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Toxic polyketides produced by *Fusarium* sp., an endophytic fungus isolated from *Melia azedarach*

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ABSTRACT

A new isocoumarin derivative named fusariumin (**1**), together with two known related resorcylic acid lactones aigialomycin D (**2**) and pochonin N (**3**), has been isolated from the cultures of *Fusarium* sp. LN-10, an endophytic fungus originated from the leaves of *Melia azedarach*. Their structures were established on the basis of extensive spectroscopic analyzes including 1D- and 2D- NMR (^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY) experiments. Compounds **1**–**3** displayed significant growth inhibitory activity against the brine shrimp (*Artemia salina*)

Recently statistical analyzes showed that 51% of the biologically active metabolites obtained from endophytes are previously unknown, compared with only 38% of novel substances from soil microflora. Endophytes have been recognized as important sources of a variety of structurally novel active secondary metabolites with anticancer, antimicrobial and other biological activities.[1,2]

Melia azedarach Linn (Meliaceae), also known as Chinaberry or Persian lilac tree, is a deciduous tree native to northwestern India and has long been recognized for its insecticidal properties.[3] This plant produces a class of modified oxygenated triterpenoids, known as limonoids.[4,5] However, little is known about secondary metabolites of endophytes harbored inside the healthy tissues of *M. azedarach*.[6]

In our ongoing search for new biologically active metabolites from microorganisms inhabiting the plants,[6–11] an endophytic fungus, identified as *Fusarium* sp. LN-10, was obtained from *M. azedarach*. Three metabolites, a previously unreported isocoumarin derivative fusariumin (**1**), and two resorcylic acid lactones, aigialomycin D (**2**) and pochonin N (**3**) (Fig. 1), were isolated from the extract of this fungus. In this Letter, we herein describe the isolation and structure elucidation of the new compound, and bioactivities of these three isolated compounds.

The fungal strain *Fusarium* sp. (internal strain no. LN-10) was separated from the leaves of *M. azedarach*, a medicinal plant growing in the campus of Northwest A&F University, Yangling, Shaanxi province, China, and has been deposited at Research Centre for Natural Medicinal Chemistry, Northwest A&F University. The fungus *Fusarium* sp. was cultivated on PDA medium for 5 days at 28 °C to provide the culture broth (20 L), which was filtered to give the mycelium and culture filtrate. The mycelium was dried at 50 °C, and ultrasonically extracted three times by ethyl acetate and acetone, respectively. The combined organic layers were defatted with cyclohexane and then dissolved in methanol to give a crude extract (3.2 g). The extract was fractionated on a silica gel column, followed by separation on Sephadex LH-20 (CH_2Cl_2/MeOH = 6:4 and MeOH), normal and reverse phase column chromatography and preparative TLC using CH_2Cl_2/MeOH (15:1), affording fusariumin (**1**, 10.1 mg), aigialomycins D (**2**, 3.2 mg) and pochonin N (**3**, 2.9 mg).

Compound **1**[12] was obtained as a white amorphous solid. The molecular formula $C_{18}H_{22}O_5$ was determined by the $[M+H]^+$ peak at *m/z* 319.1539 in positive mode HR-ESI-MS and ^{13}C NMR spectra, with eight degrees of unsaturation. Its UV spectrum with absorption maxima at λ 378.7, 360.3, 345.7, 330.1, and 270.2 nm was indicative of an conjugated chromophore. Its IR spectrum with absorption bands at 3381, 1663, 1622, and 1570 cm^{-1} suggested the presence of hydroxyl, α,β-unsaturated δ-lactone carbonyl, and olefinic functionalities, respectively. These spectra revealed the presence of typical isocoumarin moiety in comparison with known isocoumarins.[13] The ^{13}C NMR and DEPT spectra of **1** with the aid of HSQC data (Table 1) showed the presence of 18 carbon signals, which were recognized as four oxygenated olefinic/aromatic carbons containing

