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NOTE

Mycoleptione, a new chromone derivative isolated from the endophytic fungus *Mycoleptodiscus* sp. MU41

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Endophytic fungi colonizing plants produce a wide variety of compounds, possibly because the plant environment stimulates them to synthesize bioactive compounds in order to adapt or be of benefit to their hosts, and hence they have been considered an excellent source for the discovery of novel secondary metabolites.[1,2] Thailand, which is located in a tropical zone, has high biodiversity, especially in plants, and thus is one of the promising countries for obtaining endophytes. As some of the biological activity of the plants used for medicinal purposes might be attributed to the microbes inhabiting the plants, particular attention has been paid to the endophytes, and consequently several novel compounds have been obtained.[3–6]

During our preliminary screening to discover structurally novel secondary metabolites of endophytic fungi isolated from Thai medicinal plants,[7] an endophytic fungus, *Mycoleptodiscus* sp., was isolated from *Tinospora crispa*. *T. crispa* has been used as traditional medicine in rural society in Thailand in order to treat fever, cholera, snake bites, rheumatism and fever due to malaria. Furthermore, this plant is currently being investigated for its antioxidant, antidiabetic, antimalarial and cosmetic effects.[8]

The genus *Mycoleptodiscus* that is classified as a plant-associated fungus has also attracted great attention because of its several interesting abilities. For example, several reports have proposed that *Mycoleptodiscus terrestris* can be used as a biological control agent for management of the world's aquatic weeds, including hydrilla and Eurasian watermilfoil,[9–11] and that *Mycoleptodiscus indicus* produces the compound eugenitin, which can activate glucoamylase from *Aspergillus niveus*.[12] Based on these facts, *Mycoleptodiscus* spp. might be regarded as an interesting unexplored target for screening novel secondary metabolites.

The preliminary screening for novel compounds with HPLC-DAD analysis detected two unknown metabolites in *n*-BuOH extract from the static culture of *Mycoleptodiscus* sp. MU41 (cultured in 10 ml of medium 6 in a test tube (size Ø16 × 150 mm) for 21 days at 28 °C) (Supplementary S2), which have the maximum UV absorption at 210, 242, 250, 288 nm and 204, 256, 380 nm, respectively. As the comparison of those spectra with the data registered in our in-house database suggested the discovery of new compounds and only limited metabolites have been reported from *Mycoleptodiscus* sp., we determined to isolate and identify the compounds from *Mycoleptodiscus* sp. MU41.

The mycelia of *Mycoleptodiscus* sp. MU41 was inoculated into 100 ml of medium 6 (glucose 1%, mannitol 10%, pharmamedia (Southern Cotton Oil Co., Memphis, TN, USA) 3.5%, KH_2PO_4 0.9%) in 500-ml baffled flasks, and cultivated for 21 days at 28 °C under a static condition to obtain **1** and under an agitated condition on a reciprocal shaker at 120 r.p.m. to yield **2**. After static cultivation, the cultures (100 ml × 20 flasks) were extracted with an equal amount of EtOAc, which was then evaporated to afford a crude extract (brown gum, 4 g). The crude extract was separated on a C_{18} chromatograph column by stepwise elution with increasing MeOH concentrations ($MeOH/H_2O$ = 0:10, 2:8, 4:6, 5:5, 6:4, 8:2, 9:1 and 10:0 v/v). The 50% MeOH fractions containing compound **1** were evaporated (67.7 mg). Compound **1** was further purified by preparative reversed-phase HPLC with a shallow MeOH gradient in 0.1% trifluoroacetic acid (a 55–65% MeOH gradient over a period of 20 min), yielding pure **1** (6.1 mg). To obtain **2** from the agitated culture, the whole culture (200 ml) was directly filtrated by filter paper (90 mm; Advantec, Tokyo, Japan). The culture filtrate (125 ml) was subjected to HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) column chromatography and was fractionated by stepwise elution with increasing MeOH concentrations ($MeOH/H_2O$ = 2:8, 4:6, 6:4, 8:2 and 10:0 v/v). The 60 and 80% MeOH fractions containing **2** were combined and evaporated. The residue (61.8 mg) was further purified by two rounds of preparative reversed-phase HPLC; the first round was conducted with an MeOH gradient in 0.1% trifluoroacetic acid (a 30–100% MeOH

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Table 1 NMR spectroscopic data of mycoleptione (1) in CD_3OD

