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A New Xanthone from the Fermentation Products of Endophytic Fungus of *Phomopsis* Species

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A new xanthone, 1,3-dihydroxy-4-(1,3,4-trihydroxybutan-2-yl)-8-methoxy-9*H*-xanthen-9-one (**1**) was isolated from the fermentation products of a *Phomopsis* fungus. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compound **1** was also evaluated for their cytotoxicity against five human tumor cell lines and it showed high cytotoxicity against A549 and PC3 cell with IC_{50} values of 5.8 and 3.6 μM, respectively.

Keywords: Xanthone, Fermentation, *Phomopsis*, Endophytic.

INTRODUCTION

The *Phomopsis* species known as an important phytopathogenic genus contains more than 900 species named from a wide range of hosts[1]. These microorganisms produce a number of secondary metabolites with various biological activities, including antimicrobial[2,3], antifungal[4,5], antimalarial[6,7], antitumor[7-9], *etc*. The xanthone derivatives are important metabolites isolated from the fermentation products of *Phomopsis* genus and they appeals to medicinal chemists because of their pronounced pharmacological effects[10-12].

With the aim of multipurpose utilization endophytic fungus of *Phomopsis* species and identify bioactive natural products, the phytochemical investigation on fermentation products of an endophytic fungus *phomopsis* species was carried out. As a result, a new xanthone (**1**) was isolated from this fermentation product. The structure of **1** (Fig. 1) was elucidated on the basis of a comprehensive analysis of the ^{1}H NMR, ^{13}C NMR and 2D NMR spectra. In addition, the cytotoxicity of **1** was evaluated. The details of the isolation, structure elucidation and cytotoxicity of this new compound are reported in this article.

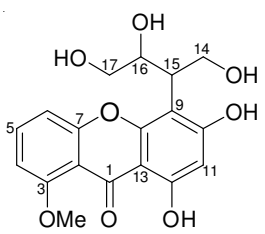

Fig. 1. Structure of compound **1**

EXPERIMENTAL

Optical rotations were measured by a JASCO DIP-1000 polarimeter. Ultraviolet absorption spectra were recorded using a Perkin-Elmer Lambda L14 spectrometer. A Perkin Elmer spectrum 100 FT-IR spectrometer was used for scanning IR spectroscopy with KBr pellets. The ^{1}D and ^{2}D NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRMS were obtained using a LC-MS/MS system, with a Acquity ultra-performance liquid chromatography (UPLC) module and a quadrupole time-of-flight (Q-TOF) spectrometer equipped with a electrospray ion source (Waters, Milford, MA) and connected to a lock-mass apparatus to perform a real-time calibration correction. Column chromatography was performed with silica gel (200-300 mesh, Qingdao Marine Chemical, Inc.), Sephadex LH-20 (Pharmacia) and reversed-phase C_{18} silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF_{254} were used. An Agilent 1100 series equipped with an Alltima C_{18} column (4.6 × 250 mm) was used for HPLC analysis and semipreparative and preparative Alltima C_{18} columns or Zorbax SB-C18 columns (9.4 × 250 and 22 × 250 mm) were used in sample preparation. Spots were visualized by heating silica gel plates sprayed with 10 % H_2SO_4 in EtOH.

The culture of *Phomopsis* amygdali was isolated from the rhizome of *Paris polyphylla var. yunnanensis* collected from ShiZhong, Yunnan, People's Republic of China, in 2007. The strain was identified by one of authors (Gang Du) based on the analysis of the ITS sequence (Genbank Accession

number KF308686). It was cultivated at room temperature for 7 days on potato dextrose agar at 28 °C. Agar plugs were inoculated into 250 mL Erlenmeyer flasks each containing 100 mL potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for five days. Large scale fermentation was carried out in 200 Fernbach flasks (500 mL) each containing 100 g of rice and 120 mL of distilled H_2O. Each flask was inoculated with 5 mL of cultured broth and incubated at 25 °C for 45 days.

Extraction and isolation: An acetone extract prepared from the fermentation products was decolorized by MCI and chromatographed on a silica gel column eluting with hexane/acetone (1:0, 4:1, 2:1, 1:1 and 0:1) to afford five fractions A-E. The further separation of fraction B3 (5.72 g), upon separation on silica gel using petroleum ether-acetone and semipreparative HPLC (65 % MeOH-H_2O, flow rate 12 mL/min) to afforded compound **1** (11.5 mg).

