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A NEW CYTOTOXIC ISOCOUMARIN FROM ENDOPHYTIC FUNGUS *Penicillium* SP. 091402 OF THE MANGROVE PLANT *Bruguiera sexangula*

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*A new isocoumarin, (3*R**,4*S**)-6,8-dihydroxy-3,4,7-trimethylisocoumarin (**1**), and five known compounds were isolated from mangrove endophytic fungus* Penicillium *sp. 091402. The structures of all the compounds were elucidated by analysis of spectrometric data.*

Key words: isocoumarin, mangrove plant, endophytic fungus.

Marine-derived microorganisms have recently come into the focus of research as one of the richest sources of new and bioactive secondary metabolites in the mangrove environment [1–3]. Our previous investigation of endophytic microorganisms of mangrove plants resulted in a number of bioactive and structurally unique metabolites [4, 5]. In our screening for cytotoxic agents from mangrove endophytic fungus, the EtOAc extract of the fermentation broth of *Penicillium* sp. 091402 from the root of the mangrove plant *Bruguiera sexangula* showed inhibitory activity towards tumor cell lines K562 and SGC-7901. Bioassay-guided fractionation led to the isolation of a new compound (3*R**,4*S**)-6,8-dihydroxy-3,4,7-trimethylisocoumarin (**1**), together with five known compounds (3*R*,4*S*)-6,8-dihydroxy-3,4,5-trimethylisocoumarin (**2**), (3*R*,4*S*)-6,8-dihydroxy-3,4,5,7-tetramethylisochroman (**3**), (*S*)-3-(3′,5′-dihydroxy-2′,4′-methylphenyl) butan-2-one (**4**), phenol A (**5**), and 3,4,5-trimethyl-1,2-benzenediol (**6**). It was reported that compounds **2**–**5** were decomposition products of citrinin [6, 7], and compound **1** possessed the same framework as compounds **2**–**5**, which indicated that **1** might be also a decomposition product of citrinin.

Compound **1** was isolated as a pale–yellow solid. The molecular formula of **1** was established as $C_{12}H_{14}O_4$ on the basis of its HREI-MS (*m/z* 223.0971, calcd 223.0970), which was supported by ^{13}C NMR and DEPT spectra. The IR spectrum exhibited bands at 3431cm^{-1} (hydroxyl group) and at 1639 (C=O), 1584, 1510 cm^{-1} (aromatic ring). The ^{13}C NMR spectrum of **1** was very similar to that of the known compound sclerotinin C [8], while the ROESY spectrum showed the stereochemistry difference between compound **1** and sclerotinin C at C-11 and C-12. *Trans*-orientations at C-11 and C-12 bond were determined by the ROESY experiment of **1**, showing that H-3 (δ 3.89) correlated with H-11 (δ 1.15) and H-4 (δ 3.08) correlated with H-12 (δ 1.14) (Table 1). This result indicated that the relative configurations at C-3 and C-4 in **1** were 3*R** and 4*S**, respectively. From this result **1** was established to be (3*R**,4*S**)-6,8-dihydroxy-3,4,7-trimethylisocoumarin.

In the primary bioassay, compound **1** showed moderate cytotoxic activity against tumor cell line K562 with IC_{50} value of 18.9 μg/mL, and **5** showed weak cytotoxic activity against tumor cell line SGC-7901 with IC_{50} value of 36.0 μg/mL.

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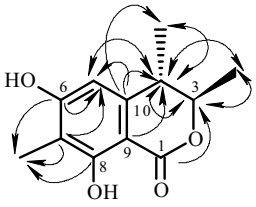
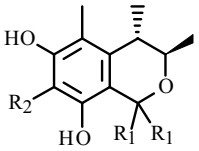
TABLE 1. NMR Data of **1** (CD_3OD, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	COSY	HMBC	ROESY
1	160.3 (s)			H-3	
3	71.9 (d)	3.89 (dq, J = 6.6, 6.5)	H-4, 12	H-4, 11, 12	H-11
4	43.5 (d)	3.08 (dq, J = 6.9, 6.9)	H-3, 11	H-3, 5, 11, 12	H-12
5	114.3 (d)	6.26 (s)		H-4	
6	160.7 (s)			H-5, 13	
7	104.2 (s)			H-5, 13	
8	178.4 (s)			H-13	
9	102.0 (s)			H-4, 5	
10	150.2 (s)			H-3, 4, 5	
11	16.5 (q)	1.15 (d, J = 7.3)	H-4	H-3, 4	
12	19.9 (q)	1.14 (d, J = 5.8)	H-3	H-3, 4	
13	10.6 (q)	2.10 (s)			



