

Article

Two New Coumarins from *Talaromyces flavus*

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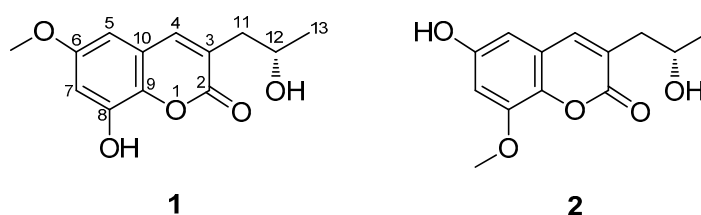
Abstract: Two new coumarins, talacoumarins A (**1**) and B (**2**), were isolated from the ethyl acetate extract of the wetland soil-derived fungus *Talaromyces flavus* BYD07-13. Their structures were elucidated by spectroscopic data (NMR, MS) analyses. The absolute configuration of C-12 in **1** was assigned using the modified Mosher's method, whereas that of C-12 in **2** was deduced via the circular dichroism data of its corresponding [Rh₂(OCOCF₃)₄] complex. Compounds **1** and **2** were evaluated for their anti-Aβ₄₂ aggregation, cytotoxic, and antimicrobial activities. The results showed that the two compounds had moderate anti-Aβ₄₂ aggregation activity, and this is the first report on the Aβ₄₂ inhibitory aggregation activity of coumarins.

Keywords: *Talaromyces flavus*; coumarin; structure elucidation; biological activities

1. Introduction

Fungi of the genus *Talaromyces* have been reported to produce a series of bioactive compounds [1–5]. Metabolites produced by *T. flavus* have also been reviewed [5]. In our searching for bioactive secondary metabolites from fungi, we have previously isolated a series of polyesters [6], as well as one sesquiterpene [7] from the wetland derived fungus *Talaromyces flavus* BYD07-13, which was collected from a soil sample in Baiyangdian (Hebei Province, China). Ongoing chemical study on this fungus has now resulted in the isolation and identification of two new coumarins, named talacoumarins A (**1**) and B (**2**) (Figure 1). In this paper, we describe the isolation, structure elucidation, as well as the anti-A β ₄₂ aggregation, cytotoxic, and antimicrobial activities of **1** and **2**.

Figure 1. Chemical structures of compounds **1** and **2**.



