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PAPER

Fusaroside, a unique glycolipid from *Fusarium* sp., an endophytic fungus isolated from *Melia azedarach*†

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Fusaroside (**1**), a unique trehalose-containing glycolipid composed of the 4-hydroxyl group of a trehalose unit attached to the carboxylic carbon of a long-chain fatty acid, was isolated from the organic extract of fermentation broths of an endophytic fungus, *Fusarium* sp. LN-11 isolated from the leaves of *Melia azedarach*. Six known compounds, phalluside (**2**), (9*R**,10*R**,7*E*)-6,9,10-trihydroxyoctadec-7-enoic acid (**3**), porrigenic acid (**4**), (9*Z*)-2,3-dihydroxypropyl octadeca-9-enoate (**5**), cerevisterol (**6**) and ergokonin B (**7**), were also isolated from this fungus. The glycolipid contains a rare branched long-chain fatty acid ($C_{20:4}$) with a conjugated diene moiety and a conjugated ketone moiety. The structure of the new compound **1** was elucidated by spectroscopic methods (1D and 2D NMR experiments, MS) and chemical degradations. The metabolites **1–5** were shown to have moderate to weak active against the brine shrimp larvae. To our knowledge, this is the first report of isolation of the first representative of a new family of glycolipids from natural sources.

Introduction

Endophytic fungi are microorganisms that live in the inter- and intracellular spaces of the tissues of apparently healthy host plants and do so in a variety of relationships, ranging from symbiotic to pathogenic.[1] Recently, statistical analyses showed that 51% of the biologically active metabolites obtained from endophytes are previously unknown, compared with only 38% of novel substances from soil microflora. During the last decade, 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] As a result, the study of fungal endophytes is currently considered a reasonable approach to the discovery of novel, bioactive natural products.[3,4]

Melia azedarach Linn (Meliaceae), also known as Chinaberry or Persian lilac tree, is a deciduous tree and has long been recognized for its insecticidal properties.[5] This plant produces a class of modified oxygenated triterpenoids, also known as limonoids.[6,7] In previous papers, we reported the isolation and structural elucidation of some new metabolites from the endophytes harboured inside the healthy tissues of *M. azedarach*.[8,9]

In our continuing search for new biologically active secondary metabolites from plant-derived fungi, we investigated chemical constituents from an endophyte *Fusarium* sp. LN-11 and isolated seven compounds including a novel glycolipid, named fusaroside (**1**), and six known compounds, **2–7**. Herein, we describe the isolation and structure characterization of the new natural glycolipid, as well as toxicity to the brine shrimp larvae of five lipids **1–5**.

Results and discussion

The fungus *Fusarium* sp. LN-11, originally obtained from the leaves of *M. azedarach* L., was grown in liquid culture at 28 °C. After 5 days, the culture broths obtained were filtered to give the mycelium. The mycelium was then dried and extracted ultrasonically by ethyl acetate and acetone successively to provide a crude extract. The extract was repeatedly subjected to column chromatography on silica gel, Sephadex LH-20, RP-18, and preparative TLC to yield five lipids (**1–5**), including a new glycolipid (**1**), and two sterols (**6** and **7**).

Fusaroside (**1**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{34}H_{54}O_{13}$ by a pseudomolecular ion peak at *m/z* 693.3457 $[M+Na]^+$ (calcd. for $C_{34}H_{54}O_{13}Na$: 693.3456) in the HRESIMS, with 8 degrees of unsaturation. The ^1H NMR spectrum recorded for **1** in C_5D_5N (Table 1) showed resonances for a series of deshielded methine groups between δ_H 4.1 and 5.9, characteristic of protons on sp^3 carbons attached to oxygen atoms, and a series of aliphatic methylene and methyl protons between δ_H 1.2 and 2.1, indicative of a branched aliphatic hydrocarbon fragment. These proton NMR features suggested that fusaroside (**1**) was a glycolipid.[10] Its positive ESI-MS/MS data generated prominent fragment ion peaks at *m/z* 693 $[M+Na]^+$,

