NOTE

Tylopilusin C, a new diphenolic compound from the fruiting bodies of *Tylopilus eximius*

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Fungi are known as producers of many important bioactive compounds, including clinical medicines and research reagents. Certain fruit bodies of fungi are used as traditional medicines especially in Asian area.^{1–3} During our screening for new metabolites from fungi, four new diphenolic compounds, named (±)-tylopilusin A and (±)-tylopilusin B, were isolated from the fruiting bodies of *Tylopilus eximius*.⁴ Continuing precise analysis of the metabolites from the fungus, one new diphenolic congener, tylopilusin C (**1**), was isolated (Figure 1). In this study, we describe the isolation, structural elucidation and biological activity of **1** in brief.

The fungus *T. eximius* was collected at Nagano, Japan in 2010. The fresh fruit body (300 g) was extracted with methanol (500 ml) for 2 months. After this extract being evaporated to aqueous solution, the residue was partitioned between water and EtOAc (ethyl ethanoate) to yield the tylopilusin mixture (1.1 g) after evaporation of the EtOAc fraction. The crude extract was dissolved in a small volume of chloroform, applied on a silica gel column (22 g, 28 × 14 cm²,

0.04–0.063 mm, MERCK, Darmstadt, Germany) and eluted stepwise with 100% chloroform, 20:1, 10:1, 5:1 and 1:1 (v/v) chloroform–methanol and 100% methanol (200 ml each). Tylopilusin C was observed in the fraction eluted with 1:1 chloroform–methanol. This fraction was further purified by reversed-phase C18 HPLC (20 × 250 mm², PEGASIL ODS, Senshu Scientific Co., Tokyo, Japan) under the following conditions; solvent, a 30-min linear gradient from 20–40% acetonitril in 0.05% trifluoroacetic acid; a flow rate of 8.0 ml min^{–1}; UV detection at 340 nm. Under these conditions, tylopilusin C was eluted as a peak with a retention time of 20.1 min. This fraction was collected and concentrated to yield pure tylopilusin C (6.0 mg) as a pale yellow solid.

The structure of **1** was mainly elucidated by analysis of NMR spectra, including 2D NMR. The molecular formula for **1** was established as C₁₈H₁₂O₇ ([M + H]⁺ *m/z* 341.0659) on the basis of high resolution ESI-MS measurement, indicating that **1** contained 13 degrees of unsaturation. (Table 1) The IR spectrum of **1** showed

Figure 1 Structures of tylopilusin C (**1**), tetramethyltylopilusin C (**2**), tylopilusins A1 and B1.

Table 1 Physico-chemical properties of tylophilus C

	Tylophilus C
Appearance	Pale yellow solid
Molecular formula	C ₁₈ H ₁₂ O ₇
Molecular weight	340
HR-ESI-MS <i>m/z</i>	(M + H) ⁺
Calcd	341.0661 (for C ₁₈ H ₁₃ O ₇)
Found	341.0659
UV _λ ^{MeOH} nm (ε)	336 (2,600), 278 (4,200), 247 (5,200)
IR _ν ^{KBr} cm ⁻¹	3259, 1685

Table 2 NMR spectroscopic data in DMSO-*d*₆ for tylophilus C

Position	δ _H ^a	δ _C ^b	HMBC
1		121.4	
2	7.93, d (<i>J</i> = 9.0)	128.8	C-4, 6, 7
3	6.92, d (<i>J</i> = 9.0)	115.6	C-1, 4, 5
4		159.0	
5	6.92, d (<i>J</i> = 9.0)	115.6	C-1, 3, 4
6	7.93, d (<i>J</i> = 9.0)	128.8	C-2, 4, 7
7		145.0	
8		141.7	
9		173.3	
10		125.5	
11		159.1	
12		162.2	
13		121.7	
14	7.06, d (<i>J</i> = 9.0)	131.0	C-10, 16, 18
15	6.75, d (<i>J</i> = 9.0)	114.0	C-13, 16, 17
16		157.3	
17	6.75, d (<i>J</i> = 9.0)	114.7	C-13, 15, 16
18	7.06, d (<i>J</i> = 9.0)	131.0	C-10, 14, 16
4-OH	10.07, s		C-3, 4, 5
8-OH	—		
12-OH	—		
16-OH	9.50, s		C15, 16, 17

Abbreviation: DMSO-*d*₆, dimethyl sulfoxide-D₆.^aChemical shifts are shown with reference to DMSO-*d*₆ as δ 2.49.^bChemical shifts are shown with reference to DMSO-*d*₆ as δ 39.7.