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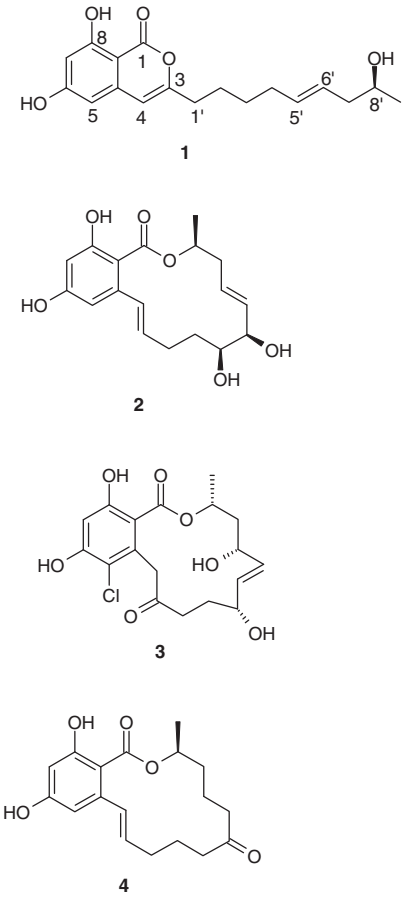

Figure 1. Structures of compounds **1**–**3** and zearalenone (**4**).

Table 1
^{1}H (500 MHz) and ^{13}C NMR data (125 MHz) of compound **1** (C_5D_5N)[a]

No.	δ_H mult. (*J* in Hz)	δ_C mult.	HMBC (H → C)
1		166.7 (s)	
3		157.8 (s)	
4	6.28 (s, 1H)	104.2 (d)	C-1′, C-3, C-4a, C-5, C-8a
4a		140.4 (s)	
5	6.64 (d, 2.1, 1H)	103.4 (d)	C-4, C-7, C-8a
6		167.4 (s)	
7	6.81 (d, 2.1, 1H)	102.5 (d)	C-5, C-6, C-8, C-8a
8		164.3 (s)	
8a		99.3 (s)	
1′	2.39 (m, 2H)	33.1 (t)	C-3, C-4, C-2′, C-3′
2′	1.61 (m, 2H)	26.5 (t)	C-3, C-4′
3′	1.37 (m, 2H)	29.0 (t)	C-1′, C-5′
4′	2.01 (m, 2H)	32.6 (t)	C-2′, C-3′, C-5′, C-6′
5′	5.54 (dt, 15.6, 7.1, 1H)	132.1 (d)	C-3′, C-6′, C-7′
6′	5.70 (dt, 15.6, 7.1, 1H)	128.2 (d)	C-4′, C-5′, C-7′, C-8′
7′	2.32 (m, 1H), 2.45 (m, 1H)	43.5 (t)	C-5′, C-6′, C-9′
8′	4.06 (dd, 6.0, 12.3, 1H)	67.3 (d)	C-6′, C-7′, C-9′
9′	1.35 (d, 6.0, 3H)	23.7 (q)	C-7′, C-8′

[a] Assigned by ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, NOESY, and HMBC experiments.

an α,β-unsaturated δ-lactone [δ166.7 (C-1)], one oxymethine carbon [δ 67.3 (C-8′)], seven non-oxygenated olefinic/aromatic carbons containing two quaternary carbons [δ 140.4 (C-4a), 99.3 (C-8a)], five methylenes, and one methyl group [δ 23.7 (C-9′)]. In the ^{1}H NMR spectra of **1** (Table 1), in addition to five methylene signals, two *meta*-coupled aromatic protons [δ 6.64 (d, *J* = 2.1 Hz, H-5), 6.81 (d, *J* = 2.1 Hz, H-7)], one singlet olefinic proton [δ 6.28, (H-4)], and two olefinic protons [δ 5.54 (dt, *J* = 15.6 and 7.1 Hz, H-5′), 5.70 (dt, *J* = 15.6 and 7.1 Hz, H-6′)], and one oxygenated methine proton [δ 4.06 (dd, *J* = 6.0 and 12.3 Hz, H-8′)] and its neighboring methyl group [δ 1.35 (d, *J* = 6.0 Hz, H-9′)] were observed. Furthermore, the HMBC spectrum (Fig. 2) of **1** showed correlations from H-4 (δ 6.28) to C-8a (δ 99.3) and C-5 (δ 103.4), from H-5 (δ 6.64) to C-8a, C-7 (δ 102.5), and C-4 (δ 104.2), and from H-7 (δ 6.81) to C-8a and C-5 (δ 103.4).

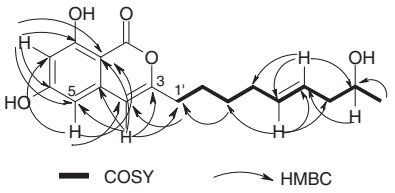

Figure 2. ^{1}H–^{1}H COSY and selected HMBC correlations of compound **1**.