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Table 1 ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR Data (ppm) of compound **1** and trehalose in C_5D_5N [a]

	1			trehalose
No.	δ_H (mult., J in Hz)	δ_C	HMBC	δ_C
1		173.7 s		
2		55.1 s		
3		196.6 s		
4	6.78 (dt, 15.0, 1.8, 1H)	126.1 d	2, 3, 6	
5	7.15 (dt, 15.0, 7.2, 1H)	148.8 d	3, 7	
6	1.91 (m, 2H)	32.4 t	4, 5, 8	
7	1.35 (m, 2H)	27.8 t	5, 8, 9	
8	1.27 (m, 2H)	29.4 t	6, 9, 10	
9	1.92 (m, 2H) [b]	32.5 t [b]	7, 11	
10	2.09 (m, 2H) [b]	33.0 t [b]	8, 11, 12	
11	5.49 (m, 1H) [c]	130.4 d [c]	9, 13	
12	5.40 (m, 1H) [c]	130.3 d [c]	10, 14	
13	5.40 (m, 1H) [c]	130.4 d [c]	11, 15	
14	5.49 (m, 1H) [c]	130.6 d [c]	12, 16	
15	2.09 (m, 2H) [b]	32.9 t [b]	13, 14, 17	
16	1.92 (m, 2H) [b]	32.9 t [b]	14, 18	
17	2.09 (m, 2H) [b]	33.0 t [b]	15, 19	
18	5.42 (dt, 15.0, 8.0, 1H)	131.3 t	16, 20	
19	5.44 (m, 1H)	125.2 t	17, 20	
20	1.61 (d, 4.8, 3H)	18.0 q	18, 19	
21	1.51 (s, 3H)	22.2 q	1, 2, 3, 22	
22	1.55 (s, 3H)	22.3 q	1, 2, 3, 21	
1′	5.87 (d, 3.6, 1H)	95.4 d	1″, 3′, 5′	95.3
2′	4.21 (dd, 9.8, 3.6, 1H)	73.4 d	1′, 4′	73.3
3′	4.69 (m, 1H)	72.5 d	1′, 4′, 5′	74.7
4′	5.77 (m, 1H)	73.5 d	1, 2′, 3′, 6′	72.1
5′	4.91 (m, 1H)	71.9 d	1′, 3′, 6′	74.1
6′	4.09 (dd, 12.0, 4.8, 1H) 4.19 (m, 1H)	62.0 t	4′, 5′	62.6
1″	5.85 (d, 4.2, 1H)	95.9 d	1′, 3″, 5″	95.3
2″	4.19 (dd, 9.8, 3.8, 1H)	73.7 d	1″, 4″	73.3
3″	4.72 (m, 1H)	74.9 d	1″, 4″, 5″	74.7
4″	4.25 (m, 1H)	72.3 d	2″, 3″, 6″	72.1
5″	4.89 (m, 1H)	74.4 d	1″, 3″, 6″	74.1
6″	4.36 (dd, 11.4, 4.8, 1H) 4.44 (dd, 12.0, 3.0, 1H)	62.8 t	4″, 5″	62.6

[a] Assigned by DEPT, COSY, HSQC, and HMBC experiments.[b,c] Assignments may be interchanged.

531 [M+Na-hexose]$^+$, 513 [M+Na-hexose-H_2O]$^+$, and 347 [M+H-2 × hexose]$^+$, suggesting **1** to contain two hexose residues.

The ^{1}H NMR data of fusaroside (**1**) (Table 1) revealed the presence of a terminal allylic methyl at δ_H 1.61 (3H, d, J = 4.8 Hz), two methyl groups H-21 (δ_H 1.51) and H-22 (δ_H 1.55) at a tertiary carbon, methylene protons as multiplets between δ_H 1.27 and 2.09, and eight olefinic protons between δ_H 5.40 and 7.15. Furthermore, the ^{13}C NMR and DEPT spectra of **1** with the aid of HSQC data (Table 1) showed the presence of 34 carbon signals, 22 of which are recognized as three methyls, eight aliphatic methylenes, eight olefinic methines, and three quaternary carbons. Among them, three quaternary carbons including an ester carbonyl at δ_C 173.7 (C-1), a ketone carbon at δ_C 196.6 (C-3) and an sp^3 carbon at δ_C 55.1 (C-2), eight olefinic carbons between δ_C 125.2 and 148.8, and two methyl groups C-21 (δ_C 22.2) and C-22 (δ_C 22.3) at a tertiary carbon, together with the terminal methyl at δ_C 18.0 (C-20) were observed. The above evidence indicated that **1** contains a long-chain fatty acid moiety with four double bonds and a geminal-dimethyl group.