Position	δ_C	δ_H	*HH-COSY*	*HMBC*
2	167.4	—		
3	112.5 (CH)	6.06 (s)		C-2, C-10, C-1′
4	182.0	—		
5	143.6	—		
6	118.0 (CH)	6.62 (dd, $J=2.2$ Hz)	H-(5-CH_3)	C-8, C-10, 5-CH_3
7	163.0	—		
8	101.7 (CH)	6.65 (d, $J=2.2$ Hz)		C6, C9
9	161.5	—		
10	115.9	—		
5-CH_3	23.1 (CH_3)	2.71 (s)	H-6	C-5, C-6, C-10
1′	42.4 (CH_2)	2.60 (dd, $J=8.7$, 14.5 Hz)	H-2′	C-2, C-3, C-2′, C-3′
		2.76 (dd, $J=4.0$, 14.5 Hz)	H-2′	C-2, C-3, C-2′
2′	71.5 (CH)	3.91 (m)	H-1′, H-3′	
3′	31.2 (CH_2)	1.57 (m)	H-2′, H-4′	C-2′, C-1′, C-4′
4′	10.3 (CH_3)	1.01 (t, $J=7.4$ Hz)	H-3′	C-3′, C-2′

^1H, ^{13}C NMR and 2D NMR spectra were obtained on JOEL JNM-ECS400 NMR spectrometers, in CD_3OD at room temperature, and the solvent peak was used as an internal standard (δ_H 3.31 and δ_C 49.0 in CD_3OD).

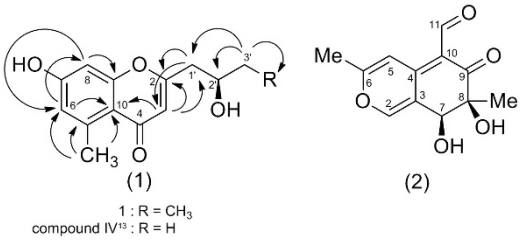

Figure 1 Structure of mycoleptione (**1**), compound VI,7-epiaustdiol (**2**).

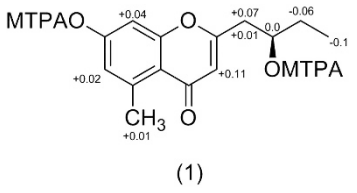

Figure 2 $\Delta\delta$S-*R* values of MTPA esters of mycoleptione (**1**).

gradient over a period of 35 min) (12.6 mg); the second round was performed with a shallow MeOH gradient in 0.1% trifluoroacetic acid (a 35–45% MeOH gradient over a period of 20 min), yielding pure **2** (8.1 mg).

Compound (**1**) was obtained as a yellow powder. The molecular formula was determined to be $C_{14}H_{16}O_4$ based on high-resolution electron impact mass spectrometry (obs. *m/z* 248.1046 $[M]^+$, calcd. 248.1043 for $C_{14}H_{16}O_4$), and ^1H and ^{13}C NMR spectra data (Table 1). The IR spectrum showed a broadened OH absorption at 3398 cm^{-1}. The ^1H NMR spectrum of **1** indicated two methyl signals at δ 1.01 (t, $J=7.4$ Hz, 3H) and 2.71 (s, 3H), two methylene protons at δ 1.57 (m, 2H), 2.60 (dd, $J=8.7$, 14.5 Hz, 1H) and 2.76 (dd, $J=4.0$, 14.5 Hz, 1H), one methine proton at δ 3.91 (m, 1H) and three aromatic protons at δ 6.06 (s, 1H), 6.62 (d, $J=2.2$ Hz) and 6.65 (d, $J=2.2$ Hz, 1H). The ^{13}C NMR and DEPT data of **1** revealed the presence of 14 carbon signals, including one carbonyl carbon at δ 182.0, five quaternary carbons at δ 115.9, 143.6, 161.5, 163.0 and 167.4, two methyl carbons at δ 10.3 and 23.1, two sp^3 methylene carbons at δ 31.2 and 42.4, one sp^3 methine carbon at δ 71.5 and three sp^2 methine carbons at δ 101.7, 112.5 and 118.0. In addition to the characteristic UV and IR spectra, such as the maximum UV absorption at 242, 250 and 288 nm, and a strong absorption band at 1645 cm^{-1} in the IR spectrum,[13] the observed carbon signals at δ 101.7, 112.5, 115.9, 118.0, 143.6, 161.5, 163.0, 167.4 and 182.0 in the ^{13}C NMR spectrum suggested the presence of a 5, 7-disubstituted chromone moiety.[14] The complete structure of **1** was deduced by comprehensive interpretation of its ^1H, ^{13}C NMR, HH-COSY, HSQC and HMBC spectra and other spectroscopic data.

