyl-1,3-dihydroisobenzofuran moiety. The vincinal proton−proton coupling constant of J = 9.6 Hz was observed among the oxygenated methine protons, H-3 and H-3′, of the two isobenzofuran moieties, clearly indicating that the two moieties could be linked via C-3 and C-3′. The direct linkage of C-3 to C-3′ was confirmed by the HMBC spectrum. Long-range couplings were observed from the proton of H-3 to the carbons C-3′ and C-3′a. Similarly, the proton of H-3′ was long-range coupled to the carbons C-3 and C-3a. In addition, H-1 and H-1′β showed four-bond couplings with C-3′ and C-3, respectively. Thus, the planar structure of **1a** was determined to be novel dimeric 1,3-dihydroisobenzofuran, as shown in Figure 1.

The ^{1}H NMR spectrum of **1b** was the same as that of **1a** except for the aromatic methyl signal, while their ^{13}C NMR data were almost identical (Table 1). The major difference was that the long-range coupling of 1.6 Hz was observed between a hemiacetal methine (δ_H 6.39, dd, J = 8.4, 1.6; δ_C 101.0) of H-1 and an oxgenated methine (δ_H 5.25, dd, J = 8.4, 1.6; δ_C 83.9) of H-3 in **1b**. The planar strucutre of **1b** was determined to be the same as that of **1a** from the HMBC spectrum, suggesting that **1b** could be an epimer at C-1 of **1a**.

The relative configurations of **1a** and **1b** were determined on the basis of the NOE correlations. In the NOESY spectrum, the methyl protons of H_3-8 exhibited NOEs with H-1 and OH-1 in both **1a** and **1b**. However, the hemiacetal methine proton of H-1 showed weak NOEs with H-3 in **1a**, but with H-3′, not H-3, in **1b** (Figure 2). These results indicated that H-1 and H-3 have a *cis* orientation in **1a** and a *trans* orientation in **1b**. **1a** is thus an epimer of **1b** at C-1, as shown in Figure 2. This relative configuration is supported by the fact that the two forms were easily exchangeable at C-1.

The molecular formula of **2** was determined to be $C_{19}H_{20}O_{10}$ on the basis of high-resolution ESIMS in combination with ^{1}H

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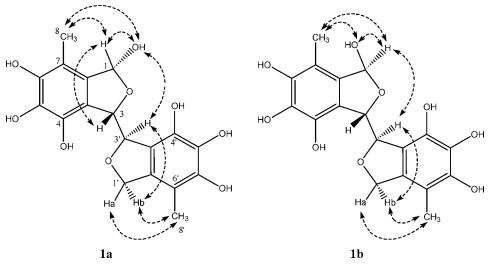

Figure 2. Relative configuration and key NOE correlations of **1a** and **1b**.

and ^{13}C NMR data (Table 1). The IR and UV spectrum were almost identical to those of **1**. The ^{1}H and ^{13}C NMR data of **2** dissolved in DMSO-d_6 were similar to those of **1**, indicating that **2** is a mixture of two epimers, **2a** and **2b**. The difference between **1a** and **2a** was a signal from the methoxymethylene group (δ_{Ha} 4.29, d, J = 10.8 and δ_{Hb} 4.32, d, J = 10.8; δ_C 83.9 and δ_H 3.20, s; δ_C 57.2) in **2a** replacing the aromatic methyl signal (δ_H 1.94, s; δ_C 12.1) of **1a**. The location of the methoxymethylene group was determined to be C-7′ by the HMBC correlations from the methylene protons H-8′ to C-6′, C-7′, and C-7′a, as well as the NOE correlations between two methylenes at H-1′ and H-8′. The minor form (**2b**) of **2** is also an epimer of **2a** at C-1 on the basis of NMR and NOE data. Thus, **2** is the C-8′ methoxymethyl analogue of **1**.

Compounds **1** and **2** are novel dimeric compounds of highly hydroxylated 1,3-dihydroisobenzofurans. The 1,3-dihydroisobenzofuran moiety has been reported only in FR198248 and FR202306, which were shown to have anti-influenza activity.[13,14] Although the 1,3-dihydroisobenzofuran unit is a known metabolite, it is rarely found from microorganisms and its dimeric form has not been previously described as far as we know.