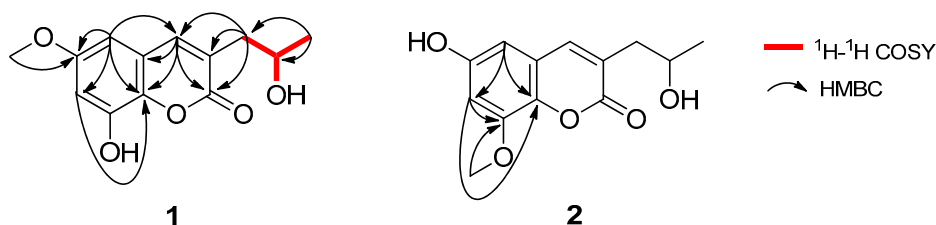
2. Results and Discussion

Compound **1** was isolated as a yellow amorphous powder, and its molecular formula was determined as C₁₃H₁₄O₅ on the basis of the HR-ESI-MS (m/z 273.0740, calcd. 273.0739 [M+Na]⁺). The UV spectrum showed an absorption band with λ_{\max} 208, 257, and 294 nm, characteristic of coumarins [8]. The IR spectrum of **1** displayed absorption bands for hydroxyl (3261 cm⁻¹), carbonyl (1684 cm⁻¹) and aromatic (1592 and 1497 cm⁻¹) groups. The ¹H-NMR spectrum (Table 1) of **1** exhibited a pair of *meta*-positioned aromatic protons at δ_{H} 6.63 and 6.61 (each, 1H, d, $J = 2.7$ Hz), an olefinic proton at δ_{H} 7.72 (1H, s). It also displayed the signals of one methoxyl at δ_{H} 3.74 (3H, s), one methyl at δ_{H} 1.10 (3H, d, $J = 6.2$ Hz), an oxygenated methyne proton at δ_{H} 3.92 (1H, m), one methylene protons at δ_{H} 2.54 (1H, dd, $J = 13.5, 4.9$ Hz), 2.45 (1H, dd, $J = 13.5, 7.8$ Hz), as well as a phenolic hydroxyl at δ_{H} 10.20 (1H, s), and an alcoholic hydroxyl at δ_{H} 4.62 (1H, d, $J = 4.5$ Hz). The ¹³C-NMR spectrum (Table 1) combined with DEPT 135 spectrum displayed 13 resonances for an ester carbonyl carbon (δ_{C} 161.0), eight aromatic/olefinic carbons (δ_{C} 155.6, 145.1, 141.1, 136.4, 126.5, 120.2, 104.9, 100.1), one oxymethine carbon (δ_{C} 64.2), one methoxyl group (δ_{C} 55.4), one methylene carbon (δ_{C} 40.3), and one methyl group (δ_{C} 23.4).

The ¹H-¹H COSY correlations between H-12 and H_{a,b}-11/H₃-13/12-OH, in combination with the HMBC correlations from H_{a,b}-11 to C-2/C-3/C-4, H-4 to C-2/C-9/C-10, H-5 to C-4/C-6/C-7/C-9, H-7 to C-9, and H₃-13 to C-11/C-12, indicating the presence of 3-propyl-6,8-dioxy coumarin moiety in **1** (Figure 2). Moreover, the methoxyl group was located at C-6 by the HMBC correlation from 6-OCH₃ to C-6. Considering the ¹³C-NMR chemical shifts of C-8 (δ_{C} 145.1) and C-12 (δ_{C} 64.2), as well as molecular formula, the two carbons should be connected with hydroxyl groups. Thus, the structure of **1** was fully elucidated to be as indicated in Figure 1, and it was named talacoumarin A.

Table 1. ^{13}C - (100 MHz) and ^1H - (400 MHz) NMR data for compounds **1** and **2** in $\text{DMSO-}d_6$ (δ in ppm).

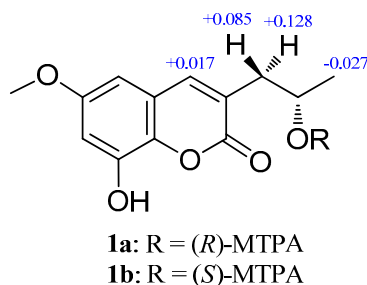
Position	1		2	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1				
2	161.0		160.9	
3	126.5		126.6	
4	141.1	7.72 (s)	140.9	7.69 (s)
5	100.1	6.63 (d, 2.7)	102.8	6.51 (d, 2.3)
6	155.6		153.9	
7	104.9	6.61 (d, 2.7)	102.4	6.66 (d, 2.3)
8	145.1		147.0	
9	136.4		135.7	
10	120.2		119.9	
11	40.3	2.45 (dd, 13.5, 7.8), H_a 2.54 (dd, 13.5, 4.9), H_b	40.2	2.44 (dd, 13.6, 7.7), H_a 2.52 (overlapped), H_b
12	64.2	3.92 (m)	64.2	3.92 (m)
13	23.4	1.10 (d, 6.2)	23.4	1.09 (d, 6.1)
8-OH		10.20 (s)		
12-OH		4.62 (d, 4.5)		
6-OCH ₃	55.4	3.74 (s)		
8-OCH ₃			55.9	3.84 (s)

Figure 2. Selected ^1H - ^1H COSY and HMBC correlations of **1** and **2**.

The absolute configuration at C-12 was determined to be *S* by comparison of its optical rotation value with that of (*S*)-orthosporin (**1**: $[\alpha]_{\text{D}}^{20} +56.0$ (*c* 0.5, CH_3OH); (*S*)-orthosporin: $[\alpha]_{\text{D}}^{22} +61.8$ (*c* 1.0, CH_3OH) [9]). As confirmation, the absolute configuration at C-12 was established by the modified Mosher's method [10]. The values of the (*R*)- and (*S*)-MTPA esters **1a** and **1b** also indicated the *S* configuration for C-12 (Figure 3).