The positions of the four double bonds in the fatty acyl chain were determined by HMBC, COSY and EI-MS spectra (Fig. 2 and 3). The HMBC spectrum of **1** displayed correlations observed between H-4 and C-2, C-3, C-6, between H-11 and C-9, C-13, H-13 and C-11, C-15, between H-18 and C-16, C-20, and between H-19 and C-17, C-20, indicating these double bonds to be located at C-4, C-11, C-13 and C-18 (Fig. 2). This deduction was further supported by fragmentations occurring along the fatty acid chain observed in the EI-MS spectrum of **1**, showing a set of diagnostic fragment ions of m/z 231, 206, 137, 110, 85 and 55 (Fig. 3). Moreover, analysis of the ^{1}H–^{1}H COSY data led to the identification of two proton spin systems shown in Fig. 2, locating the two double bonds at C-4 and C-18 of structure **1**, respectively. Likewise, these above HMBC and COSY correlations also reinforced the presence of three features present in the fatty acid moiety, namely a conjugated enone and a conjugated diene, as well as of an allylic group, which were revealed by three sets of their respective proton resonances at δ_H 6.78 (1H, dt, J = 15.0, 1.8 Hz, H-4), δ_H 7.15 (1H, dt, J = 15.0, 1.8 Hz, H-5)], and at δ_H 5.49 (each H, m, H-11 and H-14), 5.40 (each H, m, H-12 and H-13), as well as at δ_H 5.42 (1H, dt, J = 15.0, 8.0 Hz, H-18), 5.44 (1H, m, H-19), and 1.61 (3H, d, J = 4.8 Hz, H-20) in the ^{1}H NMR spectrum of **1**.

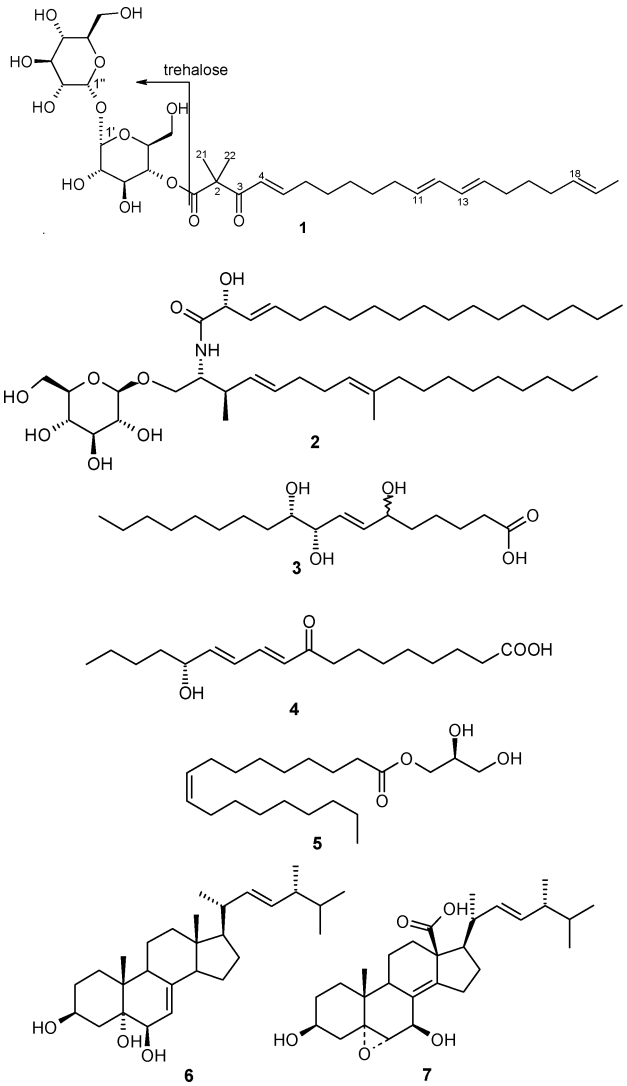


Fig. 1 Structures of metabolites **1**–**7**.

