Phenolic Derivatives from *Dioscorea bulbifera*

Guokai Wang\(^1\), Juan Zheng\(^1\), Jaising Yang\(^2\), Yunpeng Su\(^1\), Nan Zhang\(^1\), Huiwen Liu\(^1\) and Jinsong Liu\(^1\)\(^*\)

\(^1\) School of Pharmacy & Anhui University of Chinese Medicine; Anhui Innovative Team from Colleges for Scientific Research’s Platform-The Innovative Team in Researching the Key Technologies concerning the Integration of Processing Chinese Medicine Decoction Pieces in Producing Area, Hefei, Anhui 230012, P. R. China

\(^2\) Department of Medical Research, China Medical University Hospital, China Medical University, Taichung 40402, Taiwan

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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. © 2019 ACG Publications. All rights reserved.

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.

\(^*\) Corresponding author: E-Mail: jinsongliu@ahtcm.edu.cn; Phone:+86-551-68129167 Fax:+86-551-68129125
Phenolic derivatives from *Dioscorea bulbifera*

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 1 cm 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). Column chromatography (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

![Figure 1. Structures of compounds 1-8](image-url)
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 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 CHCl₃/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 CHCl₃/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. IR_{max} (KBr): 3430, 2924, 1632, 1120, 588 cm⁻¹. UV (MeOH) λ_{max} (logε): 367 (3.28), 275(2.86),1H (600 MHz, CD₂OD) and ¹³C NMR (125 MHz, CD₂OD): Table 1. HR-ESI-MS m/z: [M-H] 295.0614 (calcd. for C₁₇H₁₉O₅ 295.0612).

**Diosbulbiol B (2):** Yellow powder. [α]₂₅.₅ = -14.55 (C 0.00110, MeOH). IR_{max} (KBr): 3443, 2925, 1631, 1384, 1030, 586 cm⁻¹. UV (CHCl₃) λ_{max} (logε): 203 (3.94), 220 (3.87), 279 (3.77) nm. ¹H (400 MHz, CD₂OD) and ¹³C NMR (100 MHz, CD₂OD): Table 1. HR-ESI-MS m/z: [M+K]⁺ 367.0941 (calcd. for C₁₉H₂₃O₅K 367.0942).

![Figure 2](image-url) Key ¹H–¹H 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 C₁₇H₁₂O₅, was deduced from the HR-ESI-MS peak at m/z 295.0614 [M-H]⁻ (m/z C₁₇H₁₉O₅ 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 ¹H-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 ¹³C-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 ¹H and ¹³C 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 $\delta_H$ 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 hydroxy at $\delta_H$ 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 $\delta_C$ 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 $\delta_C$ 65.9 (C-3), and two carbonyl at $\delta_C$ 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-platyphyllyone [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 ($\delta_C$ 199.3) rather than it ($\delta_C$ 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$_3$OD) and 2 (400/100 MHz, CD$_3$OD)

| Position | $\delta_H$ (J in Hz) | $\delta_C$ | No. | $\delta_H$ (J in Hz) | $\delta_C$ |
|----------|---------------------|-----------|-----|---------------------|-----------|
| 1        | 166.3               | 1         | 199.3 | 2.78 m              | 46.3      |
| 2        | 183.9               | 2         | 4.62 m | 65.9               |
| 3        | 105.5               | 3         | 2.67 dd (6.2, 3.6) | 50.8      |
| 4        | 159.3               | 4         | 211.4 |                     |
| 5        | 6.19 br. s          | 6         | 3.05 dd (8.7, 6.3) | 46.0      |
| 6        | 165.2               | 7         | 2.78 s | 29.8                |
| 7        | 95.0                | 1'        | 130.3 |                     |
| 8        | 163.3               | 2'        | 7.87 d (8.7) | 132.0      |
| 9        | 107.9               | 3'        | 6.82 d (8.6) | 116.5      |
| 10       | 117.3               | 4'        | 164.3 |                     |
| 11       | 139.1               | 5'        | 6.82 d (8.6) | 116.5      |
| 12       | 128.0               | 6'        | 7.87 d (8.7) | 132.0      |
| 13       | 139.9               | 1''       | 133.2 |                     |
| 14       | 116.9               | 2''       | 7.01 d (8.4) | 130.3      |
| 15       | 160.9               | 3''       | 6.68 d (8.4) | 116.2      |
| 16       | 116.9               | 4''       | 156.6 |                     |
| 17       | 130.9               | 5''       | 6.68 d (8.4) | 116.2      |
| 18       | 7.54 d (8.2)        | 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], platyphenolone (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