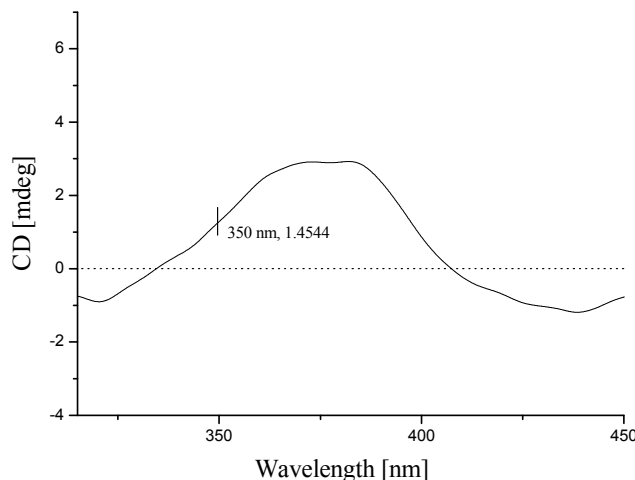
Compound **2** possessed the same molecular formula and UV absorption characteristic as that of **1**, suggesting **2** may be a coumarin isomer of **1**. The NMR spectroscopic data (Table 1) suggested **2** was very similar to **1**, except for the position of the methoxy group, which was located at C-8 for **2** instead of at C-6 based on the HMBC correlations from 8-OCH₃ to C-8, from H-5 to C-7/C-9, and from H-7 to C-8/C-9 (Figure 2). With the aid of the ^1H - ^1H COSY, HSQC, and HMBC correlations, the planar structure of **2** was established and all the ^1H - and ^{13}C -NMR signals were assigned.

Figure 3. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ obtained for (*S*)-(1a) and (*R*)-MTPA (1b) esters.



The optical rotation of **2** ($[\alpha]_D^{20} +55.6$ (*c* 0.5, CH₃OH)) was consistent with that of **1**, which suggested that **2** had the same configuration. The absolute configuration at C-12 was determined on the basis of the circular dichroism of the complex formed *in situ* with [Rh₂(OCOCF₃)₄] [11,12], with the inherent contribution of the ligand subtracted. Upon addition of [Rh₂(OCOCF₃)₄] to a solution of **2** in CH₂Cl₂, a metal complex was formed, acting as an auxiliary chromophore. It has been demonstrated that the sign of the E band (at *ca.* 350 nm) can be used to correlate the absolute configuration of a secondary and tertiary alcohol by applying the bulkiness rule. In this case, the Rh complex of **2** displayed a positive E band (Figure 4), correlating with a 12*S* absolute configuration. Hence, the structure of **2** was established as shown in Figure 1 and named to be talacoumarin B.

Figure 4. The CD spectrum of the Rh complex of **2** with the inherent CD spectrum subtracted.



So far, natural products from fungi with the 3-alkyl-6,8-dioxycoumarin scaffold are relatively rare, and only eight such compounds have been reported [8,13–15]. The inhibitory activities against A β ₄₂ aggregation of compounds **1** and **2**, along with that of the crude extract, were tested by a thioflavin T (ThT) assay [16] with epigallocatechin gallate (EGCG) as the positive control. Compounds **1** and **2** showed moderate anti-A β ₄₂ aggregation activities, with relative inhibitory rates of (49.33 \pm 3.16)% and (44.99 \pm 3.64)% [the positive control EGCG had a relative inhibitory rate of (67.23 \pm 2.51)%] at the concentration of 100 μ M, while the crude extract has no activity. This represents the first report of the anti-A β ₄₂ aggregation activity of coumarins. Compounds **1**, **2**, and the crude extract were also evaluated for the cytotoxicity against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) and the antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*,

Candida albicans, and *Aspergillus niger*. However, none of them showed any cytotoxic ($IC_{50} > 40 \mu\text{M}$) or antimicrobial activities ($MIC > 1.0 \text{ mg/mL}$).

