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Phenolic Derivatives from *Dioscorea bulbifera*

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Abstract: Two new phenolic derivatives, diosbulbiol A (**1**), diosbulbiol B (**2**), and six known compounds were isolated from *Dioscorea bulbifera*. Their structures was determined by MS, IR, UV, 1D- and 2D-NMR. The cytotoxicity of new compounds were evaluated against four cancer cell lines.

Keywords: *Dioscorea bulbifera*; dioscorea; cytotoxicity; phenolic derivatives; diosbulbiol A; diosbulbiol B.

1. Introduction

Dioscorea bulbifera L. (family Dioscoreaceae) is widely distributed in China and used to treat a variety of diseases including thyroid disease and cancer. Previous phytochemical investigations on the root of *D. bulbifera* showed the presence of clerodane diterpenoids [1-3], norclerodane diterpenoids [4], apianen lactones [5], flavonoids and anthraquinones [6]. Our prior study on the plant disclosed the presence of various types of compounds [7-9]. Further investigation resulted in the isolation of two new phenolic derivatives, diosbulbiol A (**1**), diosbulbiol B (**2**), along with six known compounds (**3**-**8**) from the ethanol extract of the tubers (Figure 1). Compound **1** is a diphenylpentadienone, the diphenylpentadienone dervative biologically activity, for example against leukaemia cells , anti-cancer, anti-allergic, activities [10]. Compound 2 is a diarylheptanoide. Diphenylpentadienone derivative has shown the anti-leishmanial activity [11]. The cytotoxicity of new compounds were evaluated against four cancer cell lines.

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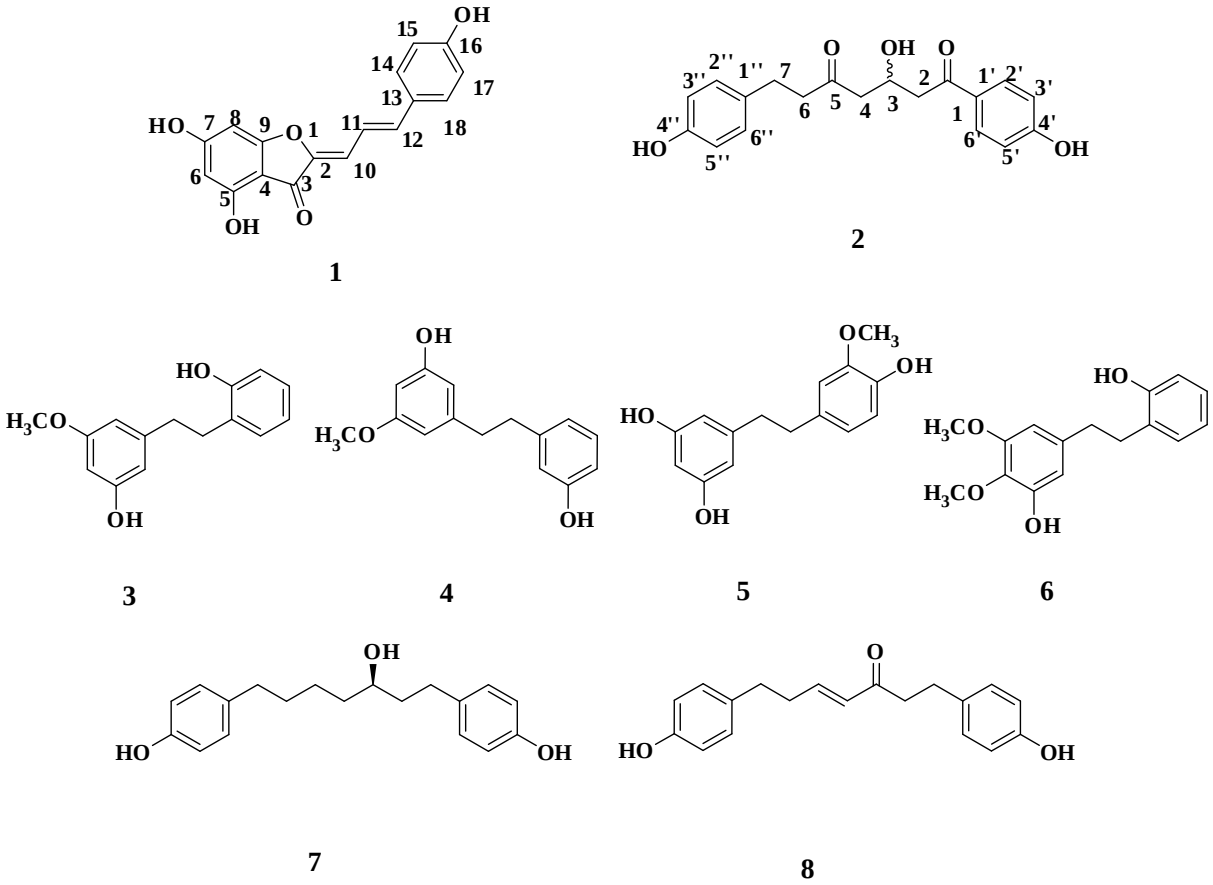