characteristic absorption at 3259 and 1685 cm⁻¹, suggesting the presence of a carbonic acid moiety. The ¹H NMR spectrum of **1** (in DMSO-*d*₆ (dimethyl sulfoxide-D₆)) showed four types of aromatic methine signals. (Table 2) Analysis of the ¹H NMR data using decoupling methods suggested two types of 1,4-disubstituted benzene rings. The ¹³C NMR spectrum (in DMSO-*d*₆) showed 18 resolved signals, which were classified into 8 *sp*² methine carbons and 10 quaternary carbons, including 2 carbonyl carbons (C-9 and C-12) and 5 oxygenated *sp*² carbons (C-4, C-7, C-8, C-11 and C-16) (Table 2). Among them, C-1 to C-6 and C-13 to C-18 were identified as 1, 4-disubstituted benzene ring carbons. Furthermore, the chemical shifts of C-7 (δ 145.0), C-8 (δ 141.7), C-9 (δ 173.3), C-10 (δ 125.5) and C-11 (δ 159.1) suggested that a 3-hydroxy-4-pyrone ring should exist.^{5,6} In additional HMBC NMR experiment, the cross peaks from 2-H and 6-H (δ 7.93) to C-7 indicated that one benzene is linked at C-7 of the pyranone ring. Furthermore, correlations from 14-H and 18-H (δ 7.06) to C-10 allowed the other benzene and the pyranone functionalities to be joined at C-10 as shown in Figure 1. Taking into consideration the molecular formula and the IR data, a carbonyl acid moiety was connected at C-11. Based on all the data, the total structure of **1** was elucidated as shown in Figure 1. Unfortunately, signals of 8-OH and 12-OH were not observed in ¹H NMR experiments. To confirm the structure, **1** was methylated with trimethylsilyl diazomethane,⁷ and the derivative **2** was analyzed by HI-ESI-MS measurements. (Methylation of tylophilus C; Tylophilus C (1.8 mg) was dissolved in MeOH (200 μl) and benzene (800 μl) and trimethylsilyl diazomethane was added (500 μl, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) at 25 °C for 30 min. The reaction mixture was evaporated *in vacuo* to obtain the products (1.8 mg). The products were purified by reversed-phase C-18 HPLC (10 × 250 mm², PEGASIL ODS), a 70% acetonitrile in 0.05% trifluoroacetic acid, at a flow rate 2.5 ml min⁻¹ with UV detection at 340 nm. Under this condition, tetramethyltylophilus C (**2**) was eluted as a peak with a

Scheme 1 Proposed biosynthetic pathway of tylophilus.

retention time of 13.2 min. This peak was collected and concentrated to yield 1.0 mg as pale yellow solid.) (Tetramethyltylopilus C (**2**): Pale yellow solid; ^1H NMR (600 MHz, CDCl_3) δ_{H} 8.07 (2H, d, $J=9.0$ Hz), 7.24 (2H, d, $J=9.0$ Hz), 7.02 (2H, d, $J=9.0$ Hz), 6.96 (2H, d, $J=9.0$ Hz), 3.92 (3H, s), 3.89 (3H, s), 3.84 (3H, s) and 3.75 (3H, s); HR-ESI-MS $[\text{M} + \text{Na}]^+ m/z$ 419.11098 ($\text{C}_{22}\text{H}_{20}\text{O}_7\text{Na}$, calcd 419.1107).) The molecular formula of **2** was established as $\text{C}_{22}\text{H}_{20}\text{O}_7$, indicating that four methyl residues were incorporated into **1**. Furthermore, four methoxyl methyl signals (δ 3.92, 3.89, 3.84 and 3.75) were observed in ^1H NMR experiments. Thus, the structure of the derivative was elucidated as **2**, supporting the structure of **1**.

The plausible biosynthetic pathway is proposed in Scheme 1. The biosynthetic precursor atromentin,⁸ which was isolated from the same broth of this strain, is biosynthesized from two L-tylosines by enzymes AtrA and AtrD.⁹ Next, atromentin can be opened in the central ring (I) and re-cyclized to afford tylopilus A and/or tylopilus C type ring systems by enzymatic reactions.

The biological activity of **1** was investigated using in-house assay system. By our routine antimicrobial assay systems,¹⁰ **1** (20 μg per 6-mm disk) showed no antimicrobial activity against Gram-positive and -negative bacteria, fungi and yeasts¹⁰ tested. However, **1** was found to inhibit the yellow pigment production by methicillin-resistant *Staphylococcus aureus* (MRSA) with a white zone (8 mm) at 10 μg per 6-mm disk.¹¹ These data suggested that **1** might selectively inhibit staphyloxanthin production by MRSA without any effect on the growth of MRSA.

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