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ISOLATION AND CYTOTOXICITY OF ISOCOUMARINS FROM THE ENTOMOGENOUS FUNGUS *Setosphaeria* sp.

**Sha-Sha Liu,[1] Wen-Bin Gao,[1] Jie Kang,[1]
Xiao-Hui Yang,[1] Fei Cao,[1] Fan-Dong Kong,[2]
You-Xing Zhao,[2*] and Du-Qiang Luo[1*]**

*A new isocoumarin compound named setosphacohol A (**1**), together with six known ones, alternariol (**2**), isoaltenuene (**3**), phomasatin (**4**), alternariol 5-*O*-methyl ether (**5**), 1-deoxyrubralactone (**6**), and rubralactone (**7**), was isolated from the entomogenous fungus* Setosphaeria *sp. The structure of the new compound was elucidated by analysis of the 1D and 2D spectroscopic data as well as MS. Compounds **2**, **5**, **6** showed moderate cytotoxicity against six human tumor cell lines MCF-7, MGC-803, H1975, Huh-7, A549, and HeLa with IC_{50} values ranging from 23.04 to 96.91 $\mu g \cdot mL^{-1}$.*

Keywords: *Setosphaeria* sp., isocoumarins, cytotoxicity.

Isocoumarins comprise a six-membered oxygen heterocycle (α-pyranone) [1], along with one benzene ring, and exhibit a wide range of biological activities including anticancer, anti-HIV, antibacterial, antifungal, anti-inflamatory, antileukemic, antimalarial, antitubercular, and hepatoprotective activity [2]. Recently, an entomogenous fungus *Setosphaeria* sp. (strain LGWB-2) was isolated from *Harmonia axyridis*, obtained from Baoding (Hebei Province), People′s Republic of China. A new isocoumarin, setosphacohol A (**1**), together with six known ones (Fig. 1), was isolated from the methanol extract of the rice medium of *Setosphaeria* sp. Herein, we report the isolation, structural elucidation, and cytotoxicity of compounds **1–7** as shown in Fig. 1.

Compound **1** was obtained as a white solid. The molecular formula of **1** was determined as $C_{16}H_{22}O_7$ by HR-ESI-MS at *m*/*z* 349.1241 $[M + Na]^+$ (calcd 349.1263). The ^1H NMR spectrum (Table 1) suggested the presence of one OH [δ 10.88 (1H, s, 8-OH)], one methyl [δ 0.97 (3H, t, J = 7.2 Hz, CH_3-13)], three oxygenated methines [δ 4.46 (1H, m, H-3), 4.87 (1H, d, J = 10.2 Hz, H-4), and 4.15 (1H, m, H-10)], one aromatic proton [δ 6.77 (1H, s, H-5)], and two methoxy groups [δ 3.96 (3H, s, CH_3O-6) and 3.89 (3H, s, CH_3O-7)]. The ^{13}C NMR spectrum (Table 1) of **1** showed 16 carbon resonances, including one methyl [δ 13.9 (q, C-13)], three methylenes, three oxygenated methine groups, two methoxy groups [56.3 (q, CH_3O-6), and 60.8 (q, CH_3O-7)], one olefinic carbon [δ_C 99.5 (d, C-5)], and six quaternary carbons (including one carbonyl δ 169.1). The NMR data of **1** showed close similarity to those in the literature [3], suggesting compound **1** possessed the same planar structure as that reported in the literature. This was further confirmed by interpretation of the HMBC long-range correlations from H-5 to C-4, C-6, C-7, C-8a and C-4a, H-4 to C-3, C-5, C-6, and C-4a, and H-9 to C-3 and C-4, and ^1H–^1H COSY H-3/H-4/H-9/H-10/H-11/H-12/H-13, as shown in Fig. 1.

The absolute configurations of compound **1** at C-3 and C-4 were determined from the NOESY and ECD spectra. The NOESY correlation of H-9/H-4 indicated the *cis*-configuration of H-3 and 4-OH. The simulated ECD spectrum for (3*R*,4*R*)-**1** via Boltzmann statistics was compared with the experimental ECD spectrum (Fig. 2). According to Fig. 2, the calculated ECD spectral curve was a mirror-image of the experimental ECD spectrum. Therefore, the absolute configurations of C-3 and C-4 in compound **1** were determined as (3*S*,4*S*).

1) College of Life Science, Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of Ministry of Education, Hebei University, 071002, Baoding, P. R. China, e-mail: duqiangluo@163.com; 2) Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, 571101, Haikou, P. R. China, e-mail: zhaoyouxing@itbb.org.cn. Published in *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2020, pp. 688–690. Original article submitted August 28, 2019.

