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 were 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.

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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 derivative 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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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 performed 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 CH2Cl2/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 an 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 CHCl3/MeOH (1:1, v/v), followed by chromatography over repeated silica gel column (petroleum ether/aceton, 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 CHCl3/MeOH (1:1, v/v), followed by chromatography over repeated silica gel column (petroleum ether/aceton, 50:50, v/v) to afford Compound 6 (8 mg).

2.4. Spectroscopic Data

*Diosbulbiol A (1)*: Yellow powder. IR
\text{max}(\text{KBr}): 3430, 2924, 1632, 1120, 588 cm\(^{-1}\). UV (MeOH) \(\lambda_{\text{max}}\) (loge): 367 (3.28), 275(2.86), \(^1\)H (600 MHz, CD\(_3\)OD) and \(^{13}\)C NMR (125 MHz, CD\(_3\)OD): Table 1. HR-ESI-MS m/z: [M-H] 295.0614 (calcd. for C\(_17\)H\(_{10}\)O\(_5\) 295.0612).

*Diosbulbiol B (2)*: Yellow powder. \([\alpha]_{D}^{25.5} = -14.55\) (C 0.00110, MeOH). IR\text{max} (KBr): 3443, 2925, 1631, 1384, 1030, 586 cm\(^{-1}\). UV (CHCl3) \(\lambda_{\text{max}}\) (loge): 203 (3.94), 220 (3.87), 279 (3.77) nm. \(^1\)H (400 MHz, CD\(_3\)OD) and \(^{13}\)C NMR (100 MHz, CD\(_3\)OD): Table 1. HR-ESI-MS m/z: [M+K]\(^{+}\) 367.0941 (calcd. for C\(_{19}\)H\(_{30}\)O\(_5\)K 367.0942).

![Figure 2. Key \(^1\)H–\(^1\)H COSY and HMBC relevant of compound 1 and 2](image)

3. Results and Discussion

3.1. Structure Elucidation

Compound 1 was obtained as a yellow powder. Its molecular formula C\(_{17}\)H\(_{10}\)O\(_5\), was deduced from the HR-ESI-MS peak at m/z 295.0614 [M-H] (m/z C\(_{17}\)H\(_{10}\)O\(_5\); Calcd 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 \(\delta_H 6.44\) (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 \(\delta_H 6.80\) (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 \(\delta_C 159.3\) (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 \(\delta_C 183.9\) (C-3), and two carbon-carbon double bonds \(\delta_C 166.3\) (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 methoxy 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 1H-1H COSY spectrum (Figure 2) to deduce the planar structure of 1. In addition, compared with 1H-NMR spectrum and coupling constant, two aromatic ring obtained meta substitution and ortho substitution, respectively. Furthermore, according to the 1H-1H 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_{20}H_{32}O_{8}, was deduced from the HR-ESI-MS peak at m/z 367.0941 [M+K]^+ (Calcd for C_{19}H_{32}O_{8} 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 1H-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 13C-NMR and DEPT of 2 exhibited 19 carbon resonances (Table 1). The signals were observed 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 1H and 13C 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 13C 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.

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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], platyrhogenone (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 (IC50 > 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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References


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