A side chain structure [–$(CH_2)_4$CH=CHCH$_2$CH(OH)CH$_3$–] of **1** was identified from the ^{1}H–^{1}H COSY experiments depicted in Figure 2, which also revealed that the secondary hydroxy group was attached to C-8′ (δ 67.3). The hydroxy substitution was further substantiated by the HMBC correlations of H-9′ (δ 1.35) and H-6′ (δ 5.70) to the oxymethine carbon C-8′ (δ 67.3), and H-8′ (δ 4.06) to the olefinic carbon C-6′ (δ 128.2). The presence of the disubstituted olefin at C-5′ was also ascertained from the HMBC correlations of H-6′ to C-4′, C-5′, and C-8′, and H-5′ to C-3′, C-6′, and C-7′ (Fig. 2). These above mentioned data suggest that compound **1** contains a 6,8-dihydroxyisocoumarin moiety with an aliphatic substituent at C-3. The HMBC correlations from H-4 (δ 6.28) and H-3′ (δ 1.37) to C-1′ (δ 33.1), from H-1′ (δ 2.39) to C-3 (δ 157.8) and C-4 (δ 104.2) established the connectivity between the isocoumarin moiety and the side chain. The gross planar structure of **1** was further confirmed by the EI-MS spectrum (Fig. 3), which gave prominent fragment ion peaks at *m*/*z* 300 (M^+–H_2O), 177 [M^+–$C_9H_{16}OH$ (side chain)] and 192 ($C_{10}H_8O_4$).

The geometry configuration of the olefin at C-5′ in **1** was determined to have an *E*-configuration based on the large coupling constant ($J_{H\text{-}5'/H\text{-}6'}$ = 15.6 Hz). In addition, this compound showed the same positive sign of the optical rotation, [α] +1.01 (*c* 0.2, MeOH), as that of a known compound, (*S*)-3-hydroxybutyl benzoate, [α] +25 (*c* 2.00, $CHCl_3$),[14] and of several analogs of known absolute configuration such as (+)-(*S*)-dichlorodiaportin, [α] +10 (*c* 0.07 $CHCl_3$).[13a] Thus, the absolute configuration at C-8′ in **1** was presumed to be *S*∗. On the basis of these data, the structure of **1** was established as 3-(8′*S*∗-hydroxy-nona-5′*E*-enyl)-6,8- dihydroxyisochromen-1-one, named fusariumin.

The structures of aigialomycin D (**2**) and pochonin N (**3**) were determined on the basis of ESI-MS and ^{1}H, ^{13}C, and 2D NMR data.

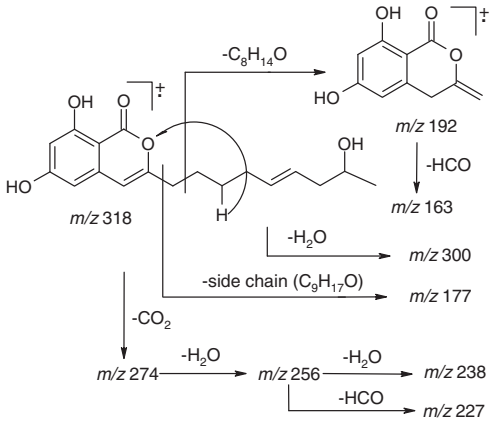

Figure 3. Proposed major EI-MS fragmentation of compound **1**.

These data were identical to those previously reported in the literature.[15,16] Recently, the absolute *S*-configuration of a methyl group in aigialomycin D (**2**) was assigned by its enantioselective total synthesis.[14] Aigialomycin D (**2**), which was first isolated from the fungus *Aigialus parvus* BCC 5311 found in the mangrove wood,[15] has been shown to possess potent antimalarial activity against *Plasmodium falciparum* K1 and antitumor activity against KB cells. Another radicicol analog, pochonin N (**3**), was newly isolated from a culture broth of the fungus *Pochonia chlamydosporia* TF-0480.[16] These two compounds, belonging to a novel family of naturally occurring 14-membered resorcylic acid lactones, also known as resorcinylic macrolides, are usually fungal metabolites with unique chemical structures and potent biological activities such as antitumor, antibiotic, antimalarial, and HSP90 inhibition.[17,18]