Figure 1. Structures of compounds **1-8**

2. Materials and Methods

2.1. General Experimental Procedures

UV spectra were respectively recorded with Shimadzu double-beam 201A equipped with a DAD and a 1cm path-length cell and IR spectra were obtained on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. Optical rotation was obtained on Jasco P-1020 digital polarimeter. 1D and 2D NMR spectra were run on Bruker DRX-500 and AV-400 spectrometer (Karlsruhe, Germany). Chemical shifts (δ) were expressed in ppm with reference to solvent signals. HREI-MS was measured on a Waters AutoSpec Primier P776 instrument (Waters, Milford, MA, USA). Preparative HPLC was per-formed using an Agilent 1260 and a reverse-phase C18 column (Agilent Zorbax SB-C18, 150 mm × 9.4 mm, 5 μm, Kyoto, Japan). Columnchromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden).

2.2. Plant Material

The tubers of *D. bulbifera* were collected from Anhui Province, P. R. China, in Sep. 2016 and identified by Qin-Shan Yang, Anhui University of Chinese Medicine. A voucher specimen (No. DB201601) has been deposited in the Department of Natural Products Chemistry, Anhui University of Chinese Medicine.

2.3. Extraction and Isolation

Dried crushed tubers (15 kg) of *D. bulbifera* were extracted with 75% EtOH two times (v/v, 2×150 L) at room temperature. The filtrate was concentrated under vacuum to give the extract, which

was suspended in 5 L water and partitioned successively with petroleum ether (6×5 L), EtOAc (6×5 L), and *n*-BuOH (10×5 L). The EtOAc soluble portion (546 g) was subjected to silica gel column chromatography eluting with CH_2Cl_2/MeOH (100:1 to 0:1, v/v) to yield nine fractions, Fr.1–9, based on TLC analysis. Fr. 4 was purified through a prep-HPLC equipped with a ODS-A column (250 × 10 mm) to yield Compound **1** (20 mg), **3** (10 mg), **4** (8 mg). Fr. 5 was subjected to a Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1, v/v), followed by chromatography over repeated silica gel column (petroleum ether/acetone, 70:30, v/v) to afford Compound **5** (14 mg) and purified a prep-HPLC equipped with a ODS-A column to yield Compound **2** (3 mg), **7** (6 mg) and **8** (7 mg). Fr.9 was subjected to a Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1, v/v), followed by chromatography over repeated silica gel column (petroleum ether/acetone, 50:50, v/v) to afford Compound **6** (8 mg).

2.4. Spectroscopic Data

*Diosbulbiol A (**1**):* Yellow powder. IRν_{max} (KBr): 3430, 2924, 1632, 1120, 588 cm^{-1}. UV (MeOH) λ_{max} (logε): 367 (3.28), 275(2.86).^{1}H (600 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD): Table 1.HR-ESI-MS *m/z*: [M-H]$^-$ 295.0614 (calcd. for $C_{17}H_{11}O_5$ 295.0612).

*Diosbulbiol B (**2**):*Yellow powder.$[\alpha]_D^{25.5}$ = -14.55 (*C* 0.00110, MeOH). IRν_{max} (KBr): 3443, 2925, 1631, 1384, 1030, 586 cm^{-1}. UV ($CHCl_3$) λ_{max} (logε): 203 (3.94), 220 (3.87), 279 (3.77) nm. ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): Table 1. HR-ESI-MS *m/z*: [M+K]$^+$ 367.0941 (calcd. for $C_{19}H_{20}O_5K$ 367.0942).