ORCID

Guokai Wang: 0000-0002-3924-6169
Juan Zheng: 0000-0003-1006-0747
Jaising Yang: 0000-0001-7302-8248
Yunpeng Sun: 0000-0001-6368-0427
Nan Zhang: 0000-0002-3360-2150
Huwen Liu: 0000-0001-8517-7507
Jinsong Liu: 0000-0002-8982-6719

References

[1] R. B. Teponno, A. L. Tapondjou, E. Abou-Mansour, H. Stoeckli-Evans, P. Tane and L. Barboni (2008). Bafoudiosbulbins F and G, further clerodane diterpenoids from Dioscorea bulbifera L. var sativa and revised structure of Bafoudiosbulbin B, Phytochemistry. 69, 2374-2379.
[2] R. B. Teponno, A. L. Tapondjou, D. Gating, J. D. Djoukeng, E. Abou-Mansour, R. Tabacchi, P. Tane, H. Stoeckli-Evans and D. Lontsi (2006). Bafoudiosbulbins A and B, two anti-salmonellal clerodane diterpenoids from Dioscorea bulbifera L. var sativa, Phytochemistry 67, 1957-1963.
[3] R. B. Teponno, A. L. Tapondjou, H. Ju-Jung, J. H. Nam, P. Tane and H. J. Park (2007). Three new clerodane diterpenoids from the bulbils of Dioscorea bulbifera L. var. sativa, Helv. Chim. Acta 90, 1599-1605.
[4] Y. Tang, Y. B. Xue, L. Zhou, J. W. Zhang, G. M. Yao, Z. W. Luo, G. Du and Y. H. Zhang (2014). New norsesodane diterpenoids from the tubers of Dioscorea bulbifera, Chem. Pharm. Bull. 62, 719-724.
[5] S. Z. Zheng, Z. Guo, T. Shen, X. D. Zhen and X. W. Shen (2003). Three new apian lactones from Dioscorea bulbifera L., Indian. J. Chem. – Sect. B 42, 946-949.
[6] S. S. Li, I. A. Iiyya, J. Z. Deng and S. X. Zhao (2000). Flavonoids and anthraquinone from Dioscorea bulbifera L., China J. Chin. Mater. Med. 25, 159-160.
[7] G. Wang, J. S. Liu, B. B. Lin, G. K. Wang and J. K. Liu (2009). Two new furanoid norterpines from Dioscorea bulbifera, Chem. Pharm. Bull. 57, 625-627.
[8] G. Wang, B. B. Lin, J. S. Liu, G. K. Wang, F. Wang and J. K. Liu (2009). Chemical constituents from tubers of Dioscorea bulbifera, China J. Chin. Mater. Med. 34, 1679-1682.
[9] J. S. Liu, W. N. Gao, J. Zheng, G. K. Wang and Q. S. Yang (2017). Chemical constituents from fresh tubers of Dioscorea bulbifera, China J. Chin. Mater. Med. 42, 510-516.
[10] D. Sharma and J. K. Makrandi (2014). Mercuric acetate mediated oxidative cyclization of (2E, 4E)-1-(2-hydroxyphenyl)-5- phenylpenta-2, 4-dien-1-ones: Synthesis of (Z)-2-((E)-3-phenylallylidene)benzofuran-3(2H)-ones, J. Heterocyclic Chem. 51, 1818-1820.
[11] C. A. C. Araujo, L. V. Alegrio and L. L. Leon (1998). Antileishmanial activity of compounds extracted and characterized from Centrolebias sclerophyllum, Phytochemistry 49, 751-754.
[12] K. Sunnerheimsjöberg and P. G. Knutsson (1995). Platylloside: Metabolism and digestibility reduction in vitro, J. Chem. Ecol. 21, 1339-1348.
[13] L. M. Li, G. Q. Li, X. Wu, G. C. Wang and Y. L. Li (2014). Stilbenoids from rhizomes of Dioscorea bulbifera, Chin. Trad. Herbal. Drugs. 45, 328-332.
[14] Y. W. Leong, C. C. Kang, L. J. Harrison and A. D. Powell (1997). Phenanthrenes, dihydrophenanthrenes and bibenzyls from the orchid Bulbophyllum vaginatum, Phytochemistry 44, 157-165.
[15] Y. G. Chen, H. Yu and X. Lian (2015). Isolation of stilbenoids and lignans from Dendrobium hongdie, Tropical J. Pharm. Res. 14, 2055
[16] N. H. Tung, S. K. Kim and J. C. Ra (2010). Antioxidative and hepatoprotective diarylheptanoids from the bark of Alnus japonica, Planta. Med. 76, 626-629

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