One New Terphenyl Glycoside From a Sponge-Derived Fungus Trichoderma reesei (HN-2016-018)

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Abstract
One new terphenyl glycoside (1), gliocladinin D, together with 4 known compounds (2-5) were isolated from the sponge-derived fungus Trichoderma reesei (HN-2016-018). The structure of the new compound was elucidated by the comprehensive spectroscopic analysis, including 1-dimensional and 2-dimensional nuclear magnetic resonance, and high-resolution electrospray ionization mass spectrometry. Compound 3 exhibited moderate Topo I inhibitory activity.

Keywords
sponge-derived fungus, Trichoderma reesei, terphenyl glycoside, phenolics, Topo I inhibitory activity

In the current era, marine-derived fungi have attained high focus as a promising source for biologically and pharmaceutically active secondary metabolites and have grown into the third largest source of marine natural products.¹² In this perspective, fungi associated with sponges yield numerous molecules with diverse structures and potential biological activities,³ like glycosides,⁴ terpenoids,⁵ alkaloids,⁶ and polyketides.⁷ Terphenyls are a class of aromatic compounds possessing an 18-carbon tricyclic core skeleton, categorized into o-, m-, and p-subclasses.⁸ p-Terphenyls are the leading class of terphenyls reported from the natural sources compared with the other terphenyl groups.⁹ The majority of terphenyls have been isolated from fungal sources; therefore, they are considered as fungal secondary metabolites,⁹,¹⁰ although, few of terphenyls have also been reported from moss and actinomycetes.¹¹,¹² Moreover, the terphenyl family exhibited extensive activities, including antitumor, antibacterial, antiaging, anticoagulant, neuroprotective, immunosuppressive, and α-glucosidase inhibitory effects.⁹ A literature survey revealed that p-terphenyl glycosides exhibited cytotoxic⁰,¹³ and antibacterial activities.¹⁴ For example, gliocladinin C, a p-terphenyl glycoside isolated from a potato-derived fungus Chaetomium subaffine L01 showed strong cytotoxic activity against human tumor cell lines Hep-2 and HepG-2.¹³

Large number of terphenyl derivatives have been reported from marine sources. For example, 2 new terphenyl derivatives, terphyl acid, and terphyl diacid, along with 6 known terphenyls, possessing cytotoxic activity were reported from the marine-derived Aspergillus sp. AF119.¹⁵ Ivanets et al reported terphenyl derivative 3″-hydroxyterphenyllin from a fungus Aspergillus sp. collected from an unknown colonial ascidian.¹⁶ Aspergillus candidus IF10 obtained from marine environment produced 3 new terphenyls, 4-deoxy-isoterprenin, 4-de-oxyprenylterphenyllin, and prenyl-terphenyllin, exhibiting strong cytotoxic activities.¹⁷ As a part of our current investigation on exploring for new marine natural bioactive products, 5 p-terphenyl glycosides were isolated from a coral derived-fungus Aspergillus candidus.¹⁸ During our continuous study on new bioactive p-terphenyl glycosides, the chemical profile of the crude extract obtained from Trichoderma reesei also draws our attention due to the presence of p-terphenyl characteristic UV pattern. One new
terphenyl glycoside gliocladinin D (1), along with four previously reported secondary metabolites, 2-(2′-hydroxypropyl)-5-methyl-7-hydroxychromone (2), pachybasin (3), emodin (4), and vertinolide (5), were obtained from the ethyl acetate (EtOAc) extract of T. reesei by employing different isolation techniques. Herein, we reported their chromatographic separation, structural elucidation, and bioactivity evaluation of these compounds.