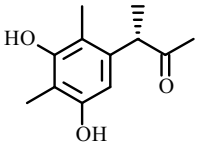
1



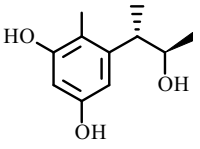
2, 3

2: R_1 = O, R_2 = H

3: R_1 = H, R_2 = CH_3



4



5

EXPERIMENTAL

General Procedures. The NMR spectra were run on Bruker AV-400 MHz and DRX-500 MHz spectrometers, using TMS as an internal standard. The HR-EIMS spectra were measured with a VG Auto-3000 spectrometer. The IR spectra were measured on a Nicolet 380 FT-IR instrument as KBr pellets. The UV spectra were measured on a Beckman DU800 spectrometer. Optical rotation was measured at room temperature using a Rudolph Autopol III polarimeter.

Fungus Material. The fungus *Penicillium* sp. 091402 was isolated from the root of the mangrove *Bruguiera sexangula* Linn. from the mangrove reserve zone of Qinglan Port, Hainan, China, and identified as *Penicillium* sp. by Prof. Kui Hong. A voucher of this species was lodged at the Marine Microorganism Laboratory, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou, China.

Fermentation, Extraction, and Isolation. The strain *Penicillium* sp. 091402 was cultured in 70 L of a 50% seawater-based medium (100 L fermenter) containing 20.0 g/L soluble starch, 15.0 g/L soybean extract, 5.0 g/L yeast extract, 2.0 g/L peptone, 4.0 g/L $CaCO_3$, and 4.0 g/L NaCl, and the pH of the medium was adjusted to 7.0. The fermentation was incubated at room temperature for 7 days.

The fermentation broth (140 L) of *Penicillium* sp. 091402 was separated by filter, and the filtered broth was evaporated to 5 L and then extracted with EtOAc. The EtOAc extract (14.0 g) was subjected to VLC by gradient elution utilizing petroleum ether–acetone to give 12 fractions (Fr. 1-Fr. 12). Fraction 9 (1.9 g) was further chromatographed on a silica gel column eluted with $CHCl_3$–MeOH (7:1) to give 12 fractions (Fr. 9-1–Fr. 9-12). Fraction 9-12 (931.5 mg) was purified on a silica gel column eluted with $CHCl_3$–MeOH (8:1) to yield compound **1** (64.8 mg). Fraction 6 (2.7 g) was subjected to a Sephadex LH-20 column eluted with 95% EtOH to give nine fractions (Fr. 6-1–Fr. 6-9). Fraction 6-4 (179.9 mg) was further chromatographed on a silica gel column eluted with petroleum ether–acetone (6:1) to afford compound **2** (43.0 mg). Fraction 6-6 (310.1 mg) was purified on a silica gel column eluted with petroleum ether–ethyl acetate (13:2) to afford compounds **3** (4.5 mg) and **4** (6.7 mg). Fraction 7 (5.2 g) was further chromatographed on a silica gel column eluted with $CHCl_3$–MeOH (15:1) to yield compound **5** (155.6 mg). Fraction 5 (2.1 g) was purified on a silica gel column eluted with petroleum ether–ethyl acetate (9:1) to give compound **6** (38.9 mg).

(3*R,4*S**)-6,8-Dihydroxy-3,4,7-trimethylisocoumarin (1):** pale-yellow solid, mp 222–223°C, $[\alpha]_D^{25}$ –33.5° (*c* 2.9, MeOH–CH_3Cl 1:1). UV spectrum (CH_3OH, λ_{max}, nm) (log ε): 323 (4.30), 259 (3.92), 226 (3.36). IR spectrum (KBr, ν, cm^{-1}): 3431 (OH), 1639 (O–C=O), 1584, 1510 (Ar), 1411, 1268, 1187, 1097, 1058, 840.