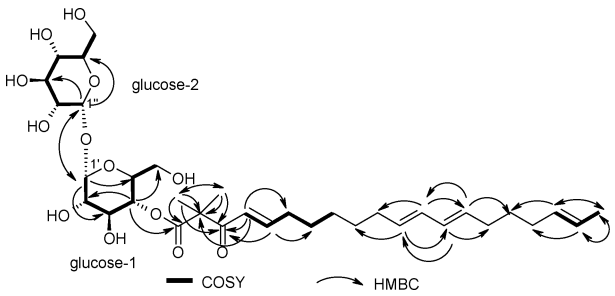


Fig. 2 Key COSY and HMBC correlations in **1**.

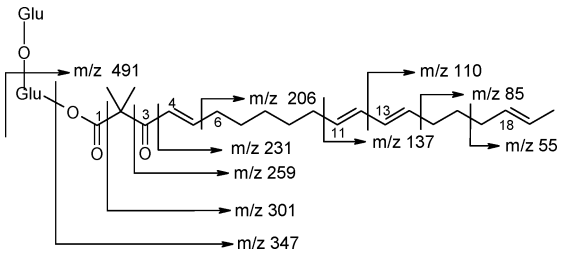


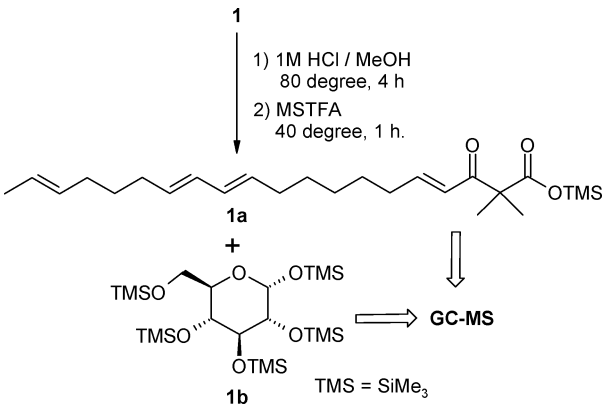
Fig. 3 Proposed major EI-MS fragmentation pathway of **1**.

On the other hand, the location of one carbonyl group at C-3 and two additional tertiary methyl branches at C-2 on the alkene chain were ascertained from the HMBC spectrum, showing correlations of H-21 with C-22, C-1, C-2, C-3, of H-22 with C-21, C-1, C-2, C-3, of H-4 with C-3, C-2, and of H-5 with C-3 (Fig. 2). This was further confirmed from characteristic fragment ions at *m/z* 231, 259, and 301 of the EI-MS spectrum of **1** (Fig. 3).

The limited amounts of **1** prevented the use of NOE spectroscopy to determine the configuration of the double bonds. Thus, the geometry of the double bonds of the long-chain alkene moiety in structure **1** can be determined from the ^{13}C NMR chemical shifts of the methylene carbon next to the olefinic carbon, namely, the carbon signals observed between δ_C 27–28 in *cis* type and between δ_C 32–33 in *trans* type.[11,12] The *trans* configuration of these double bonds was deduced from the chemical shifts of C-6 (δ_C 32.4), C-10 (δ_C 33.0), C-15 (δ_C 32.9) and C-17 (δ_C 33.0). In addition, the 4*E*,18*E* configurations of **1** were also in agreement with the large vicinal coupling constants ($^3J_{H\text{-}4\text{-}H\text{-}5}$ and $^3J_{H\text{-}18\text{-}H\text{-}19}$ values of *ca*. 15 Hz) (Table 1). Therefore, it is clear that **1** possesses a fatty acyl chain with the all-*E* geometry of the double bond moiety.