3. Experimental Section

3.1. General Procedures

Optical rotations were measured using a JASCO P-1020 polarimeter (JASCO Corporation, Tokyo, Japan). The IR spectra (KBr) were recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer (JASCO Corporation). The UV spectra were recorded in CH_3OH using a JASCO V-550 UV/Vis spectrophotometer (JASCO Corporation). ^1H - (400 MHz), ^{13}C - (100 MHz), DEPT 135 (100 MHz), and 2D (^1H - ^1H COSY, HSQC, and HMBC) NMR spectra were recorded in $\text{DMSO-}d_6$ on a Bruker AV 400 spectrometer using solvent signals ($\text{DMSO-}d_6$: $\delta_{\text{H}} 2.50/\delta_{\text{C}} 39.5$) as an internal standard (Bruker Corporation, Fallanden, Switzerland). HR-ESI-MS were measured on a Waters Synapt G2 TOF mass spectrometer (Waters Corporation, Manchester, UK). Column chromatographies (CCs) were carried out on silica gel (200–300 mesh, Marine Chemical Group Corporation, Qingdao, China), and ODS (60–80 μm , YMC, Tokyo, Japan). Silica gel GF₂₅₄ (Marine Chemical Group Corporation) was used for analytical TLC. The analytical HPLC was performed on a Shimadzu HPLC system equipped with a LC-20AB pump, and a SPD-20A diode array detector (Shimadzu, Kyoto, Japan), using a Phenomenex Gemini C18 column (5 μm , 4.6 mm \times 250 mm, Phenomenex Inc., Torrance, CA, USA). The Preparative HPLC was performed on Shimadzu LC-6AD system equipped with a LC-6AD pump, and a SPD-M20A detector (Shimadzu), using an RP-18 column (5 μm , 21.2 mm \times 250 mm, Gemini, Phenomenex Inc.; detector set at 220 and 254 nm).

3.2. Fungal Material and Culture

The strain of *Talaromyces flavus* (No.BYD07-13) was identified on the basis of the morphological characters and gene sequence analyses. The ITS, beta-tubulin, and calmodulin sequences of the strain have been deposited at GenBank as KF917583, KF917584, and KF917585, respectively. It was deposited in the culture collection at the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou, China. The fermentation of No.BYD07-13 was described in our previous paper [6].

3.3. Extraction and Isolation

The fermented material was extracted three times with EtOAc (3 \times 6.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (46.2 g), which was dissolved in 90% v/v aqueous CH_3OH (500 mL) and partitioned against an equal volume of cyclohexane. The aqueous CH_3OH layer was evaporated to dryness under reduced pressure to give an aqueous CH_3OH extract (w, 28.1 g), which was subjected to ODS column chromatography (CC) using a $\text{CH}_3\text{OH-H}_2\text{O}$ gradient elution (30:70, 50:50, 70:30, and 100:0, v/v) to give four fractions (w1 to w4). The fraction w1 (574 mg) was further separated by ODS CC using a $\text{CH}_3\text{OH/H}_2\text{O}$ gradient elution (30:70, 40:60, and 50:50, v/v, each 0.5 L) to give three subfractions (w1a to w1c). The subfraction w1a (351 mg) was

purified by preparative HPLC eluting with CH₃CN/H₂O (v/v 15:85, flow rate: 8 mL/min) to afford compounds **1** (37.9 mg, *t_R* 21.2 min) and **2** (5.0 mg, *t_R* 16.1 min).

