

CHEMICAL CONSTITUENTS OF THE FUNGUS

Glomerella cingulata

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Endophytes are microorganisms that reside in the internal tissues of living plants without having any immediate overt negative effects on its hosts [1–3]. As it lives in the special eco-environment, the endophytic fungus seems to be an especially fruitful producer of biologically active secondary metabolites with which it probably regulate its interactions with its hosts [4–11]. The research on endophytes showed that they are rich sources of diverse and bioactive metabolites [12, 13]. During a program of investigation on potentially bioactive secondary metabolites from Formosan endemic plant endophytes, an endophytic fungal strain, *Glomerella cingulata* (Polyatigmataceae) BCRC 09F0211, was isolated from the leaves of *Syzygium formosanum* (Hayata) Mori (Myrtaceae). Previous chemical investigations of the genus *Glomerella* have received less attention, and only a few articles had been reported [14–16]. In the course of our search for potential diverse secondary metabolites from natural fungal sources, and to further understanding of the minor metabolites of *Glomerella*, we examined an EtOAc-soluble fraction of *G. cingulata*, which showed inhibitory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) release in RAW 264.7 murine macrophages, as determined by our primary screening (approximately 75% inhibition at a concentration of 10 µg/mL). Current phytochemical investigation of the abovementioned fungus has led to the isolation of nine metabolites, ubiquinone Q9 (**1**) [17], α -tocopheryl quinone (**2**) [18], *trans*-phytol (**3**) [19], indole-3-carbaldehyde (**4**) [20], a mixture of 24-methylenecycloartanol (**5**), cycloartenol (**6**) [21], β -sitosterol (**7**) [22], β -sitostenone (**8**) [23], and ergosterol peroxide (**9**) [24]. The structures of the isolated metabolites were determined by extensive analysis of their spectroscopic data as well as by comparison with literature data. The chemistry and bioactivity of *Glomerella cingulata* have seldom been contacted previously. All compounds **1–9** were isolated for the first time from the genus *Glomerella*.

General Experimental Procedures. TLC: silica gel 60 F₂₅₄ precoated plates (Merck). Column chromatography (CC): silica gel 60 (70–230 or 230–400 mesh, Merck) and Spherical C18 100A Reversed Phase Silica Gel (RP-18) (particle size: 20–40 µm; Silicycle). UV spectra: Jasco UV-240 spectrophotometer. Optical rotation: Jasco DIP-370 polarimeter. IR spectra: Perkin-Elmer-2000 FT-IR spectrophotometer. ¹H, ¹³C, and 2D NMR spectra: Varian-VNMRS-600, and Varian-Mercury-400 spectrometers. EI-MS: VG-Biotech Quatro-5022 mass spectrometer; (*m/z*, (*I*_{rel.}, %)). HR-EI-MS spectra were recorded on a Finnigan/Thermo Quest NAT mass spectrometer. ESI and HR-ESI-MS: Bruker APEX-II mass spectrometer.

Microorganism, Cultivation, and Preparation of the Fungal Strain. The fungus used in this study was collected on May, 2013 in Pingtung County Townships, Taiwan, and identified by one of the authors, Dr. Sung-Yuan Hsieh. The strain is preserved with the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (FIRDI), under the ID No. 09F0211. Fourteen-day-old colonies of the *Glomerella* sp. strain on PDA medium in a 9-cm Petri dish were cut into the bottle and blended for 30 s with 100 mL distilled water to prepare the fungal inoculum for solid-state fermentation. Fermentation was carried out in 50 Erlenmeyer flasks (100 mL), each containing about 100 g of rice. Distilled H₂O (105 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 120°C for 30 min.

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