Determination of the length of the long-chain fatty acyl moiety of compound **1** was carried out by chemical degradation. A very small amount of **1** was subjected to methanolysis with HCl in MeOH, followed by silylation with MSTFA (*N*-methyl-*N*-ethyldimethylsilyltrifluoroacetamide) (Scheme 1),[13] leading to products, silylated fatty acid (**1a**) and silylated glucose (**1b**). The silylated fatty acid **1a** was subjected to GC-MS analysis, allowing us to identify **1a** as the sole product of the reaction due to typical fragment ions at *m/z* 419 $[C_{22}H_{33}O_3SiMe_3+H]^+$. The structure of the fatty acid moiety in **1** was accordingly determined to be (4*E*,11*E*,13*E*,18*E*)-2,2-dimethyl-3-oxo-4,11,13,18-tetra-eneicosanoic acid, as indicated in structure **1**. This rare branched fatty acid is first to be discovered from natural sources.

The ^{1}H and ^{13}C NMR and HSQC NMR data revealed two anomeric proton resonances at δ_H 5.87 of glucose-1 (H-1′) and 5.85 of glucose-2 (H-1″), attached to the corresponding anomeric



Scheme 1 Microscale chemical degradation procedure of **1**.

carbon resonances at δ_C 95.4 of glucose-1 (C-1′) and 95.9 of glucose-2 (C-1″), in addition to a number of oxymethines and oxymethylenes between δ_H 4.09 and 5.77, and between δ_C 62.0 and 74.9. 2D NMR (^{1}H–^{1}H COSY, TOCSY, HSQC, and HMBC) experiments (Fig. 2 and Table 1) allowed the assignment of the proton and carbon shifts for each of the two monosaccharides in the molecule. Starting from the anomeric protons H-1′ and H-1″, interpretation of COSY and TOCSY spectra revealed two sugar spin systems corresponding to two hexoses. HMBC correlations observed between H-1′ and C-5′ (δ_C 71.9) of the glucose-1, and between H-1″ and C-5″ (δ_C 74.4) of the glucose-2, indicated that the hexoses existed in the pyranose forms. The lack of dispersion in the methine proton resonances between δ_H 4.1 and 5.9 regions of the sugar moiety could not resolve vicinal coupling constants for the H-3 to H-5 regions in both systems, but only vicinal coupling constants ($J_{H\text{-}1'\text{-}H\text{-}2'}$ = 3.6, $J_{H\text{-}1''\text{-}H\text{-}2''}$ = 4.2 Hz) for the H-1 to H-2 regions observed are all small, consistent with all equatorial–axial relationships of these protons (Table 1). This discloses that both monosaccharides were glucose residues having α anomeric configurations.

In order to identify the absolute configuration of the sugar, the GC-MS retention times of the resulting silylated sugar **1b** were compared with those of silylated authentic sugars using the reaction conditions described above for transformation of the natural product.[13] Analysis without further purification of **1b** showed three peaks attributable to isomeric α/β-pyranone/furanone derivatives (Fig. S11, ESI†). These were identical, within error limits, to peaks observed for TMS-glucose. All other investigated sugars were clearly different. In addition, the configuration of natural glucose is essentially D. Therefore, the results confirmed the presence of the glucoses in **1** and showed that the absolute configurations of both glucose residues were determined as D.

HMBC data provided evidence for the nature of the linkages between the two monosaccharides. The anomeric proton of the glucose-1 (H-1′) of **1** showed cross-peaks with C-3′ (δ_C 72.5), C-5′ (δ_C 71.9) and C-1″, and the other anomeric proton of the glucose-2 (H-1″) with C-3″ (δ_C 74.9), C-5″ (δ_C 74.4) and C-1′, in comparison to the ^{13}C NMR data of an authentic sample of sugar α,α-trehalose in C_5D_5N, which established a 1,1-glycosidic linkage between the two glucose residues (Fig. 2 and Table 1). These data confirmed the disaccharide chain to be α-D-glucopyranosyl-(1-1)-α-D-glucopyranosyl residue, also known as α,α-trehalose residue.