(3*R*,4*S*)-6,8-Dihydroxy-3,4,5-trimethylisocoumarin (2): colorless needles, mp 216–218°C, $[\alpha]_D^{23}$ −15.5° (*c* 0.26, MeOH). PMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 6.25 (1H, s, H-7), 4.69 (1H, q, J = 6.6, H-3), 3.08 (1H, q, J = 7.0, H-4), 2.06 (3H, s, H-13), 1.28 (3H, d, J = 6.1, H-11), 1.27 (3H, d, J = 6.9, H-12). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 170.4 (s, C-1), 164.9 (s, C-8), 163.6 (s, C-6), 144.2 (s, C-10), 115.6 (s, C-5), 101.3 (d, C-7), 99.7 (s, C-9), 81.5 (d, C-3), 35.7 (d, C-4), 19.9 (q, C-12), 19.8 (q, C-11), 10.0 (q, C-13).

(3*R*,4*S*)-6,8-Dihydroxy-3,4,5,7-tetramethylisochroman (3): pale-yellow solid, mp 175–177°C, $[\alpha]_D^{25}$ –13.3° (*c* 1.1, MeOH). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.68 (2H, m, H-1), 3.93 (1H, dq, J = 6.6, 6.5, H-3), 2.61 (1H, dq, J = 6.9, 6.4, H-4), 2.13 (3H, s, H-13), 2.12 (3H, s, H-14), 1.25 (3H, J = 6.92, H-11), 1.23 (3H, J = 6.6, H-12). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 151.1 (s, C-6), 147.7 (s, C-8), 134.7 (s, C-10), 113.0 (s, C-5), 112.8 (s, C-9), 106.8 (s, C-7), 74.3 (d, C-3), 59.2 (t, C-1), 35.1 (d, C-4), 20.6 (q, C-11), 17.9 (q, C-12), 10.5 (q, C-14), 8.0 (q, C-13).

(*S*)-3-(3′,5′-Dihydroxy-2′,4′-methylphenyl) butan-2-one (4): colorless needles, mp 98–100°C. PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.13 (1H, s, H-6′), 3.93 (1H, q, J = 6.9, H-3), 2.22 (3H, s, H-1), 2.14 (3H, s, Me-4′), 2.04 (3H, s, Me-2′), 1.29 (3H, d, J = 6.8, H-4). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 210.8 (s, C-2), 153.5 (s, C-3′), 153.2 (s, C-5′), 137.6 (s, C-1′), 113.6 (s, C-2′), 109.0 (s, C-4′), 105.9 (d, C-6′), 49.8 (d, C-3), 28.6 (q, C-1), 16.8 (q, C-4), 11.4 (q, 2′-CH_3), 8.4 (q, 4′-CH_3).

Phenol A (5): pale-yellow solid, mp 129–131°C. PMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 6.28 (1H, d, J = 1.8, H-6), 6.18 (1H, d, J = 2.0, H-4), 3.86 (1H, dq, J = 6.2, 6.3, H-3′), 3.06 (1H, dq, J = 6.8, 6.9, H-2′), 2.08 (3H, s, Me-2), 1.14 (3H, d, J = 7.8, H-1′), 1.12 (3H, d, J = 7.2, H-4′). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 157.0 (s, C-3), 156.3 (s, C-5), 145.8 (s, C-1), 115.4 (s, C-2), 106.0 (d, C-6), 101.3 (d, C-4), 72.1 (d, C-3′), 43.1 (d, C-2′), 19.6 (q, C-4′), 16.5 (q, C-1′), 10.9 (q, 2-CH_3).

3,4,5-Trimethyl-1,2-benzenediol (6): colorless needles, mp 112–114°C. PMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 6.25 (1H, s, H-6), 2.13 (3H, s, H-9), 2.06 (3H, s, H-7), 2.06 (3H, s, H-8). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 154.4 (s, C-1), 153.9 (s, C-2), 135.4 (s, C-3), 115.8 (s, C-4), 110.3 (s, C-5), 109.9 (d, C-6), 20.3 (q, C-7), 11.9 (q, C-8), 9.1 (q, C-9).

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