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A NEW ISOCHROMAN DERIVATIVE FROM THE MARINE FUNGUS *Phomopsis* sp. (No. ZH-111)

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*A new isochroman, (3*R*,4*S*)-3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin (**1**), and two known compounds were isolated from the marine fungus* Phomopsis *sp. (No. ZH-111). Their structures were determined by spectroscopic methods, mainly 1D and 2D NMR. Preliminary pharmacological test revealed that compound **1** and exumolide A (**3**) can accelerate the growth of subintestinal vessel plexus (SIV) branches markedly and compound **2** can inhibit the growth of subintestinal vessel plexus (SIV) branches.*

Keywords: natural product, isochroman derivative, marine fungus, bioassays, NMR assignment.

Marine-derived fungi have proven to be rich sources of structurally novel and biologically active secondary metabolites, which have become interesting as significant resources for new chemicals in drug discovery [1]. In our search for new metabolites from marine mangrove endophytic fungi from the South China Sea, many significant new bioactive compounds have been isolated [2–5]. The investigation of the mangrove endophytic fungus (zh-111) led to the isolation of a new isochroman named (3*R*,4*S*)-3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin (**1**) and two known compounds **2** and **3** [6, 7]. Their structures were determined by spectroscopic methods, mainly 1D and 2D NMR.

Compound **1** was obtained as a colorless solid. The molecular formula was deduced as $C_{10}H_{10}O_5$ through high-resolution electron ionization mass spectrometric (HR-EI-MS) studies, which showed an M^+ peak at *m*/*z* 210.0522 (calcd for 210.0523). The ^{1}H NMR displayed signals of a methyl at δ 1.34, two methine signals [4.81 (dq, J = 2.8, 6.8), 4.99 (br.d, J = 4.4 Hz)], and two benzene protons at δ 7.16 (d, J = 8.8 Hz) and 6.81 (d, J = 8.8 Hz); the latter indicate the *ortho*-coupled protons in benzene ring. One of the exchangeable proton signals was observed at δ 10.66, which indicated that the hydroxyl group was chelated with the carbonyl group. The ^{13}C NMR and DEPT spectra indicated there were one methyl, four methines, and five quaternary carbons, including one carbonyl carbon (δ 170.3) (Table 1). In the ^{1}H–^{1}H COSY spectrum the correlation between H-3 and H-11 located H_3-11 at C-3. In the HMBC spectrum, correlations from H-3 to C-4, C-10 and H-4 to C-3, C-9, C-10, and C-11 indicated that the connection points of the lactone ring and benzene ring were C-9 and C-10 (Fig. 1). The relative stereochemistry of **1** was elucidated by NOE experiments. NOE correlations between H-3 and 4-OH and H-4 and 11-H_3 indicated that H-3 and H-4 were on the opposite side. So the structure of compound **1** was established to be (3*R*,4*S*)-3,4-dihydro-4,5,8-trihydroxy-3-methylisocoumarin.

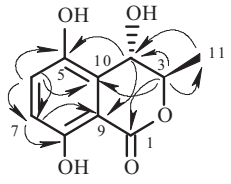

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Fig. 1. The structure and key HMBC correlations of **1**.

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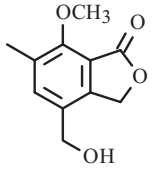
TABLE 1. NMR Data (400 MHz, acetone-d_6) of **1**

C atom	δ_C (DEPT)	δ_H, J/Hz	HMBC	^1H–^1H COSY
1	170.3 (C)			
3	82.5(CH)	4.81 (dq, J = 4.4, 6.8)	C-1, 4, 10	H-11
4	66.3 (CH)	4.99 (br.d, J = 4.4)	C-3, 5, 9, 10, 11	
5	149.2 (C)			
6	127.2 (CH)	7.16 (d, J = 8.8)	C-5, 8, 10	H-7
7	119.4 (CH)	6.81 (d, J = 8.8)	C-5, 8, 9	H-6
8	157.0 (C)			
9	108.9 (C)			
10	125.7 (C)			
11	19.1 (CH_3)	1.34 (d, J = 6.8)	C-3, 4	H-3
4-OH		4.52 (br.s)		
5-OH		4.58 (br.s)		
8-OH		10.66 (s)		



