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NOTE

8′-epimer of herbicidin F and its congeners from *Streptomyces* sp. YIM 66142

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Herbicidins are isolated from different strains of *Streptomyces*, and they exhibit several interesting biological activities.[1–5] Herbicidins A and B indicated no activity at a concentration of 100 μg mL^{-1} against *Xanthomonas oryzae in vitro*, but protection of the leaves of rice plant from bacterial leaf blight disease was caused by *X. oryzae*.[1] Herbicidins A and B have also been shown to reduce seed germination[1] and although they exhibit selective toxicity toward dicotyledonous plants, no toxicity against animals is observed.[1] In our effort to search for new biological compounds from endophytes, a new antibiotic 8′-epimer of herbicidin F (**1**), along with four known compounds, herbicidin F (**2**),[5] herbicidin A (**3**),[5] herbicidin B (**4**)[5–7] and 2′-*O*-demethylherbicidin F (**5**)[8] were isolated from the broth of *Streptomyces* sp. YIM 66142, a *Dendrobium chrysotoxum* endophyte (Figure 1). The absolute configuration of the 8′-epimer of herbicidin F (**1**) was established using its electronic CD (ECD) spectrum, density functional theory (DFT)-ECD calculations and NOESY spectrum. An antibiotic activity assay showed that herbicidin B (**4**) and 2′-*O*-demethylherbicidin F (**5**) had antibacterial activity against *Bacillus subtilis* as well as their inhibition of germination of the plant seeds, such as cabbage.

Streptomyces sp. YIM 66142 was cultivated in 250 mL Erlenmeyer flasks containing 50 mL of seed medium (0.4% yeast extract, 0.4% glucose, 0.5% malt extract, decavitamin, 0.03% alanine, pH 7.2). The flasks were shaken at 300 r.p.m. at 28 °C for 2 days using a rotary shaker. The seed culture (10%) was transferred into 1 L Erlenmeyer flasks containing 250 mL of fermentation medium (0.3% soybean powder, 2% glucose, 0.2% yeast extract, 0.5% starch, 0.2% peptone, 0.4% NaCl, 0.05% K_2HPO_4, 0.05% $MgSO_4$ and 0.2% $CaCO_3$, pH 7.2). The flasks were shaken at 300 r.p.m. for 6 days at 28 °C using a rotary shaker. The fermented whole broth (60 L) was centrifuged to separate the mycelia, and the fermented solution was extracted three times by an equivalent volume of EtOAc, to yield the EtOAc extract (29.1 g). The mycelia were extracted three times with acetone, yielding the acetone extract (8.0 g). The two extracts were then suspended in distilled H_2O and extracted three times with EtOAc, to concentrate the total sample in solution (13 g). This sample was separated into seven fractions (A1–A7) using a silica gel column with petroleum ether to MeOH. The fifth fraction, A5 ($CHCl_3$-MeOH 20:1), was separated using a Sephadex LH-20 column and eluted with methanol to yield six fractions (A5-I to A5-VI). Fraction A5-III was again fractionated with a Sephadex LH-20 column and eluted with methanol to obtain compound **2** (100 mg). Fraction A5-IV was subsequently separated into nine fractions (fractions A5-IV–I to A5-IV–IX) with a Sephadex LH-20 column. Fraction A5-IV–VI was further fractionated on silica gel with chloroform/acetone elution solvent (12:1–12:4, v/v) to obtain **1** (1.2 mg), and fraction A5-IV–VII was separated on silica gel with chloroform/methanol (45:1, v/v) to obtain **4** (1.5 mg). The A6 part was separated into four fractions (A6-I to A6-IV) using a Sephadex LH-20 column and eluted with methanol. The fraction A6-IV was again fractionated with a Sephadex LH-20 column and eluted with methanol to obtain **3** (4.2 mg) and **5** (5.1 mg).

