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SECONDARY METABOLITES ISOLATED FROM THE FUNGUS *Biscogniauxia cylindrospora* BCRC 33717

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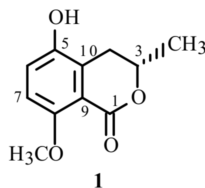
*One new dihydroisocoumarin, (3*S*)-5-hydroxy-8-*O*-methylmellein (**1**), as well as nine known compounds, 5-formylmellein (**2**), mellein-5-carboxylic acid (**3**), 5-hydroxymethylmellein (**4**), 3β-hydroxystigmast-5-en-7-one (**5**),* N-trans-*feruloyltyramine (**6**),* N-cis-*feruloyltyramine (**7**), vanillic acid (**8**), methyl paraben (**9**), and syringaldehyde (**10**), were isolated from the* n-*BuOH-soluble fraction of the 70% EtOH extract of rice fermented with the endophytic fungus* Biscogniauxia cylindrospora *(BCRC 33717). Their structures were elucidated by 1D and 2D NMR spectroscopy together with HR-ESI-MS analysis and comparison of the spectroscopic data with those reported for structurally related compounds.*

Keywords: *Biscogniauxia cylindrospora*, Xylariaceae, secondary metabolites.

Endophytic fungi living in the intracellular spaces of plants are thought to possess diverse biological properties, and are expected to produce many novel bioactive secondary metabolites [1–4]. An endophytic fungal strain, named BCRC 33717, from the bark of the medicinal plant *Cinnamomum* sp., was collected from Sun-shei, Taipei, Taiwan, during September of 2000. This strain was determined to be *Biscogniauxia cylindrospora* (family Xylariaceae) based on their cultural and anamorphic data by Dr. Y.-M. Ju [5].

The Xylariaceae is a large family (Xylariales, Ascomycotina) of 36 or more genera. Secondary metabolites produced by representatives from at least one-third of these genera have now been isolated and identified [6]. A variety of structurally diversified compounds, including terpenoids [7], cyclopeptides [8, 9], polyketides [10, 11], cytochalasins [12], xanthones [13, 14], and unique unclassified xyloketals [15], is widely distributed in the *Xylaria* sp. Contrary to *Xylaria* sp., the metabolites of the genus *Biscogniauxia* have received less attention until now. To further understand the chemotaxonomy of the genus *Biscogniauxia* and to continue searching for novel bioactive metabolites from Xylariaceae, *B. cylindrospora* was chosen for a phytochemical investigation. Careful examination of the above title fungus has resulted in the isolation of one new dihydroisocoumarin, named (3*S*)-5-hydroxy-8-*O*-methylmellein (**1**), together with 9 known constituents **2**–**10**. Compounds **2**–**9** were found for the first time from this species. The structures of these compounds were established by means of spectral experiments. We herein report the isolation and characterization of the new compound.



Compound **1**, obtained as an optically active oil, showed the molecular formula $C_{11}H_{12}O_4$ as determined by HR-ESI-MS at *m/z* 231 $[M + Na]^+$ (calcd 231.0633). UV absorption bands at 217 (4.02), 270 (3.81), and 308 (3.60) nm demonstrated that **1** was structurally related to a dihydroisocoumarin group [16]. Its IR spectrum showed absorption bands for a hydroxyl at

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3400 cm^{-1}, a conjugated carbonyl at 1652 cm^{-1}, and a benzene ring at 1600 and 1510 cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectral data displayed a hydroxyl group [δ 4.77 (1H, br.s)], a tetrasubstituted benzene [δ 6.79 (1H, d, J = 9.0 Hz, H-7) and δ 111.2 (C-7), 6.98 (1H, d, J = 9.0 Hz, H-6) and δ 120.9 (C-6), δ 155.5 (C-8), 113.7 (C-9), 128.3 (C-10), 144.8 (C-5)], and a methoxyl [δ 3.89 (3H, s, 8-OMe) and δ 56.4]. Additionally, the AB portion of a typical ABX system [δ 4.53 (1H, m, H-3) and δ 74.0 (C-3), δ 2.62 (1H, dd, J = 16.8, 11.7 Hz, H-4a), 3.10 (1H, dd, J = 16.8, 3.0 Hz, H-4b) and δ 29.5 (C-4)] was observed. These features were similar to those of the known compound (3*R*)-5-hydroxy-8-*O*-methylmellein [17]. This deduction was confirmed by correlations between signals at H-3 and C-4 and C-10, and H-4a, 4b and C-10, C-5, C-3, and C-9 in the HMBC spectrum. The location of the methoxyl and hydroxyl were assigned on the basis of HMBC correlations from methoxyl protons to C-8 and hydroxyl proton to C-5. The absolute configurations at C-3 of compound **1** was determined as 3*S* by the positive value of its $[\alpha]_D$ and the positive Cotton effect shown at 275 nm, which is similar to those of known related compounds [18, 19].

From the above data, compound **1** was characterized as (3*S*)-5-hydroxy-8-methoxy-3-methylisochroman-1-one, named (3*S*)-5-hydroxy-8-*O*-methylmellein, and its structure was illustrated as **1**, which was further confirmed by COSY, NOESY, HSQC, and HMBC experiments. Although compound **1** is a stereoisomer of (3*R*)-5-hydroxy-8-*O*-methylmellein [17], the structure of **1** has not yet been reported from natural sources.