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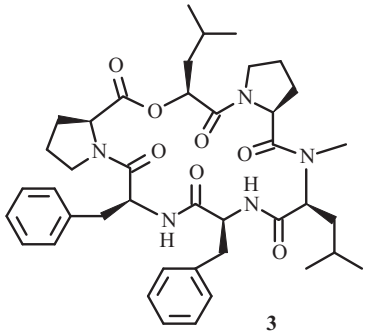

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Primary bioassays showed that compound **1** and compound **3** can accelerate the growth of blood vessels markedly, and compound **2** can inhibit the growth of blood vessel (Fig. 2). The three compounds exhibited weak cytotoxicity against Hep-2 and HepG2 cells (IC_{50} > 50 mg/mL).

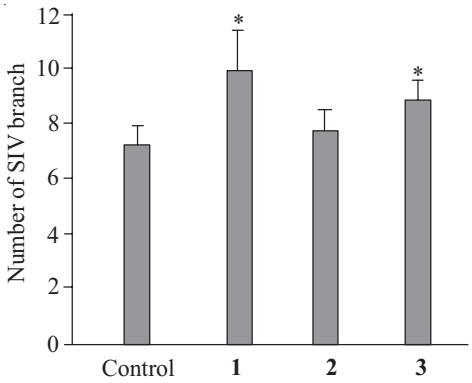

Fig. 2. The effect of three compounds on embryonic angiogenesis [zebrafish embryos were incubated with three compounds at final concentration 100 μM for 72 h. Data expressed as mean ± SD. Results were obtained from three independent experiments. Asterisks: significant differences (*$p < 0.05$)].

EXPERIMENTAL

Melting points were determined on an X-4 micro-melting point apparatus and were uncorrected. Optical rotations were measured on a Schmidt + Haensch Polartronic HH W5 polarimeter and were uncorrected. IR spectra were measured on a Bruker Vector 22 spectrophotometer. ^{1}H and ^{13}C NMR data were recorded on a Varian Inova 400 MB NMR spectrometer operating at 400 and 125 MHz for ^{1}H and ^{13}C, respectively (TMS as internal standard). HR-EI-MS were measured on a Thermo MAT95XP high resolution mass spectrometer, and EI-MS on a Thermo DSQ EI-mass spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd) was used for column chromatography (CC).

Fungus Material and Culture Conditions. *Phomopsis* sp. (No. zh-111) was isolated from the mangrove sediment of ZhuHai, Guangdong, China and deposited in the Department of Applied Chemistry, Zhongshan University, Guangzhou, Guangdong, China. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 500 mL Erlenmeyer flask containing 200 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L, pH 6.0). The flask was incubated at 28°C. After being shaken on a rotary shaker for 3–5 days, the mycelium was aseptically transferred to a 1000 mL Erlenmeyer flask containing the culture liquid (600 mL). The flask was then incubated at 20°C for 21 days. The cultures (200 L) were filtered through cheesecloth. The filtrate was concentrated to 5 L below 50°C and extracted three times with an equal volume of ethyl acetate. The combined organic extracts were applied to a silica gel column, eluting with a gradient of petroleum ether to ethyl acetate to yield **1** (5 mg), **2** (4 mg), **3** (10 mg).

(3*R*,4*S*)-3,4-Dihydro-4,5,8-trihydroxy-3-methyl-1H-2-benzopyran-1-one (1). Colorless solid, $[\alpha]_D^{20}$ + 40.0° (*c* 0.31, MeOH), mp 280–281°C.

FT-IR (KBr, ν_{max}, cm^{-1}): 3237 (st), 2922 (m), 2853, 2728, 2678, 1731 (st), 1615 (st), 1463, 1378, 1305, 1180, 1105, 1062, 1047, 991, 994, 851, 814, 722 (st). For ^{1}H NMR, ^{13}C NMR, and 2D NMR data, see Table 1.

EI-MS *m/z* (EI$^+$, I_{rel}, %): 210 (17.0), 192 (100), 174 (9.0), 150 (25.0), 137 (24.5), 122 (14.5), 109 (9.5), 97 (10.0), 81 (12.5), 69 (17.0), 57 (18.0), HR-EI-MS (HR-EI$^+$, *m/z*): 210.0522 (calcd for $C_{10}H_{10}O_5$ 210.0523).