The molecular mass was determined by electrospray ionization mass spectrometry (ESI-MS) in positive mode, and the molecular formula $C_{23}H_{29}N_5O_{10}$ was obtained by high-resolution electron ionization mass spectrometry (HREIMS) of compound **1** (*m/z* 535.1939; calcd for $C_{23}H_{29}N_5O_{10}$ at *m/z* 535.1914). The ^{1}H NMR (Table 1) spectrum of **1** displayed two three-proton signals at δ1.83 (3H, d, J = 6.9 Hz), 1.87 (3H, s) for methyl groups attached to a double bond and 3.50 (3H, s) and 3.74 (3H, s) for the methyloxy groups; for one methylene group at δ 2.24 (2H, m); and for 11 methine groups at δ 3.82 (1H, m), 3.83 (1H, d, 3.9 Hz), 4.23 (1H, s), 4.47 (1H, s), 4.55 (1H, s), 4.58 (1H, s), 5.63 (1H, d, 4.0 Hz), 6.12 (1H, s), 7.06 (1H, d), 8.16 (1H, s) and 8.22 (1H, s). The ^{13}C NMR spectrum (Table 1) and DEPT spectra of **1** showed the following 23 carbon signals: 2 methyls, 1 methylene, 11 methines, 2 methyloxy and 7 quaternary carbons. The 1D NMR data and molecular formula of **1** demonstrated that the structure of **1** has the

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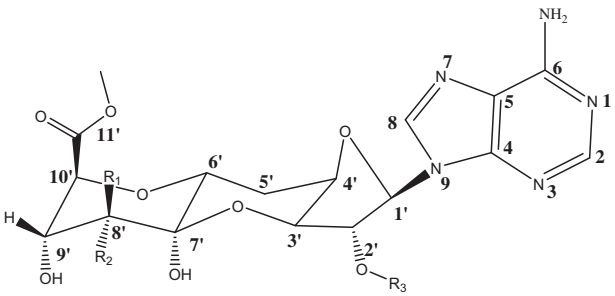

1 R_1=H, R_2=(E)-OCO(CH_3)C=CHCH_3, R_3=CH_3

2 R_1=(E)-OCO(CH_3)C=CHCH_3, R_2=H, R_3=CH_3

3 R_1=(E)-OCO(CH_2OH)C=CHCH_3, R_2=H, R_3=CH_3

4 R_1=OH, R_2=H, R_3=CH_3

5 R_1=(E)-OCO(CH_3)C=CHCH_3, R_2=H, R_3=H

Figure 1 Herbicidin derivatives from the *Streptomyces* sp. YIM 66142.

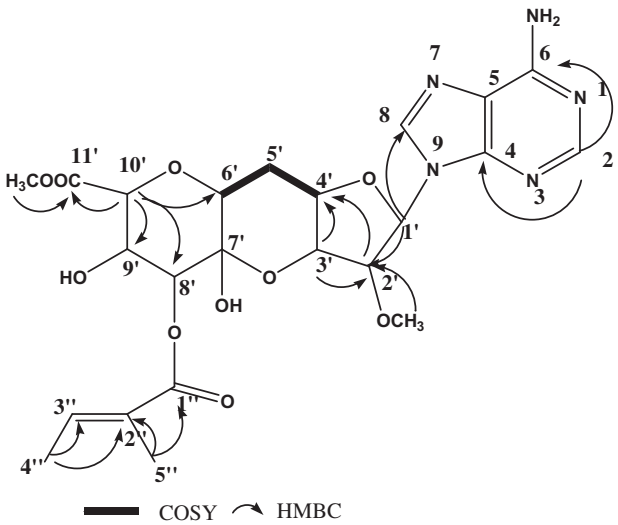

Figure 2 Key HMBC and ^{1}H-^{1}H COSY correlations for compound **1.**

Table 1 ^{1}H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) data of compounds 1 and 2

	Proton		*Carbon*	
Position	***1***	***2***	***1***	***2***
2	8.22, s	8.26, s	153.9	154.1
4	–	–	150.5	150.5
5	–	–	119.9	119.8
6	–	–	157.2	157.5
8	8.16, s	8.00, s	140.3	140.5
1′	6.12, s	6.10, d (1.4)	89.3	88.8
2′	4.23, s	4.11, s	90.8	91.8
3′	4.47, s	4.54, d (1.9)	73.9	75.7
4′	4.55, s	4.44, d (2.1)	79.9	78.9
5′	2.24, m	2.29, m	26.3	26.6
6′	3.82, m	4.58, dd, (6.2, 4.4)	69.3	66.4
7′	–	–	94.8	93.4
8′	5.63, d (4.0)	5.05, d (3.1)	72.4	71.9
9′	3.83, d (3.9)	4.37, d (3.0)	71.0	70.5
10′	4.58, s	4.51, s	78.0	78.3
11′	–	–	170.7	171.2
2′-OCH_3	3.50, s	3.45, s	58.3	58.4
11′-OCH_3	3.74, s	3.66, s	53.0	52.7
1″	–	–	169.0	167.2
2″	–	–	129.7	128.5
3″	7.06, d (6.8)	6.76, q (7.1)	139.9	141.7
4″	1.83, d (6.9)	1.95, d (7.1)	14.3	15.1
5″	1.87, s	1.92, s	12.0	12.3