The other known isolates, 5-formylmellein (**2**) [20], mellein-5-carboxylic acid (**3**) [21], 5-hydroxymethylmellein (**4**) [22], 3*β*-hydroxystigmast-5-en-7-one (**5**) [23], *N-trans*-feruloyltyramine (**6**) [24], *N-cis*-feruloyltyramine (**7**) [25], vanillic acid (**8**) [26], methyl paraben (**9**) [26], and syringaldehyde (**10**) [26] were readily identified by comparison of their spectral data (UV, IR, ^{1}H NMR, MS) with the data from the corresponding values in the literature. Among them, all known compounds were isolated from *Biscogniauxia* species for the first time.

EXPERIMENTAL

General Experimental Procedures. All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. Optical rotations were measured on Jasco P-1020 digital polarimeter, UV spectra were obtained on a Jasco UV-240 spectrophotometer in MeOH, and IR spectra (KBr or neat) were taken on a Perkin–Elmer System 2000 FT-IR spectrometer. 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectra using $CDCl_3$ as solvent were recorded on a Varian Unity Plus 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) and 600 (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR) spectrometers. Chemical shifts were internally referenced to TMS as the internal standard. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems) spectrometer and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer. Low-resolution EI-MS spectra were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Silica gel (70–230, 230–400 mesh) (Merck) was used for column chromatography, and silica gel 60 F-254 (Merck) was used for TLC and preparative TLC.

Fungus Material. *Biscogniauxia cylindrospora* (BCRC 33717) was used throughout this study, and specimens have been deposited at the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute. *B. cylindrospora* BCRC 33717 was maintained on potato dextrose agar (PDA), and the strain was cultured on potato dextrose agar slants at 25°C for 7 days and the spores harvested by sterile water. The spores (5×10^5) were seeded into 300 mL shake flasks containing 50 mL RGY medium (3% rice starch, 7% glycerol, 1.1% polypeptone, 3% soybean powder, 0.1% $MgSO_4$, 0.2% $NaNO_3$), and cultivated with shaking (150 rpm) at 25°C for 3 days. After the mycelium enrichment step, an inoculum mixing 100 mL mycelium broth and 100 mL RGY medium was inoculated into plastic boxes (25 cm × 30 cm) containing 1 kg sterile rice and cultivated at 25°C for producing the rice, and the above-mentioned RGY medium was added to maintain the growth. After 28 days of cultivation, the rice was harvested and used as a sample for further extraction.

Extraction and Separation of Compounds. The rice of the *B. cylindrospora* BCRC 33717 (1 kg) was extracted three times with 70% EtOH at room temperature. The ethanol syrup extract was partitioned between *n*-BuOH–H_2O (1:1) to afford *n*-BuOH (fraction A, 1.5 g) and H_2O (fraction B, 880 mg) soluble fractions. The *n*-BuOH-soluble fraction (1.5 g) was chromatographed over silica gel (70–230 mesh), eluting with CH_2Cl_2, and enriched with acetone to produce 12 fractions (A1–A12). Fraction A-2 (150 mg) was chromatographed over silica gel, eluting with CH_2Cl_2–EtOAc (15:1→1:1), to obtain 5 fractions (A-2-1–A-2-5). Fraction A-2-3 (21 mg) was purified by preparative TLC to afford 3*β*-hydroxystigmast-5-en-7-one (**5**) (1.5 mg). Fraction A-3 (230 mg) was chromatographed over silica gel, eluting with CH_2Cl_2–MeOH (15:1→0:1), to obtain 10 fractions (A-3-1–A-3-10). Fraction A-3-3 (26.3 mg) was repeatedly purified by preparative TLC to afford (3*S*)-5-hydroxy-

8-*O*-methylmellein (**1**) (1.5 mg). Fraction A-3-7 (41 mg) was purified by preparative TLC (*n*-hexane–EtOAc 1:1) to give 5-hydroxymethylmellein (**4**) (3.5 mg). Fraction A-4 (165 mg) was resubjected to silica gel column chromatography (CH_2Cl_2–MeOH 20:1→1:1) to afford 8 fractions (A-4-1–A-4-8). Fraction A-4-3 (45 mg) was repeatedly purified by preparative TLC (CH_2Cl_2–EtOAc, 10:1) to afford *N-cis*-feruloyltyramine (**7**) (1.1 mg). Fraction A-6 (263 mg) was resubjected to silica gel column chromatography (CH_2Cl_2–MeOH 12:1→1:1) to afford 7 fractions (A-6-1–A-6-7). Fraction A-6-3 (27 mg) was repeatedly purified by preparative TLC (CH_2Cl_2–EtOAc 10:1) to afford syringaldehyde (**10**) (2.4 mg). Fraction A-6-6 (32 mg) was repeatedly purified by preparative TLC (CH_2Cl_2–MeOH 20:1) to afford vanillic acid (**8**) (2.4 mg). Fraction A-7 (150 mg) was chromatographed over silica gel, eluting with CH_2Cl_2–acetone (10:1), to obtain 10 fractions (A-7-1–A-7-10). Fraction A-7-4 (40 mg) was repeatedly purified by preparative TLC to afford methyl paraben (**9**) (2.4 mg). Fraction A-8 (112 mg) was chromatographed over silica gel, eluting with CH_2Cl_2–acetone (18:1), to obtain 5 fractions (A-8-1–A-8-5). Fraction A-8-4 (15 mg) was repeatedly purified by preparative TLC to afford 5-formylmellein (**2**) (1.9 mg). Fraction A-10 (450 mg) was chromatographed over silica gel, eluting with CH_2Cl_2–MeOH (10:1→1:1), to obtain 10 fractions (A-10-1–A-10-10). Fraction A-10-4 (40 mg) was repeatedly purified by preparative TLC to afford mellein-5-carboxylic acid (**3**) (1.7 mg) and *N-trans*-feruloyltyramine (**6**) (1.8 mg).

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