Table 2 Toxicity of metabolites **1–5** with mortality rates (%)

Sample	1	2	3	4	5
mortality	47.6	64.8	26.2	20.9	18.7

Finally, the relatively downfield chemical shift of H-4′ (δ_H 5.77) suggested that the trehalose is esterified at C-4′, as confirmed by a cross-peak between the proton H-4′ of the glucose-1 and the ester carbonyl carbon C-1 of the fatty acyl group in the HMBC experiment. This indicated the fatty acyl chain to be linked to C-4′ of the sugar chain *via* an ester linkage. The structure of **1** was thus unambiguously established as 4′-(4*E*,11*E*,13*E*,18*E*)-2,2-dimethyl-3-oxo-4,11,13,18-tetraeneeicosanoyl α-D-glucopyranosyl-(1–1)-α-D-glucopyranoside, named fusaroside, as shown in Fig. 1.

The structures of compounds **2–7** were determined on the basis of ESI-MS and ^{1}H, ^{13}C, and 2D NMR data, as well as by comparison with data reported in the literature, as phalluside-1 (**2**),[14] (9*R**,10*R**,7*E*)-6,9,10-trihydroxyoctadec-7-enoic acid (**3**),[15] porrigenic acid (**4**),[16] (9*Z*)-2,3-dihydroxypropyl octadeca-9-enoate (**5**),[17] cerevisterol (**6**)[18] and ergokonin B (**7**).[19] Compounds **2–7** (except **6**) are reported from endophytic fungi for the first time.

The isolated metabolites **1–5** were tested for *in vitro* toxicity toward brine shrimp (*Artemia salina*) using the reported assay in which fusariumin, an isocoumarin obtained from the same fungal strain, was used as a positive control (mortality rate: 78.2%).[8] They were found to possess weak to moderate growth inhibitory effects on brine shrimp larvae at concentrations of 10 μg ml^{-1}, as given in Table 2. The glycolipids **1** and **2** exhibited moderate toxicity, with mortality rates of 47.6% and 64.8%, respectively.

It is noteworthy that phalluside-1 (**2**) is a glucosphingolipid that was isolated in 1998 from the marine ascidian *Phallusia fumigata*;[14] oxylipin **3** is a trihydroxylated fatty acid that was reported from a traditional herb, *Dracontium loretense*, with toxicity to human peripheral blood mononuclear cells;[15] porrigenic acid (**4**), a conjugated ketonic fatty acid, was isolated as a cytotoxic constituent of the higher mushroom *Pleurocybella porrigens*;[16] and fatty acid ester **5** is a monoglyceride present in a medicinal plant *Rhazya stricta*.[17] Furthermore, with respect to polyoxygenated sterols cerevisterol (**6**) and ergokonin B (**7**), the former is commonly found in fungi, which has been previously reported as a cytotoxic agent, and inhibited the activity of DNA polymerase alpha,[19] and the latter has been found to be an antifungal antibiotic which was isolated in 1991 from the fungus *Trichoderma koningii*.[20] Biogenetically, both steroids have been known to be biosynthezied from ergosterol, a ubiquitous fungal sterol.

Glycolipids are membrane components and occur in all kingdoms of living organisms, *i.e.*, bacteria, plants, and animals. They are usually divided into three major groups, glycoglycerolipids, glycosphingolipids, and isoprenoid glycosides, dependent on their lipid moiety. A number of glycolipids occur, however, that cannot be classified in any of these groups. These compounds are made of a glycosyl residue attached to the hydroxyl group of a fatty alcohol or a hydroxy fatty acid, or to the carboxyl group of a fatty acid (ester linkage). They typically exist in bacteria and yeast and exhibit interesting biological properties.[21] To the best of our knowledge, in the present study, fusaroside (**1**), which is a glycolipid made of an unusual branched fatty acid and an α,α-trehalose, is quite rare and has not been reported previously. Notably, this family of glycolipids is previously unreported. In addition, trehalose is a disaccharide widely present in bacteria, yeast, and fungi, as well as some plants.[22] Recent studies have shown that trehalose does not only primarily function as a reserve carbohydrate, but also as a highly efficient protectant, enhancing the resistance of cellular components against adverse conditions such as high temperature, freezing, and low dehydration.[21] The fungal metabolites that may be formed in fungi constitute their chemical defense system against parasites, as well as various predators such as bacteria, fungi, animals, and insects.[23] This newly discovered fungal glycolipid **1**, in combination with other lipids, might be presumed to act as phytoalexins occurring in endophytic fungi. It should be pointed out that the biological functions of the new glycolipid and other substances present in the endophytic *Fusarium* sp. remains unclear, and that further investigation needs to be performed.