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

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	–	169.1 (C)	9	2.09 (m)	38.1 (CH_2)
3	4.46 (m)	81.2 (CH)	10	4.15 (m)	68.0 (CH)
4	4.87 (d, J = 10.2)	66.8 (CH)	11	1.55 (m)	39.7 (CH_2)
4a	–	139.1 (C)	12	1.40 (m); 1.46 (m)	18.6 (CH_2)
5	6.77 (s)	99.5 (CH)	13	0.97 (t, J = 7.2)	13.9 (CH_3)
6	–	159.1 (C)	6-OCH_3	3.96 (s)	56.3 (OCH_3)
7	–	135.6 (C)	7-OCH_3	3.89 (s)	60.8 (OCH_3)
8	–	155.8 (C)	8-OH	10.88 (s)	
8a	–	101.0 (C)			

TABLE 2. Cytotoxic Activities of Compounds **1–7*** (IC_{50}, μg·mL^{-1})

Compound	MGC-803	MCF-7	H1975	Huh-7	A549	HeLa
2	56.37 ± 15.74	39.05 ± 3.83	77.69 ± 12.86	40.93 ± 12.75	36.59 ± 3.68	96.91 ± 13.71
5	46.16 ± 16.88	64.05 ± 3.92	> 100	72.46 ± 5.06	23.04 ± 4.06	90.92 ± 3.80
6	> 100	> 100	> 100	92.68 ± 6.13	90.71 ± 12.99	> 100
Cisplatin	102.81 ± 8.41	25.05 ± 3.39	71.94 ± 3.36	3.22 ± 0.45	47.58 ± 6.74	71.91 ± 13.39

*Compounds **1**, **3**, **4**, and **7** have IC_{50} values greater than 100 μg.mL^{-1}.

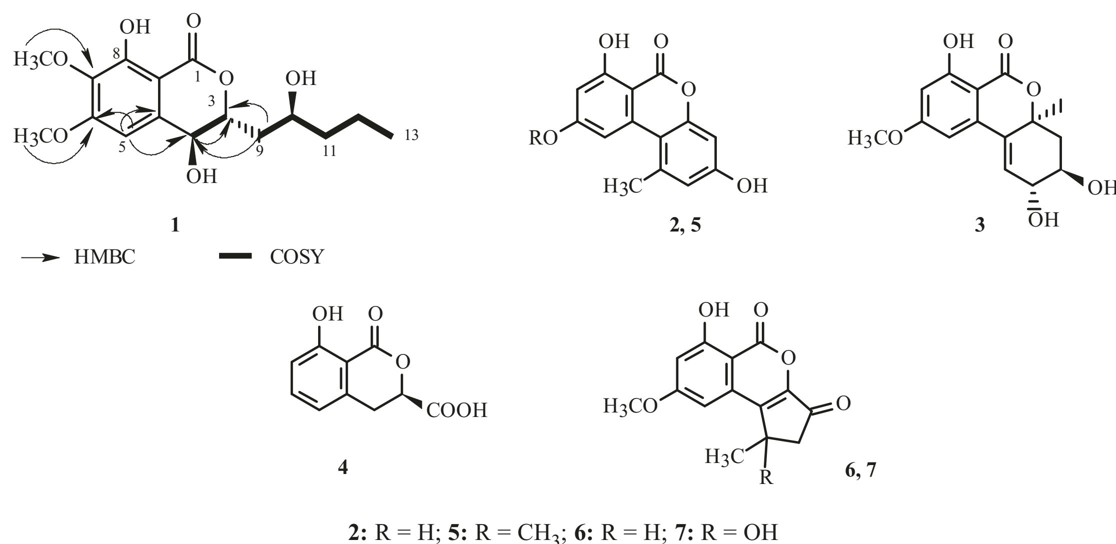

Fig. 1. Chemical structures of compounds **1**–**7** and key HMBC and COSY correlations for compound **1**.

Compounds **2–7** were identified as alternariol (**2**) [4], isoaltenuene (**3**) [5], phomasatin (**4**) [6], alternariol 5-*O*-methyl ether (**5**, djalonensone) [7], 1-deoxyrubralactone (**6**) [8], and rubralactone (**7**) [9] by comparison of their NMR data with those reported in the literature. All compounds were evaluated for their cytotoxic activities against six human tumor cell lines MCF-7, MGC-803, H1975, Huh-7, A549, and HeLa by the MTT method, with cisplatin as positive control. Table 2 shows that compounds **2**, **5**, and **6** have moderate cytotoxicities with IC_{50} values ranging from 23.04 to 96.91 μg·mL^{-1}, while compounds **1**, **3**, **4**, and **7** were inactive against all the tested cancer cell lines with IC_{50} values over 100 μg·mL^{-1}.

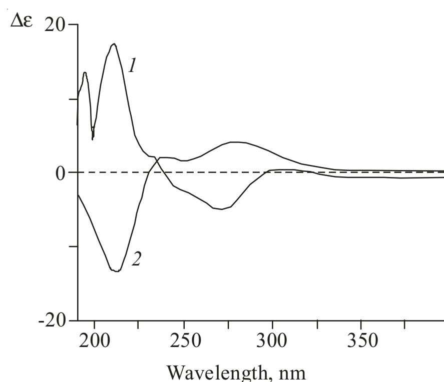

Fig. 2. The ECD curve of compound **1**: exp. ECD for **1** (*1*); calcd ECD for (3*R*,4*R*)-**1** (*2*).