1,3-Dihydroxy-4-(1,3,4-trihydroxybutan-2-yl)-8-methoxy- 9*H*-xanthen-9-one (1): Obtained as yellow gum, $[\alpha]_D^{22.5}$ + 13.6 (*c* =13.6, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.26), 230 (3.85), 252 (3.68), 332 (3.70) nm; IR (KBr, ν_{max}, cm^{-1}): 3420, 2917, 1602, 1519, 1487, 1405, 1328, 1270, 1203, 1160, 1095, 872, 758; ^{1}H- and ^{13}C NMR: see Table-1; ESIMS (positive ion mode): *m/z* 385 $[M + Na]^+$; HRESIMS (positive ion mode): *m/z* 385.0893 $[M + Na]^+$ (calcd $C_{18}H_{18}O_8Na$ for 385.0899).

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{18}H_{18}O_8Na$ on the basis of positive HRESIMS $[M + Na]^+$ *m/z* 385.0893 (calcd 385.0899) with 10 ° of unsaturation. The UV absorptions at 332, 252, 230 and 210 nm showed an extended chromophore and a substituted benzene ring. The IR spectral data of **1** showed the presence of hydroxyl groups (3420 cm^{-1}) and phenyl groups (1602, 1519, 1487 cm^{-1}). The ^{1}H NMR spectrum (Table-1) displayed three coupled aromatic methines (δ_H 6.62 (1H, d, *J* = 8.3 Hz, H-4), 7.55 (1H, t, *J* = 8.3 Hz , H-5), 6.97 (1H, d, *J* = 8.3 Hz, H-6)) featuring one 1,2,3-trisubstituted aromatic ring deduced from their coupling constants, one singlet aromatic methine (δ_H 6.61 (1H, s, H-11) indicative of one 1,2,3,4,5-pentasubstituted aromatic ring, one methoxy group (δ_H 3.75, -OMe-3), two methylene groups (δ_H 4.20, H_2-17 and δ_H 4.75, H_2-14) and two methine groups including one which was oxygenated at C-16 (δ_H 4.43, H-15 and δ_H 5.26, H-16). Further analysis of its ^{13}C NMR (DEPT) data (Table-1) revealed the presence of one ketone carbonyl, twelve aromatic carbons (four of which were protonated) indicative of two substituted phenyl rings, two methoxy, two methylene and two methine groups including an oxygenated one. By careful analysis of NMR data, we found that compound **1** should be a xanthone derivative and its NMR spectral data were similar to those of a known compound, secosterigmatocystin[12]. They possessed the same substitution pattern, except for the substituents variation on the aromatic rings. For compound **1**, a methoxy group located at C-3 was deduced from the HMBC correlations (Fig. 2) of the methoxy proton signal (δ_H 3.75) with C-3 (δ_C 166.5). Furthermore, tow phenolic hydroxy groups located at C-10 and C-12 were supported by the HMBC correlations of one phenolic hydroxy proton signal (δ_H 13.50) with C-9 (δ_C 110.2), C-10 (δ_C 158.7) and C-11 (δ_C 99.3); and of another phenolic hydroxy proton signal (δ_H 13.86) with C-11 (δ_C 99.3), C-12 (δ_C 162.5) and C-13 (δ_C 104.8), respectively. The structure of **1** was established as 1,3-dihydroxy-4-(1,3,4-trihydroxybutan-2-yl)-8-methoxy-9*H*-xanthen-9-one. It is very difficult to determine the relative configurations of C-15 and C-16 only through spectroscopic analysis. We tried to determine the relative configuration of **1** by Mosher's method and X-ray. Unfortunately, we failed to obtain the desired results. Thus, the relative configurations of C-15 and C-16 remain unassigned.

Since certain xanthones exhibit potential cytotoxicity[10,13,14], the cytotoxicity of compound **1** was tested using a previously reported procedure[15]. All treatments were performed in triplicate. In the MTT assay, the IC_{50} valve was defined as the concentration of the test compound resulting in a 50 % reduction of absorbance compared with untreated cells. Compounds **1** was also evaluated for their cytotoxicity against five human tumor cell lines and it showed high cytotoxicity against A549 and PC3 cell with IC_{50} values of 5.8 and 3.6 μM, respectively.

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TABLE-1
^{1}H- (500 MHZ) AND ^{13}C-NMR (125 MHZ) DATA FOR 1 (OBTAINED IN C_5D_5N)

No.	δ_C (m)	δ_H (m, *J* = Hz)	No.	δ_C (m)	δ_H (m *J* = Hz)
1	181.9 s		11	99.3 d	6.61, s
2	106.2 s		12	162.5 s	
3	166.5 s		13	104.8 s	
4	110.7 d	6.62, d (8.3)	14	61.5 t	4.72, m
5	136.2 d	7.55, t, (8.3)	15	42.4 d	4.43, m
6	116.8 d	6.97, d, (8.3)	16	72.8 d	5.26, m
7	158.0 s		17	66.6 t	4.20, m
8	155.2 s		OMe-3	55.8 q	3.75, s
9	110.2 s		Ar-OH-10		13.50, s
10	158.7 s		Ar-OH-12		13.86, s

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