Conclusions

In conclusion, fusaroside (**1**), the first example of a novel class of glycolipids, which was produced by the endophytic fungus *Fusarium* sp., has been revealed to consist of an uncommon polyunsaturated long-chain fatty acid with three separate olefinic structure elements, a conjugated diene, a conjugated ketone and a monoene, and a trehalose residue. Moreover, six previously reported metabolites **2–7** were also obtained from this fungus. Compounds **2–7** (except **6**) have been described from endophytic fungi for the first time. The five lipids **1–5** were found to exert some toxic effects on brine shrimp larvae.

Experimental section

General

The optical rotations were measured on a Perkin-Elmer polarimeter 241 polarimeter at the sodium D line. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer with KBr pellets, peaks are reported in cm^{-1} on Perkin–Elmer 1600 Series FT-IR. UV/vis spectra were recorded on a Varian Cary 100 UV-visible spectrophotometer; peak wavelengths are reported in nm. ^{1}H NMR spectra were recorded on Varian Inova 500 (499.8 MHz), and Varian Inova 600 (600 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d = doublet, dd = doublet doublet, t = triplet, q = quartet, m = multiplet, br = broad. ^{13}C NMR spectra were recorded with Bruker Avance 500 (125.7 MHz). Chemical shifts were measured relative to tetramethylsilane (TMS) as the internal standard. 2D NMR spectra: H, H COSY spectra (^{1}H,^{1}H Correlated Spectroscopy), HMBC spectra (Heteronuclear Multiple Bond Connectivity), HSQC spectra (Heteronuclear Multiple Quantum Coherence) and NOESY spectra (Nuclear Overhauser Effect Spectroscopy). Mass spectra were recorded with EI MS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosine as the standard. ESI mass spectra were recorded on a Quattro Triple Quadrupol mass spectrometer, with a Finnigan TSQ 7000 with nano-ESI API ion source. High-resolution ESI mass spectra were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC with a diode array detector.

GC-MS was performed on a Thermo Finnigan Trace GC-MS system (Autosampler AS 2000), using a Varian column CP-Sil

8CB for amines (30 m × 0.25 mm i.d., 0.25 μm film thickness). The temperature was programmed from 40 °C (held for 1 min) to 300 °C (held for 4 mins), ramping the temperature up at 10 °C min^{-1}. The derivatized sugar residues of **1** were identified by comparison of their retention times and fragmentation pattern with standards.

Silica gel (Merck) 60–120 mesh for column chromatography and pre-coated TLC sheets (layer thickness 0.2 mm) and preparative TLC plate (layer thickness 1.25 mm) of silica gel 60 GF_{254} were used. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in methanol.

Fungal material

The endophytic fungal strain was isolated from the fresh leaves of the tree *M. azedarach* L. growing in the campus of Northwest A&F University, Yangling, Shaanxi province, China. The isolate was identified as *Fusarium* sp. LN-11 by morphological analysis and was deposited at the Research Centre for Natural Medicinal Chemistry, Northwest A&F University.

Cultivation

After growing on potato dextrose agar (PDA) medium at 28 °C for 5 days, the fungus was inoculated in liquid medium containing: $CaCl_2$ 0.5 g, KH_2PO_4 0.1 g, KCl 0.05 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, glucose 20.0 g, peptone 15.0 g, 1000 ml H_2O. The pH was adjusted to 6.0 before autoclaving. Fermentation was carried out in 1000 ml flasks each containing 200 ml medium on a rotary shaker at 150 rpm at 28 °C for 5 days.