backbone of the undecose antibiotic herbicidin. Comprehensive NMR spectral data showed that the structure of **1** was similar to herbicidin F (**2**) except for the chemical shift differences at C-6′, C-7′, C-8′, C-11′, C-1″, C-2″ and C-3″. An analysis of COSY spectroscopic data revealed the $C_{4'}$-$C_{5'}$-$C_{6'}$ structural connection. The connection of C-1′ to N-9 was determined by the HMBC H-1′ to C-8 correlation. The $C_{1'}$-$C_{2'}$-$C_{3'}$ fragment was determined by the HMBC correlations from H-1′ to C-2′ and H-2′ to C-4′, H-3′ to C-2′ and C-4′. The $C_{7'}$-$C_{8'}$-$C_{9'}$-$C_{10'}$-$C_{11'}$ segment was determined by the HMBC correlations between H-10′ and C-6′, C-8′, C-9′ and C-11′. The (*E*)-2-methyl-2-butenoic acid moiety was determined by the following HMBC correlations: 4″-H to 2″-C and 3″-C, 5″-H to 2″-C and 1′ ′-C. Further structural data were obtained using 2D NMR (Figure 2). ROESY correlations were found between H-1′ and H-2′, H-3′ and H-4′, H-2′ and 8-H, H-3′ and H-5′, H-5′and H-6′ in compound **1**. ROESY correlations between H-8 and H-3″, H-3″ and H-6′ were not observed (Figure 3). However, ROESY correlation peaks for herbicidin F (**2**) between H-3″ and H-6′ were observed, suggesting that the (*E*)-2-methyl-2-butenoic acid group connected to C-8′ is oriented differently in compounds **1** and **2**. The configuration of **1** was confirmed to be 8'-*epi*-herbicidin F. Another 8′-*epi*-herbicidin was reported recently in *Streptomyces*.[8] To identify its absolute configurations of compound **1**, ECD spectrum was used in combination with DFT-ECD calculations.

The experimental ECD spectrum of herbicidin F (**2**) contains a broad negative signal centered at 268 nm and a strong positive signal centered at 220 nm with a shoulder at 203 nm. The experimental ECD spectrum of 8-*epi*-herbicidin F (**1**) exhibits a broad negative signal centered at 260 nm, a weakly positive signal centered at 223 nm, and a weakly negative signal centered at 213 nm. The change in ECD spectra was caused by the 8'-(*E*)-2-methyl-2-butenoic acid moiety located at the equatorial bond in compound **1**. The calculated spectrum of 8'-*epi*-herbicidin F (**1**) and herbicidin F (**2**) (Figure 4) was well matched with the experimental spectra. This comparison allowed us to identify compound **1** as 8'-*epi*-herbicidin F.

Compounds **2**, **3** and **5** showed no marked cytotoxicity on human leukemia cells (HL-60), human lung cancer cells (A549), hepatocellular carcinoma cells (SMMC-7721), human breast adenocarcinoma (MCF-7) or human colorectal carcinoma cells (SW480) at 40 μM. These compounds were tested for antimicrobial activities against five strains of microorganisms (*B. subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *X. oryzae*) at 512 μg mL^{-1}. Compounds did not show significant antibacterial activity except for **4** and **5** showed MICs of 32 μg mL^{-1} against *B. subtilis* (Table 2). Much more potent inhibitory effect of herbicidins occurred against germination of the plant seeds, such as cabbage. The MICs of 8'-*epi*-herbicidin F (**1**), herbicidin F (**2**), herbicidin A (**3**),

