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A NEW 3,4-DIHYDROISOCOUMARIN ISOLATED FROM *Botryosphaeria* sp. F00741

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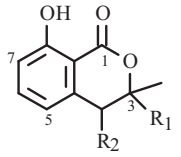
*A new 3,4-dihydroisocoumarin, named botryoisocoumarin A (**1**), was obtained from the fermentation culture of* Botryosphaeria *sp. F00741, together with two known ones, 3,8-dihydroxy-3-methylisochroman-1-one (**2**) and 4,8-dihydroxy-3-methylisochroman-1-one (**3**). Their structures were elucidated by spectroscopic analyses, including 1D and 2D NMR experiments and ESI mass spectrometry, and by comparison with those reported. Compound **1** was evaluated for its antitumor and antimicrobial activities* in vitro.

Keywords: *Botryosphaeria* sp., 3,4-dihydroisocoumarin, botryoisocoumarin A.

The fungal strain F00741 was originally obtained from the plant epidermis of *Avicennia marina* and was identified as *Botryosphaeria* sp. based on its complete ITS1-5.8S-ITS2 gene sequences. Only less than 50 compounds have been reported from *Botryosphaeria* sp. [1–6]. In our search for new bioactive metabolites from this fungus, a new 3,4-dihydroisocoumarin, named botryoisocoumarin A (**1**), was isolated, together with two known ones **2**, **3**. Here we report the isolation and structure elucidation of compound **1**. The *in vitro* antimicrobial and antitumor screenings of compound **1** are also described.

Compound **1** was obtained as a colorless crystal. The molecular formula of **1** was determined to be $C_{11}H_{12}O_4$ on the basis of ESI-MS and NMR data. The ^{13}C NMR spectrum of **1** (Table 1) revealed the presence of 11 signals corresponding to two Me, one CH_2, three CH, and five quaternary C-atoms (three being oxygenated). Analysis of the ^1H NMR spectrum of **1** (Table 1) indicated the presence of one Me signal at δ 1.69 and one OMe signal at δ 3.41. HMBC correlations from Me(3a) to C-3, C-4, and C-1, from OMe(3b) to C-3, and from H-4 to C-3, together with the AB coupling system of H-4 [δ 3.22 (d, J = 16.3), 3.15 (d, J = 16.3)], indicated that Me(3a) and OMe(3b) were connected to the chiral carbon atom C-3 and the *O*-bearing carbon of C-3 (δ_C 105.5) was connected to C-1 in the form of a lactone according to their chemical downshift.

The ^1H–^1H COSY cross-peak between H-5/H-6, H-6/H-7 and the HMBC correlations from H-5, H-6, and H-7 to the corresponding carbons (Table 1) establish the benzene fragment. The HMBC experiments also showed ^1H, ^{13}C long-range correlations from H-5 to C-4, and from H-4 to C-4a and C-8a in the benzene fragment, which established the connection of the two fragments. The hydroxy substituted at C-8 was confirmed by the chemical downshift of C-8 at δ 162.1. Thus, from the data above, the structure of compound **1** was determined to be 8-hydroxy-3-methoxy-3-methylisochroman-1-one, named botryoisocoumarin A (**1**).



1 - 3

1: R_1 = OMe, R_2 = H
2: R_1 = OH, R_2 = H
3: R_1 = H, R_2 = OH

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TABLE 1. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), HMBC, and COSY Spectral Data of **1** ($CDCl_3$, δ in ppm, multiplicity, J/Hz)

C atom	δ_H	δ_C	HMBC (C/H)	^{1}H–^{1}H COSY
1		168.9 s		
3		105.5 s		
3a	1.69 (s)	22.8 q	C-3, C-4, C-1	
3b	3.41 (s)	50.4 q	C-3	
4	3.22 (d, J = 16.3)	38.7 t		
	3.15 (d, J = 16.3)		C-3, C-5, C-8a, C-4a	H-5
4a		137.7 s		
5	6.70 (dd, J = 0.5, 7.3)	118.5 d	C-4, C-7, C-8a	H-6, H-4
6	7.43 (dd, J = 7.3, 8.8)	136.3 d	C-4a, C-8	H-5, H-7
7	6.90 (d, J = 8.4)	116.2 d	C-5, C-8, C-8a	H-6
8		162.1 s		
8a		107.8 s		

Compounds **2** and **3** were identified as 3,8-dihydroxy-3-methylisochroman-1-one (**2**) [7] and 4,8-dihydroxy-3-methylisochroman-1-one (**3**) [8, 9] according to their NMR data and comparison with those reported.

EXPERIMENTAL

General Experimental Procedures. Column chromatography (CC): silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, P. R. China), silica gel GF_{254} (Merck), RP-18 (Merck), and Sephadex LH-20 (Amersham Biosciences). TLC: precoated silica gel GF_{254} plates (0.20–0.25 mm, Qingdao Marine Chemical Factory). ^{1}H and ^{13}C NMR spectra: Bruker DRX-600 spectrometer, at 600/150 MHz, in $CDCl_3$; δ in ppm relative to Me_4Si, J in Hz. ESI-MS: Thermo-Finnigan Advantage LCQ mass spectrometer, in *m/z*.

Microbial Material. The fungus strain *Botryosphaeria* sp. F00741 (Genbank registered No. FJ037758.1) was isolated from *Avicennia marina* obtained in Fujian Province, Southeast China.

Extraction and Isolation. A stock of *Botryosphaeria* sp. F00741 was cultivated on PDA medium with a total volume of 10 L for 14 days at 28°C. The mycelium together with the culture medium was first extracted with equal volumes of EtOAc–MeOH–AcOH 80:15:5 (v/v/v) at room temperature. The crude extract was partitioned again with EtOAc and H_2O. The organic layer was dried over Na_2SO_4 (anhydrous) and the solvent evaporated under reduced pressure to afford 3.38 g of a crude organic extract (brown oil).

The extract was subjected to MPLC (80 g RP-18; 30%, 50%, 70%, and 100% MeOH in water, 2 L for each gradient) to afford three fractions: Fr.1–3.

Fr. 2 (232.6 mg) was further subjected to Sephadex LH-20 eluted with acetone to afford Fr. 2a (84.8 mg). Fr. 2a (84.8 mg) was subjected to SiO_2 chromatography using petroleum ether (PE) to afford **1** (15.6 mg) and Fr. 2a-1 (44.8 mg). Fr. 2a-1 (44.8 mg) was further subjected to Sephadex LH-20 eluted with acetone to afford Fr. 2a-1a (14.1 mg) and Fr. 2a-1b (18.7 mg). Fr. 2a-1a was further purified by CC (silica gel, PE–$CHCl_3$ 1:2) to yield **2** (4.5 mg), and Fr. 2a-1b was further purified by CC (silica gel, PE–$CHCl_3$ 1:1) to yield **3** (10.6 mg).

Bioassay Procedures. The cytotoxicities against HeLa, HepG-2, and A-549 cell lines were measured 72 h posttreatment by the MTT method [10] at a concentratin of 10 μg/mL. The results revealed that **1** displayed no activities against tumor cells.

The antibacterial activity of **1** was tested at a concentration of 50 μg/mL against bacteria (*Escherichia coli*, *Bacillus subtilis*, *Bacillus Pumilus*, and *Staphylococcus aureus*) and yeasts (*Aspergillus niger* and *Candida albicans*) using a similar MIC method with 96-well microplates [11]. The results showed no inhibitory activities.

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