Interestingly, in the present study, fusariumin (**1**) and two related resorcinylic macrolides aigialomycin D (**2**) and pochonin N (**3**) were isolated as co-metabolites of *Fusarium* sp. Biogenetically, this suggests that they could be similar biosynthetic polyketide pathway in the same fungus. It has been reported that zearalenone (**4**) and its related members are nonaketide mycotoxins produced by a variety of different *Fusarium* fungal strains.[19] Zearalenone (**4**) was first isolated from *Gibberella zeae* (perfect stage of *Fusarium graminearum*) in 1962.[20] Accordingly, the three compounds are likely to originate from the zearalenone-type precursors. Notably, a hypothetical biosynthetic pathway for compound **1** is proposed in Figure 4. The isocoumarin formation can be explained by two routes: (i) enolization of carbonyl group (path a) and subsequent intramolecular *trans*-lactonization of the resulting enol hydroxyl, and (ii) initial macrolactone hydrolysis (path b) and subsequent esterification of the resulting carboxylic acid with the enol hydroxyl group.

The growth inhibitory activity of compounds **1–3** was evaluated against brine shrimp (*Artemia salina*). Chaetomugilin A was used as a positive control, a fungal metabolite isolated from an endophyte *Chaetomium globosum*.[11] After incubation for 24 h, three compounds **1–3** were found to display significant toxicity toward brine shrimp larvae at a concentration of 10 μg/ml, with mortality rates (%) of 78.2, 76.7 and 82.8, respectively, while chaetomugilin A showed mortality rate of 78.3% at the same concentration.

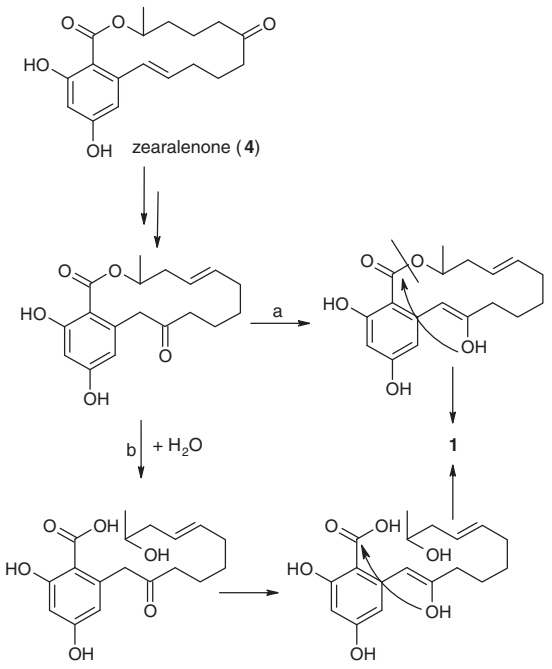

Figure 4. Hypothetical biosynthetic pathway for compound **1**.

In conclusion, during our chemical and bioactivity research into the plant-derived endophytic fungus *Fusarium* sp., a new naturally occurring isocoumarin, fusariumin (**1**), together with two known metabolites aigialomycin D (**2**) and pochonin N (**3**), was characterized. The isolated compounds showed remarkable growth inhibitory activity against the brine shrimp.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.043.

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12. *Data for fusariumin* (**1**): amorphous solid; $[\alpha]_D^{20}$ +1.01 (*c* 0.2, MeOH); UV λ_{max}(MeOH) nm (log ε) 378.7(4.23), 360.3(4.42), 345.7(4.33), 330.1(4.29), 270.2(4.80); IR (KBr) ν_{max} 3381, 1663, 1622, 1570, 1504, 1459, 1364, 1240, 1162, 1080, 974 cm^{-1}; EI-MS (70 eV): *m*/*z* (%) 318(2) [M^+], 300 (9), 274(75), 256 (100), 238 (21), 227(24), 215 (14), 192(62), 177(21), 163 (44), 150 (48), 121(46), 107(27), 91(8), 79(20), 69(23), 55(26); ESI-MS (180 °C): *m*/*z* (%) 319 $[M+1]^+$, 341 $[M+Na]^+$, 659 $[2M+Na]^+$; HRMS (ESI): calcd for $C_{18}H_{23}O_5$ 319.1540; found 319.1539 $[M+H]^+$; ^1H and ^{13}C NMR data, see Table 1.
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