The physicochemical data of **1** resembled those of the first chromone compound (compound IV; Figure 1) reported by Kashiwada *et al.*,[13] except for the signals (C-3′ δ 42.4 and C-4′δ 10.3) originating from the ethyl group connected to the C-2′, while a methyl group was attached in compound IV. The substitution of the methyl group in compound IV for an ethyl group at the 2-hydroxybutyl chain was further confirmed by HH-COSY correlation of H-3′ (δ 1.57) with H-2′ (δ 3.91), and also by long-range correlation of H-3′ (δ 1.57) with C-2′ (δ 71.5). Judging from these data, the structure of compound **1** was assigned as a chromone bearing a 2-hydroxybutyl chain.

The absolute configuration at C-2′ was deduced by the Mosher ester method.[15] Negative Δ δ S-R values were observed for H-3′(−0.06) and H-4′(−0.1), while positive Δ δ S-R values were observed for H-1′ (+0.01, +0.07), H-3 (+0.11), H-6 (+0.02), H-8 (+0.04) and H-5-CH_3(+0.01), indicating a 2′R absolute configuration for **1** (Figure 2 and Supplementary S8). Consequently, the structure of **1** was determined to be (*R*)-7-hydroxy-2-(2-hydroxybutyl)-5-methyl-4*H*-chromen-4-one and compound **1** was designated as mycoleptione (Figure 1).

Compound (**1**): a yellow powder; $[\alpha]_D^{26}$ -26 (*c* 0.10, MeOH); m.p. 158–162 °C; UV (MeOH) λ_{max} (log ε) 210 (4.34), 242 (4.21), 250 (4.23), 288 (4.08) nm; high-resolution electron impact mass spectrometry *m/z* $[M]^+$ 248.1046 (calcd. for $C_{14}H_{16}O_4$, 248.1043). IR ν_{max} (film) 3151–3398 (OH group), 2972, 2931, 2881, 1645 (C=O), 1577, 1396, 1340, 1276, 1159 cm^{-1}. For ^1H (CD_3OD_3, 400 MHz), ^{13}C (CD_3OD_3, 100 MHz), HH-COSY and HMBC, see Table 1 and Supplementary Figures S3 and S4.

The antimicrobial activity of mycoleptione was evaluated by the procedure of the Clinical and Laboratory Standards Institute (CLSI) using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29923, *Enterococcus faecalis* ATCC 29212, *Saccharomyces cerevisiae* ATCC 6275, *Candida albicans* OUT 6266, *Aspergillus niger* ATCC 6275, *Rhizopus oryzae* ATCC10404 and *Geotrichum candidum* IFO4598 as indicator strains.[16] However, no activities against these strains were detected (MIC > 512 μg ml^{-1} for antibacterial and > 128 μg ml^{-1} for anti-yeast and antifungal assays). Subsequently, the antioomycetes activity against *Aphanomyces cochlioides* AC-5 and *Phytophthora sojae* P6497 was measured by the agar plug method of Strobel with some modifications.[17] Mycoleptione showed moderate activity against *A. cochlioides*, with an MIC value of 6.25 μg ml^{-1}, but had no effect against *P. sojae* even at 100 μg ml^{-1}.

In addition to the determination of **1**, compound **2** was identified as 7-epiaustdiol based on its spectroscopic data (Figure 1 and Supplementary Figures S5, S6, S7 and S9).[18,19] The antioomycetes assay revealed that **2** displayed strong activity against both

A. cochlioides and *P. sojae*, with MIC values of <0.39 and $1.56\,\mu g\,ml^{-1}$, respectively. This is the first report that 7-epiaustdiol (**2**) is produced in this genus and has the antioomycete activities.

Antioomycetes assay: the MIC of **1** and **2** against two plant pathogenic oomycetes, *A. cochlioides* AC-5 and *P. sojae* P6497, was determined with a 96-well microplate (Zellkultur Test Plate 96F; Zellkultur, Basel, Switzerland) using the method modified from Strobel *et al.*[17] The compound dissolved in methanol by serial two-fold dilution (3 µl) was dispensed into a microplate containing 300 µl of potato dextrose broth and V8 for *A. cochlioides* and *P. sojae*, respectively, for final concentration ranging from 0.39 to $100\,\mu g\,ml^{-1}$. An agar plug (5 mm diameter) from the 7-day-old culture of the oomycetes was placed into each well. The antioomycetes activity was evaluated after incubation for 5–6 days by comparing with a negative control containing culture broth and microorganisms without the compounds. Hygromycin B dissolved in MeOH was used as the positive control, and 3 µl of MeOH was used as the negative control. The lowest concentration of the compound under which the microorganism could not grow was considered the MIC.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)