Extraction and isolation

The culture broth (20 L) of *Fusarium* sp. LN-11 was filtered to give the mycelium and water phase. The mycelium was dried at 50 °C, and smashed directly with ultrasonic extraction by ethyl acetate and acetone three times. These two parts were combined after TLC, and defatted with cyclohexane after being dissolved in methanol to get a crude extract (2.7 g). The crude extract was subjected to a CC over silica gel and eluted with CH_2Cl_2–MeOH (100 : 0–50 : 50) in a gradient elution manner to provide seven fractions (Fr1.–Fr7.). Fr.6 (0.3 g) was purified by Sephadex LH-20 column chromatography (MeOH) and reversed phase RP-18 column chromatography (MeOH–H_2O) to yield compounds **1** (1.8 mg) and **2** (5.1 mg). Fr.5 (0.5 g) was separated by Sephadex LH-20 CC using MeOH, followed by preparative TLC with CH_2Cl_2–MeOH (6 : 1) to afford compounds **3** (4 mg), **4** (6 mg) and **5** (5 mg). Fr.4 (0.4 g) was rechromatographed on a Sephadex LH-20 column (CH_2Cl_2–MeOH, 6 : 4), and further purified by preparative TLC (CH_2Cl_2–MeOH, 9 : 1) to give compounds **6** (7 mg) and **7** (8 mg).

Absolute configuration of sugar

A solution of about 0.1 mg of **1** in 50 μL of MeOH and 50 μL of 1 M HCl was hydrolyzed at 80 °C. After 4 h, the sample was taken to dryness at 0.1 mbar at room temperature, and the residue was derivatized with 50 μL of MSTFA (*N*-methyl-*N*-ethyldimethylsilyltrifluoroace-tamide) at 40 °C for 60 min. GC-MS analysis showed three signals, with retention times of 18.60, 19.02 and 19.85 min. D-Glucose (18.58, 19.16, 19.93 min), D-mannose (18.20, 19.26, 20.01 min), and D-galactose (18.30, 18.76, 19.22 min) as standards were analyzed as above.

Fusaroside (1). Amorphous solid. $[\alpha]_D^{20}$ = +179 (*c* = 0.186, MeOH); EI-MS (70 eV): *m/z* (%) 491(4), 347 (2), 301(4), 275 (3), 259 (8), 231 (9), 232(13), 175 (6), 163 (19), 145 (27), 137 (17), 127 (18), 109 (30), 110 (16), 111 (8), 85 (34), 81 (43), 73 (75), 55 (100); MS (ESI) (180 °C): *m/z* (%) 693 $[M+Na]^+$, 705 $[M+Cl]^+$, 1363 [2 M+Na]$^+$. ESI-MS/MS: *m/z* (%) 693 (27), 532 (10), 531 (100), 513 (34), 347 (12), 231 (5). HR-MS (ESI): *m/z* calcd. for $C_{34}H_{54}O_{13}Na$: 693.3456; found: 693.3457 $[M+Na]^+$; ^1H- and ^{13}C-NMR spectroscopic data, see Table 1.

Brine shrimp (*Artemia salina*) bioassay

The brine shrimp toxicity was assayed by small modified microtiter-plate method using brine shrimp *Artemia salina* as a test organism. Briefly, approximately 30 nuclei larvae hatched from eggs of *A. salina* in 0.2 ml of artificial sea water which were incubated with a sample (5 μl in DMSO solution) in a deep-well microtiter plate at room temperature. After 24 h, the dead larvae were determined by counting the number of the dead animals in each well under a microscope. To each test row, a blind sample was accompanied by adding DMSO. The mortality rate was calculated using the formula: M = [(A–B–N)/(G–N)]×100

M = percent of the dead larvae after 24 h; A = number of the dead larvae after 24 h; B = average number of the dead larvae in the blind samples after 24 h; N = number of the dead larvae before starting the test; G = number of selected larvae for test.

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