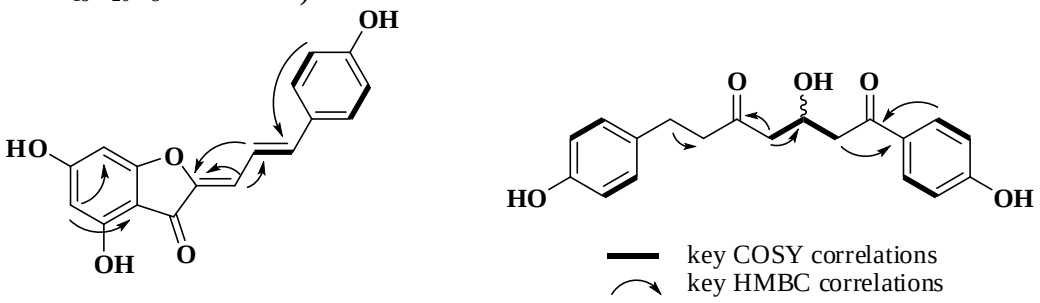

Figure 2. Key ^{1}H–^{1}H COSY and HMBC relevant of compound **1** and **2**

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a yellow powder. Its molecular formula $C_{17}H_{12}O_5$, was deduced from the HR-ESI-MS peak at *m/z* 295.0614 [M-H]$^-$ (*m/z* $C_{17}H_{11}O_5$ Calcd for 296.0612), consistent with twelve degrees of unsaturation. The IR spectrum showed absorption bands at 3430 cm^{-1} and 1632 cm^{-1} ascribed to hydroxyl and benzene ring groups, respectively. The ^{1}H-NMR spectrum exhibited also signals for benzene ring at δ_H 6.44 (1H, br.s, H-8), 6.19 (1H, s, H-6) and 7.54 (2H, d, *J* = 8.2 Hz, H-14, H-18), 6.83 (2H, d, *J* = 8.2 Hz, H-15, H-17). Furthermore, the characteristic signals of two double bonds at δ_H 6.80 (1H, m, H-11), 7.58 (1H, m, H-12), and 6.18 (1H, m, H-10).

The ^{13}C-NMR and DEPT spectrum of **1** exhibited 17 carbon resonances. including two benzene rings at δ_C 159.3 (C-5), 165.2 (C-7), 100.0 (C-6), 95.02 (C-8), 105.5(C-4), 163.3 (C-9) and 130.9 (C-15, 17), 128.0 (C-13), 116.9 (C-14, 18), 160.9 (C-16), a carbonyl at δ_C 183.9 (C-3), and two carbon-carbon double bonds δ_C 166.3 (C-2),107.9 (C-10) and 117.3 (C-11), 139.1 (C-12). The ^{1}H and ^{13}C NMR spectra (Table 1) of compound **1** was very similar to those of (*Z*)-4, 6-dimethoxy-2-((*E*)-3-phenylallylidene) benzofuran-3(2H)-one [10] with the major differences that a methoxyl group was absent in **1**. After correlation of all the protons with their directly bonded carbon partners via a HSQC

spectrum, it was possible from the HMBC and ^{1}H-^{1}H COSY spectrum (Figure 2) to deduce the planar structure of **1**. In addition, compared with ^{1}H-NMR spectrum and coupling constant, two aromatic ring obtained meta substitution and ortho substitution, respectively. Furthermore, according to the ^{1}H-^{1}H COSY spectrum, the following cross-peaks H-11/H-12, H-14/H-15 and H-17/H-18 were displayed, for another, in the HMBC spectrum, key long-range correlations were assigned by the HMBC correlations from H-6/C-4, C-8 and H-10/C-2, C-11 and H-1/C-12 and H-15/C-12. Accordingly, the structure of **1** was established as shown in Figure 1 and named Diosbulbiol A.