EXPERIMENTAL

General. Optical rotations were measured on a Perkin-Elmer 341 spectropolarimeter. Electronic circular dichroism spectra were measured using a JASCO J-715 circular dichroism spectrometer. IR spectra were acquired on a Perkin-Elmer 577 instrument. NMR spectra were recorded on a Bruker AM-600 spectrometer. HR-MS spectra were recorded on a Bruker apex-ultra 7.0T spectrometer. Column chromatography (CC) was conducted over silica gel (SiO_2, 200–300 mesh; Yantai Zhi Fu Chemical Co., P. R. China), and Sephadex LH-20 gel (25–100 μm, GE Healthcare Co., Ltd., Sweden). TLC was conducted with silica gel GF_{254} plates (Yantai Zhi Fu Chemical Co., Ltd., P. R. China).

Fungus Material. The strain LGWB-2 was isolated from *Harmonia axyridis* collected in Baoding, Hebei Province of China. A voucher specimen of the fungus was deposited at the Key Laboratory of Medicinal Chemistry and Molecular Diagnosis of the Ministry of Education, College of Life Science of Hebei University. *Setosphaeria* sp. was inoculated into a 500 mL Erlenmeyer flask containing 200 mL of PD medium (20.0 g of glucose, 200.0 g of potato in 1 L of distilled H_2O). Flask cultures were incubated at 25°C on a rotary shaker at 120 rpm/min for 4 days. Fermentation was carried out in 100 Erlenmeyer flasks (500 mL) each containing 80 g of rice, and 5 mL of culture liquid was transferred as seed into each flask and incubated at 27°C for 30 days.

Extraction and Isolation. The fermented material was extracted three times with methanol (25 L for each time), and the methanol extract was concentrated *in vacuo* to yield a yellow oily residue (132.0 g). This residue was subjected to SiO_2 CC with gradient elution of petroleum ether (PE)–EtOAc (9:1, 6:1, 4:1, 2:1, and 1:1) to obtain six fractions (Frs. 1–6). Fraction 4 (4.3 g) was eluted with PE–EtOAc (30:1, 20:1, 15:1, 10:1, 5:1, and 1:1) to obtain six subfractions. Subfraction 4-3 was repeatedly purified by SiO_2 CC and Sephadex LH-20 to afford compounds **1** (5.6 mg) and **2** (4.3 mg); the same method was used to obtain **3** (6.8 mg), and from Subfrs. 4-4 to obtain **4** (3.3 mg). Fraction 5 (3.7 g) was eluted with CH_2Cl_2–MeOH (20:1, 10:1, 5:1, 2:1, and 1:1) to obtain five subfractions. Subfraction 5-1 was repeatedly purified by SiO_2 CC and recrystallized from MeOH to afford compounds **5** (10.3 mg), **6** (9.3 mg), and **7** (8.5 mg).

Setosphacohol A (1), $C_{16}H_{22}O_7$, white powder; $[\alpha]_D^{25}$ –36° (*c* 0.1, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3406, 2924, 2850, 1668, 1423, 1276, 1115. For ^1H and ^{13}C NMR data, see Table 1. HR-ESI-MS at *m/z* 349.1241 $[M + Na]^+$ (calcd for $C_{16}H_{22}O_7Na$, 349.1263).

Cytotoxity Assay. The cells were cultured at Roswell Park Memorial Institute (RPMI1640, Hyclone, Logan, UT, USA, MCF-7, H1975) using Dulbecco′s modified Eagle′s medium (DMEM, Hyclone, Logan, UT, USA, MGC-803, HeLa, Huh-7, A549) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA) at 37°C under an atmosphere of 5% CO_2 and were seeded on each well of 96-well plates containing 100 μL of tumor cell suspension (5×10^4 cells/mL). After 48 h, 2 μL (2 $\mu g \cdot mL^{-1}$) of the test compounds dissolved in an appropriate amount of DMSO (Sigma, St. Louis, MO, USA) was added to each well to a final concentration of 3.125, 6.25, 12.5, 25, 50, 100 $\mu g \cdot mL^{-1}$, with 0.5% DMSO as controls, to eliminate the effect of methanol on cells, and the whole was incubated for another 24 h. Then 20 μL of MTT solution (1 $mg \cdot mL^{-1}$, Beijing Cellchip Biotechnology Co., Ltd.) was added to each well, and the plate was incubated for 4 h under the same condition. Then 100 μL of SDS-HCl was added to each well and the whole incubated in a carbon dioxide incubator overnight. The absorbance in the control and drug-treated wells was measured using a microplate reader (Thermo Scientific,

USA) at 570 nm (emission) wavelength. All experiments were carried out in triplicate and repeated twice. The cytotoxicity was expressed as IC_{50} value (50% inhibitory concentration calculated by the modified Karber method). The results were analyzed using SPSS 19.0.

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