Gliocladinin D (1) was attained as a white amorphous powder and its high-resolution electrospray ionization mass spectrometry (HR-ESIMS) presented the mass ion peak of 543.1872 [M – H]−, establishing its molecular formula as C28H32O11 (13 degrees of unsaturation). 1H-nuclear magnetic resonance (NMR) spectrum displayed four signals of methoxy groups (δH 3.79 (×2), 3.40, 3.23) and a pair of phenyl ring system substituted at para positions (δH 6.91 [2H, d, J = 8.5 Hz], 7.31 [2H, d, J = 8.5 Hz], and 6.97 [2H, d, J = 8.5 Hz], 7.41 [2H, d, J = 8.5 Hz]). The 13C NMR spectrum presented 28 carbons including 4 methoxy, 8 aromatic methine, and 10 quaternary carbons, indicating a typical terphenyl ring system. In addition, a glucose residue was revealed in the structure of 1 based on the NMR characteristic resonances for glucose moiety. These NMR spectral features of 1 were in close resemblance to that of the reported gliocladinin C from the fungus Chaetomium subaffine L01 isolated from a potato tissue.13 The obvious difference is the addition of a methoxy signal, which was connected to C-6′ based on the heteronuclear multiple bond correlation (HMBC) (Figure 1) from the proton signal (δH 3.23) to C-6′. In addition, the methoxy group (δC 60.4, δH 3.40) and aromatic quaternary carbon (C-3′, δC 141.4; C-5′, δC 143.7) in 1 replaced the methoxy group (δC 54.9, δH 3.70) and aromatic quaternary carbon (C-3′, δC 148.0; C-5′, 154.6) in gliocladinin C. The HMBC (Figure 1) from the proton signal (δH 3.40) to C-3′ indicated that the methoxy group was attached to C-3′ rather than C-5′. The HMBC linkage (Figure 1) from the anomeric proton of glucose ring H-1″′ (δH 4.81) to C-2′ (δC 139.0) of terphenyl moiety indicated that the glucose ring was positioned at C-2′. Moreover, the two remaining methoxy groups (δH 3.79 × 2) should be connected with phenyl rings at C-4 and C-4″ respectively, based on the HMBC (Figure 1). Thus, the structure of compound 1 could be portrayed (as shown in Figure 2) and given the name as gliocladinin D.

The downfield chemical shift of glucose carbon C-1″′ (δC 102.3) and the coupling constant of anomeric H-1″′ (J = 7.6 Hz) recommended that the glucose unit was connected with terphenyl ring through β-linkage.14 To identify the absolute configuration of glucose moiety, acid hydrolysis of 1 was performed with 6 N hydrochloric acid (HCl), resulting that glucose moiety was released. Compared with the optical rotations of standard samples of d-glucose ([α]20D +100) and l-glucose ([α]20D −80), the optical rotation of the released glucose was [α]20D +20, indicating the glucose residue in 1 should be d-glucose.

Figure 1. 1H-1H correlated spectroscopy and key heteronuclear multiple bond correlation of compound 1.
The isolated compounds were evaluated for their cytotoxic, topoisomerase I inhibitory, and antibacterial activities. Unfortunately, all the tested compounds including the terphenyl glycoside gliocladinin D (1) displayed no cytotoxicity. Only compound 3 displayed moderate deoxyribonucleic acid (DNA) topoisomerase I inhibitory activity with the minimum inhibitory concentrations (MIC) value of 100 µM. None of the isolated compounds displayed antibacterial activity against the tested pathogenic bacteria.

**General Methods**

Optical rotation was measured with a JASCO P-1020 digital polarimeter. Ultraviolet (UV) spectrum was recorded on a UH 5300 UV-Vis spectrophotometer in methanol (MeOH). Infrared (IR) spectrum was recorded on a Nicolet-Nexus-470 FT-IR spectrometer with potassium bromide (KBr) pellets. \(^1\)H, \(^{13}\)C NMR spectra were recorded on a JEO-LJNM-ECP NMR spectrometer (500/125 MHz for \(^1\)H and \(^{13}\)C), using tetramethylsilane as an internal standard. High-performance liquid chromatography (HPLC) separation was attained by utilizing a Hitachi LA-2000 preparative-HPLC system, along with a Hitachi L-2455 photodiode array detector. ESIMS spectra were measured on a Thermo DSQ Elmass spectrometer and HR-ESIMS on MAT95XP high-resolution mass spectrometer. Silica gel (200-300 mesh size) (Qing Dao Hai Yang Chemical Group Co.) and Sephadex LH-20 (Amershm Biosciences) were used for column chromatography. Precoated silica gel (GF254) plates (Yantai Zifu Chemical Group Co., Yantai, China) were used for thin-layer chromatography. Sea salt (Qingdao Salt Industry Co., Ltd.) was used in fermentation.