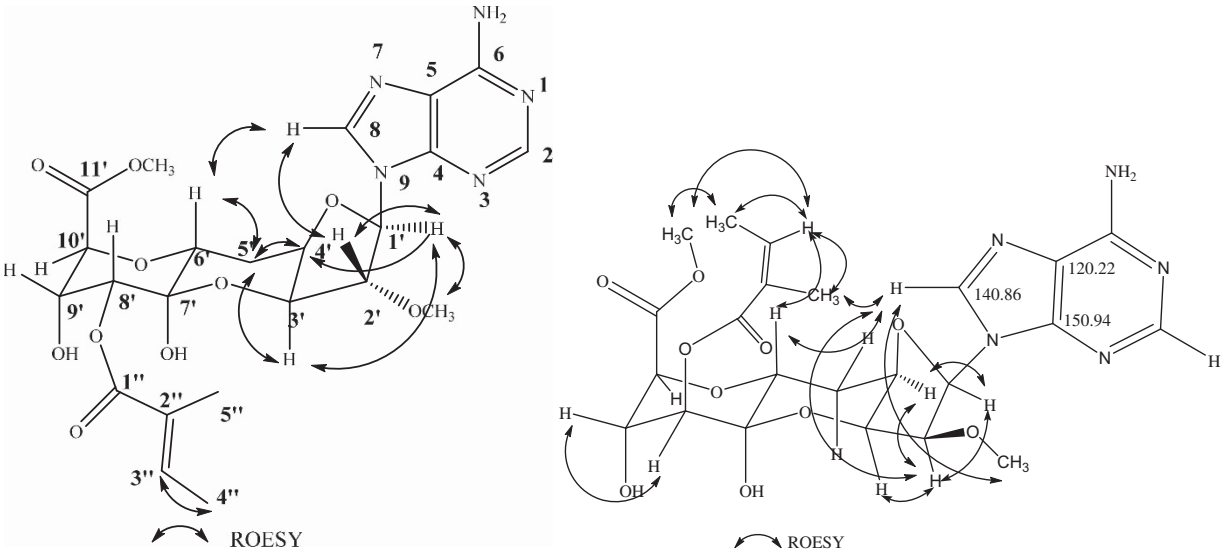

Figure 3 ROESY correlations of compounds **1** (left) and **2** (right).

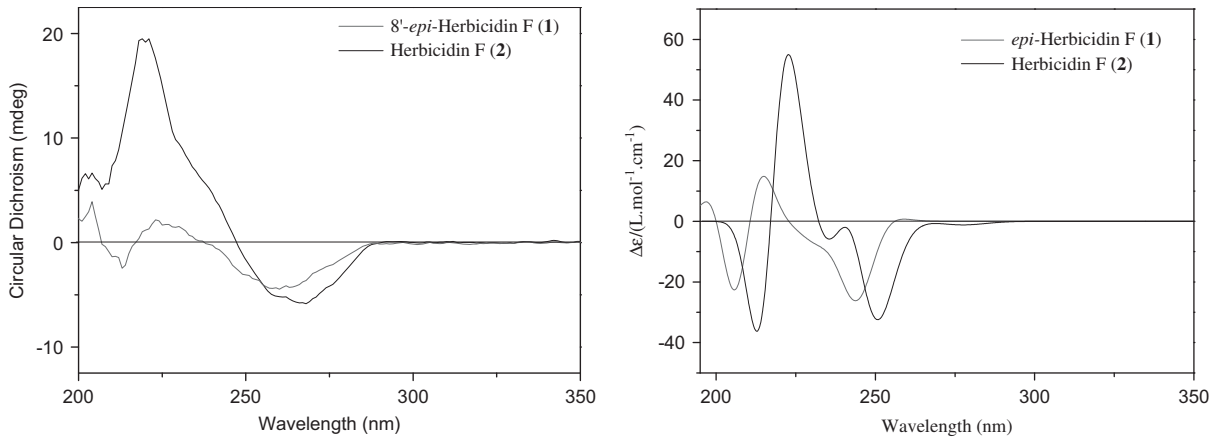

Figure 4 Experimental ECD (left) and calculated ECD (right) spectra of 8'-*epi*-herbicidin F (**1**) and herbicidin F (**2**). σ is 0.2 eV. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 2 MICs (μg mL^{-1}) of 1, 2, 3, 4 and 5 in screening of antimicrobial activity

Sample	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*	*Xanthomonas oryzae*
Compound **1**	/	/	/	/	256
Compound **2**	512	>512	512	128	512
Compound **3**	>512	>512	>512	128	256
Compound **4**	32	>512	>512	256	256
Compound **5**	32	>512	>512	512	256
Kanamycin	2	4	0.5	/	8
Nysfungin	/	/	/	0.5	/

"/" means no test.

herbicidin B (**4**) and 2′-*O*-demethylherbicidin F (**5**) for germination of the seeds of cabbage were 12.5–50 , <3.1 , 3.1–12.5 , 12.5–50 and 12.5–50 μg mL^{-1}.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)