3.4. Spectroscopic Data

Talacoumarin A (**1**). Yellow amorphous powder, $[\alpha]_D^{20} +56.0$ (*c* 0.5, CH₃OH); UV (CH₃OH) λ_{\max} (log ϵ) 208 (4.65), 257 (4.07), 294 (4.27) nm; IR (KBr): ν_{\max} 3261, 2962, 2920, 2853, 1684, 1592, 1510, 1454, 1392, 1351, 1203, 1164, 1109, 1053, 855, 840 cm⁻¹; ¹H- (DMSO-*d*₆) and ¹³C-NMR (DMSO-*d*₆) data, see Table 1. HR-ESI-MS: *m/z* 273.0740 ([M+Na]⁺, C₁₃H₁₄O₅Na; calcd. 273.0739).

Talacoumarin B (**2**). Yellow amorphous powder, $[\alpha]_D^{20} +55.6$ (*c* 0.5, CH₃OH); UV (CH₃OH) λ_{\max} (log ϵ) 208 (4.65), 257 (4.07), 294 (4.27) nm; IR (KBr): ν_{\max} 3261, 2962, 2920, 2853, 1684, 1592, 1510, 1454, 1392, 1351, 1292, 1203, 1109, 1053, 855, 841 cm⁻¹; ¹H-NMR (DMSO-*d*₆) and ¹³C-NMR (DMSO-*d*₆) data, see Table 1. HR-ESI-MS: *m/z* 273.0737 ([M+Na]⁺, C₁₃H₁₄O₅Na; calcd. 273.0739).

3.5. Preparation of (*S*)- and (*R*)-MTPA Esters of **1** (**1a** and **1b**)

A solution of **1** (0.5 mg) in pyridine-*d*₅ (0.5 mL) was treated with (*S*)-MTPA chloride (8 μ L) under an atmosphere of nitrogen in an NMR tube. The mixture was stirred at room temperature for 20 h to obtain the (*R*)-MTPA ester (**1b**). The same procedure was used to prepare the (*S*)-MTPA ester (**1a**) with (*R*)-MTPA chloride.

3.6. Absolute Configuration of the Secondary Alcohol in **2**

According to a published procedure [11,12], **2** (0.5 mg) was dissolved in a dry solution of the stock [Rh₂(OCOFCF₃)₄] complex (1.0 mg) in CH₂Cl₂ (200 μ L). The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at *ca.* 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-12 secondary alcohol moiety.

3.7. ThT Fluorescence Assay

The ThT fluorescence assay was performed as described in our previous paper [16], with EGCG as the positive control. Biological activity was determined as relative inhibitory activity (*V_i*) for each sample according to the formula: $V_i = 100 - [(F_i - F_b)/F_0] \times 100$, where *F_i* is the fluorescence value of the sample, *F_b* is its blank value, and *F₀* is the fluorescence value for free aggregation of a sample of A β ₄₂ incubated in the same buffer/HFIP/DMSO system and in absence of inhibitors.

3.8. Cytotoxicity Assay

Cytotoxic activity was tested against five human cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) using the MTT method as described in our previous paper [6]. Cisplatin and paclitaxel were used as the positive controls.

3.9. Antimicrobial Assay

The antimicrobial activity against *E. coli*, *S. aureus*, *C. albicans*, and *A. niger* were evaluated by an agar dilution method, which described in our previous paper [7]. Tobramycin was used as the positive control.

4. Conclusions

Our investigation on the metabolites of the wetland soil-derived fungus *T. flavus* BYD07-13 resulted in the isolation of two new coumarins, named talacoumarins A (**1**) and B (**2**). Compounds **1** and **2** both have the 3-alkyl-6,8-dioxycoumarin moiety, which is relatively rare in fungal metabolites. The absolute configurations of **1** and **2** were determined by the modified Mosher's method and the CD analysis of the *in situ* formed $[\text{Rh}_2(\text{OCOCF}_3)_4]$ complex. It is noteworthy that the absolute configuration of branched chain alcohol of 3-alkyl-6,8-dioxycoumarin was determined for the first time. Compounds **1** and **2** showed moderate anti-A β_{42} aggregation activity, making this the first report on the A β_{42} inhibitory aggregation activity of coumarins.

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Author Contributions

XSY, HG, YY and JWH designed research; JWH, DPQ, RQK and XZL performed research and analyzed the data; JWH and YY wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Not available.