**Fungal Materials**

The fungus *T. reesei* (HN-2016-018) was isolated from a piece of inner fresh tissue of an unidentified sponge, which was collected from the South China Sea in October 2016, and recognized on the basis of its morphological characteristics and by amplification and sequencing of the DNA sequences of the ITS region. The fungus was identified as *T. reesei* whose 500 base pair ITS sequence had 99% sequence identity to that of *T. reesei*. The strain was deposited in the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, PR China, with the GenBank (NCBI) accession number MN209898.

**Extraction and Isolation**

The fungal strain *T. reesei* (HN-2016-018) was cultivated on a solid rice media using 100 L Erlenmeyer flasks for 28 days (each flask containing 100 g of rice, 110 mL of water, and 3.3 g of sea salt) at 28°C. After 28 days, each flask of the fermented medium was extracted repeatedly (3 times) with 300 mL of EtOAc. The collected organic extract was dried by means of a rotary evaporator to attain a residue (114 g). The residue was fractioned into 5 fractions (Fr. A–Fr. E) by subjecting it into vacuum liquid chromatography eluting with EtOAc–petroleum ether (PE) (0%-100%) and methyl alcohol (MeOH)–dichloromethane (CH₂Cl₂) (0%-100%). Fr. B was further processed by Sephadex LH-20 column chromatography (CC) eluting with a mixture of CH₂Cl₂–MeOH (1:1). The ultimate purification was carried out on HPLC eluted with 70% MeOH/H₂O to attain compounds 1 (6 mg) and 2 (4.0 mg). Fr. C was applied to CC on silica gel (PE–EtOAc, v/v; 9:1) and then further purified by Sephadex LH-20 CC with a mixture of CH₂Cl₂/MeOH (1:1) to attain compounds 3 (20 mg), 4 (15.0 mg), and 5 (90 mg), respectively.

**Gliocladinin D (1)**

White amorphous powder.
UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 220 (4.35), 256 (4.43) nm.

IR (KBr) $v_{max}$: 3344, 2929, 2360, 1612, 1540, 1458, 1246, 1031 cm$^{-1}$.

$1^H$-NMR and $13C$-NMR: see Table 1.

(–)-HR-ESIMS $m/z$ 543.1872 [M – H]$^–$ (calcd for C$_{28}$H$_{31}$O$_{11}$, 543.1872).

**Determination of the Configuration of the Sugar Residue**

Compound 1 (1.5 mg) was dissolved in acetone (300 µL) along with 6 N HCl (700 µL) and added to the hydrolysis tube. The reaction was remained at 100°C for 24 hours and stopped by adding 3 mL of water. Following that aglycone was separated from glucose by extracting with 2 mL of EtOAc, and the aqueous phase was separated to examine its optical rotation. The configuration of the glucose moiety in compound 1 was determined by comparing the specific optical rotations of released glucose residue versus standard D- and L-Glucose.

**DNA Topo I Inhibitory Activity Assay**

Topoisomerase I inhibitory activity was judged by evaluating the easing of the super-coiled pBR-322 plasmid of DNA. Camptothecin was used as a positive control. Ciprofloxacin was used as a positive control.

**Cytotoxicity Assay**

The cytotoxicity was measured with the sulforhodamine B method by using liver hepatocellular carcinoma (HepG2), human cancer of the colon (HCT116), human lung cancer (A-549), human cervical cancer (HeLa), and human pancreatic cancer cells (PANC-1), human colon cancer cell (SW480). The half-maximal inhibitory concentration value was analyzed by software GraphPad Prism 5. The adriamycin was used as a positive control.

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**Supplemental Material**

Supplemental material for this article is available online.

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