The inhibitory activity of **1** and **2** against *S. aureus* PDF was evaluated according to our previously reported method (Supporting Information Table S1).[15] The antibacterial activity of **1** and **2** against Gram-positive bacteria including *S. aureus* RN4220, methicillin-resistant *S. aureus* (MRSA) strain CCARM3167, and quinolone-resistant *S. aureus* (QRSA) strain CCARM 3505 was determined using the microdilution broth method.[16] Compounds **1** and **2** inhibited *S. aureus* PDF dose dependently with IC_{50} values of 35.8 and 100.1 μM, respectively, while that of actinonin, a hydroxamate peptidic compound, as a positive control was 0.02 μM. Consistent with their PDF inhibition, **1** prevented cell growth of *S. aureus*, MRSA, and QRSA with MIC values of 32−64 μg/mL, while **2** showed weaker antibacterial activity with MIC values of 64−128 μg/mL. The MIC for actinonin was 4 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-1601 UV−visible spectrophotometer. IR spectra were obtained using a Bruker EQUINOX 55 spectrophotometer. NMR spectra were recorded on a Bruker Biospin Advance 500 spectrometer (Korea Basic Science Institute). HRESIMS data were recorded on a JEOL JMS-HX110/110A mass spectrometer.

Fungal Material. Fungal strain F543 was isolated from soil collected in Chungbuk Province, South Korea. The strain was identified as *Aspergillus flavipes* (Bain. & Sart.) Thom & Church 1926 on the basis of standard biological and physiological tests and taxonomic determination and the 18S rDNA sequence by staff at the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. The strain was deposited in the Korean Collection for Type Cultures, Daejeon, with accession number KCTC 10880BP.

Fermentation and Isolation. Fermentation was carried out in a liquid culture medium containing 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 the strain F543 from a mature 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 was transferred into 1000 mL Erlenmeyer flasks containing 300 mL of the YPS medium and cultivated on a rotary shaker (150 rpm) for 7 days at 28 °C. The culture broth (15 L) 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 pooled EtOAc layers were concentrated *in vacuo*. The resultant residue (17.75 g) was subjected to SiO_2 (Merck Art No. 7734.9025) column chromatography followed by stepwise elution with $CHCl_3$−MeOH (20:1, 10:1, 5:1). The active fractions eluted with $CHCl_3$−MeOH (20:1) were pooled and concentrated *in vacuo* to give an oily residue. The active fraction was dissolved in 50% DMSO and further purified by reversed-phase HPLC column (20 × 150 mm, YMC C_{18}) chromatography. The column was eluted with CH_3CN−H_2O (20:80) at a flow rate of 4.0 mL/min to afford **1** (26.6 mg) and **2** (4.1 mg) with retention times of 26.1 and 18.0 min, respectively.

Flavimycin A (1): white powder; $[\alpha]^{25}_D$ 3.2 (*c* 0.2, DMSO); UV (MeOH) λ_{max} (log ε) 211 (4.38), 271 (3.14) nm; IR (KBr) ν_{max} 3397 (OH), 1631, 1317, 1021 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS $[M - H]^-$ m/z 377.0874 (calcd for $C_{18}H_{17}O_9$, 377.0873).

Flavimycin B (2): white powder; $[\alpha]^{25}_D$ 12.5 (*c* 0.2, DMSO); UV (MeOH) λ_{max} (log ε) 210 (4.59), 272 (3.28) nm; IR (KBr) ν_{max} 3413 (OH), 1631, 1319 (CO), 1026 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS $[M - H]^-$ m/z 407.0983 (calcd for $C_{19}H_{19}O_{10}$, 407.0978).

Determination of Antibacterial Susceptibility. Methicillin-resistant *S. aureus* (MRSA) CCARM 3167 and quinolone-resistant *S. aureus* (QRSA) CCARM 3505 were obtained from the Culture Collection of Antimicrobial Resistant Microbes of Korea. *S. aureus* was grown to mid-log phase in Mueller-Hinton broth and diluted 1000-fold in the same medium. Cells (10^5/mL) were inoculated into Mueller-Hinton broth and dispensed at 0.2 mL/well in 96-well microtiter plates. Since test compounds were soluble in DMSO and methanol, they were prepared in DMSO, the final concentration of which did not exceed 0.05%. Cells were treated with either 0.05% DMSO as vehicle control or test samples. MICs were determined in triplicate by serial dilution of test compounds. The minimum inhibitory concentration was the lowest antibiotic concentration that completely prevented visible growth after incubation at 37 °C for 18 h.

ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra of flavimycins A (**1a** and **1b**) and B (**2a** and **2b**) and their antibacterial activity. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 82-42-860-4298. Fax: 82-42-860-4595. E-mail: wgkim@kribb.re.kr.

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