4-(Hydroxymethyl)-7-methoxy-6-methyl-1(3*H*)-isobenzofuranone (2). Yellow power, mp 265–266°C.

^{1}H NMR spectrum (400 MHz, acetone-d_6, δ, ppm): 2.29 (3H, s), 2.60 (1H, br), 3.99 (3H, s), 4.69 (2H, s), 5.33 (2H, s), 7.48 (1H, s).

^{13}C NMR spectrum (125 MHz, acetone-d_6): 170.2 (C), 158.0 (C), 147.1 (C), 137.1 (CH), 133.2 (C), 132.8 (C), 118.7 (C), 70.1 (CH_2), 63.2 (CH_3), 62.9 (CH_2), 16.4 (CH_3).

Exumolide A (3). White solid. EI-MS *m/z* (EI$^+$, %): 70 (100), 120 (34), 194 (30.5), 281 (15), 491 (13), 566 (12), 638 (9), 729 (24).

^{1}H NMR spectrum (400 MHz, acetone-d_6, δ, ppm): 0.83 (3H, d), 0.85 (3H, d), 0.86 (3H, d), 0.91 (3H, d), [1.36 (1H, m), 1.72 (1H, m)], 1.37 (1H, m), [1.40 (1H, m), 1.97 (1H, m)], 1.58 (1H, m), 1.66 (2H, m), [1.80 (1H, m), 1.42 (1H, m)], [1.82 (1H, m), 2.12 (1H, m)], [2.16 (1H, m), 1.96 (1H, m)], 2.38 (3H, s), [2.73 (1H, dd), 3.17 (1H, dd)], [3.16 (1H, dd), 2.97 (1H, dd)], 3.28 (1H, d), [3.37 (1H, ddd), 3.55 (1H, m)], [3.54 (1H, m), 3.78 (1H, ddd)], 4.35 (1H, m), 4.37 (1H, m), 4.72 (1H, dd), 5.03 (1H, dd), 5.11 (1H, d), 6.92 (1H, d), 7.14 (4H, m), 7.24 (4H, m), 7.26 (2H, m), 8.17 (1H, d).

^{13}C NMR spectrum (125 MHz, acetone-d_6): 172.0 (C), 171.3 (C), 170.1 (C), 169.6 (C), 169.4 (C), 167.4 (C), 136.5 (C), 136.4 (C), 129.3 (2CH), 129.0 (2CH), 128.5 (2CH), 128.4 (2CH), 127.0 (CH), 126.6 (CH), 70.8 (CH), 59.4 (CH), 58.9 (CH), 56.4 (CH), 54.5 (CH), 53.7 (CH), 47.2 (CH_2), 46.0 (CH_2), 40.3 (CH_2), 38.9 (CH_2), 37.2 (CH_2), 36.5 (CH_2), 31.2 (CH_2), 28.9 (CH_2), 28.8 (CH_3), 25.4 (CH_2), 24.6 (CH), 24.5 (CH), 23.3 (CH_3), 22.2 (CH_2), 21.8 (CH_3), 21.3 (CH_3).

Bioassays. The antibacterial activities of these three metabolites were tested on zebrafish embryo collections. Compounds **1** and **3** can accelerate the growth of blood vessel markedly, and compound **2** can inhibit the growth of blood vessel. The cytotoxic assays were performed using the MTT assay method [8]. The three compounds inhibited the growth of Hep-2 and HepG2 cells at a concentration above 50 μmol/mL.

Embryo Collection and Drug Treatment. Zebrafish embryos were used to examine the effect of different compounds on embryonic angiogenesis. Embryos were maintained in embryo water (0.2 g/L of Instant Ocean Salt in distilled water) at 27°C, and the embryos were sorted for viability and developmental stage (shield stage) at 6 hpf. Three embryos were placed into each well of a 96-well plate containing 100 μL embryo water ± drug treatment. The embryos were examined daily for viability, gross morphological abnormalities, and blood vessel development using an inverted Olympus DP70 epifluorescence microscope (Olympus, Tokyo, Japan). At 72 hpf, the embryos were anesthetized using 0.05% 2-phenoxyethanol in embryo water, and each embryo was examined for the presence of ectopic vessels in the subintestinal vessel plexus (SIV).

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