Compound **2** was obtained as a yellow powder. Its molecular formula $C_{19}H_{20}O_5$, was deduced from the HR-ESI-MS peak at m/z 367.0941 $[M+K]^+$ (Calcd for $C_{19}H_{20}O_5$ 367.0942), consistent with ten degrees of unsaturation. The IR spectrum showed absorption bands at 3443 cm^{-1} and 1631 cm^{-1} ascribed to hydroxyl and benzene ring groups. The ^{1}H-NMR spectrum exhibited also signals for benzene ring at δ_H 7.87 (2H, d, *J* = 8.7 Hz, H-2', H-6'), 6.82 (2H, d, *J* = 8.6 Hz, H-3', H-5') and 7.01 (2H, d, *J* = 8.4 Hz, H-2", H-6"), 6.68 (2H, d, *J* = 8.4 Hz, H-3", H-5"). The characteristic signals of a hydroxyl at δ_H 4.62 (1H, m, H-3).

The ^{13}C-NMR and DEPT of **2** exhibited 19 carbon resonances (Table 1). The signals were observed due to two benzene rings at δ_C 130.3 (C-1'), 132.0 (C-2', C-6'), 116.5 (C-3', C-5'), 164.3 (C-4') and 133.2 (C-1"), 130.3 (C-2", C-6"), 116.2 (C-3", C-5"), 156.6 (C-4"), a oxymethene at δ_C 65.9 (C-3), and two carbonyl at δ_C 199.3 (C-1), 211.4 (C-5). The ^{1}H and ^{13}C NMR spectra (Table 1) of compound **2** was very similar to those of 5-hydroxy-3-platyphyllone [12]. In addition, comparing that the HRESIMS with **1**, there is 14 mass units more than that of it and suggestive of an carbonyl group of **2**. According to the ^{13}C NMR spectrum, compound **2** showed that the obvious changes of the chemical shifts were appeared at the C-1 (δ_C 199.3) rather than it (δ_C 29.8) in 5-hydroxy-3-platyphyllone This deduction was corroborated by the 2D NMR spectra, in particular the key correlations from H-2' and H-6' to C-1 in the HMBC spectrum. Thus, the planar structure of compound 2 was determined.

Table 1. NMR data of compound **1** (500/125 MHz, CD_3OD) and **2** (400/100 MHz, CD_3OD)

Position	**1**		No.	**2**	
	δ_H (*J* in Hz)	δ_C		δ_H (*J* in Hz)	δ_C
1			1		199.3
2		166.3	2	2.78 m	46.3
3		183.9	3	4.62 m	65.9
4		105.5	4	2.67 dd (6.2, 3.6)	50.8
5		159.3	5		211.4
6	6.19 br. s	100.0	6	3.05 dd (8.7, 6.3)	46.0
7		165.2	7	2.78 s	29.8
8	6.44 br. s	95.0	1'		130.3
9		163.3	2'	7.87 d (8.7)	132.0
10	6.18 m	107.9	3'	6.82 d (8.6)	116.5
11	6.80 m	117.3	4'		164.3
12	7.58 m	139.1	5'	6.82 d (8.6)	116.5
13		128.0	6'	7.87 d (8.7)	132.0
14	7.54 d (8.2)	130.9	1"		133.2
15	6.83 d (8.2)	116.9	2"	7.01 d (8.4)	130.3
16		160.9	3"	6.68 d (8.4)	116.2
17	6.83 d (8.2)	116.9	4"		156.6
18	7.54 d (8.2)	130.9	5"	6.68 d (8.4)	116.2
			6"	7.01 d (8.4)	130.3

We had done the experience about the ECD and mosher reactions for identifying this absolutely configuration. The result implied that the absolute configuration at C-3 in **2** was not confirmed due to that was small amounts after separation and purification. Accordingly, the structure of **2** was established as shown in Figure 1 and named Diosbulbiol B.

The known phenolic derivatives was identified as 2', 3- dihydroxy-4, 5-dimethoxybibenzyl (**3**) [13], 2', 3-dihydroxy-5-methoxybibenzyl (4) [14], batatasin Ⅲ (5) [15], tristin (6) [13], 3-hydroxy-1, 7-bis-(4', 4''-dihydroxyphenyl)-heptane (**7**) [11], platyphyllenone (**8**) [16] by analysis of its spectroscopic and MS data with those reported in this literature.

The new compounds were evaluated *in vitro* for the cytotoxic activities against four cancer cell lines (including SMMC7721, MCF-7, K562 and A549). Unfortunately, none of selected compounds showed obviously inhibitory effect against four cancer cell lines (IC_{50} > 40 μM).

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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