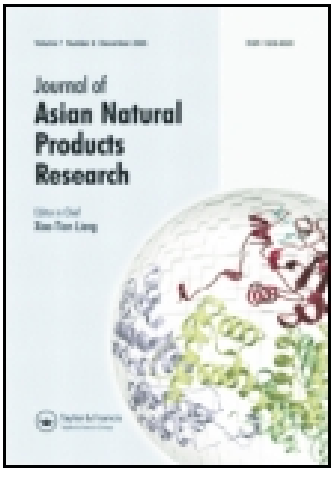


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

New phenanthrene glycosides from Dioscorea opposita

Ken Yu-Zhong Zheng[abc], Zhen-Xia Zhang[b], Wenliang Zhou[a], Hui Cao[c] & Fei-Jun Xiang[cd]

[a] The School of Life Science, Sun Yat-Sen University, Guangzhou, 510275, China

[b] Department of Biology, Hanshan Normal University, Chaozhou, 521041, China

[c] Research and Development Department, Guangdong Kangmei Pharmaceutical Research Institute Company Limited, Guangzhou, 510006, China

[d] Research and Development Department, Kangmei Pharmaceutical Company Limited, Puning, 515300, China

Published online: 29 Oct 2013.

To cite this article: Ken Yu-Zhong Zheng, Zhen-Xia Zhang, Wenliang Zhou, Hui Cao & Fei-Jun Xiang (2014) New phenanthrene glycosides from Dioscorea opposita, Journal of Asian Natural Products Research, 16:2, 148-152, DOI: 10.1080/10286020.2013.837459

To link to this article: http://dx.doi.org/10.1080/10286020.2013.837459

Journal of Asian Natural Products Research, 2014
Vol. 16, No. 2, 148–152, http://dx.doi.org/10.1080/10286020.2013.837459

New phenanthrene glycosides from *Dioscorea opposita*

Ken Yu-Zhong Zheng[a,b,c]*, Zhen-Xia Zhang[b], Wenliang Zhou[a], Hui Cao[c] and Fei-Jun Xiang[c,d]

[a]*The School of Life Science, Sun Yat-Sen University, Guangzhou 510275, China;* [b]*Department of Biology, Hanshan Normal University, Chaozhou 521041, China;* [c]*Research and Development Department, Guangdong Kangmei Pharmaceutical Research Institute Company Limited, Guangzhou 510006, China;* [d]*Research and Development Department, Kangmei Pharmaceutical Company Limited, Puning 515300, China*

(*Received 18 June 2013; final version received 20 August 2013*)

Two new phenanthrene glycosides, dioscopposide A and dioscopposide B (**1** and **2**), were isolated from the rhizomes of *Dioscorea opposita*. Their structures were determined primarily on the basis of 1D and 2D NMR techniques, MS studies, and chemical methods. All the isolates were evaluated for their inhibitory effects on the lipopolysaccharide-induced nitric oxide production using murine macrophage RAW 264.7 cells. The IC_{50} values of dioscopposide A and dioscopposide B were 5.8 and 7.2 μM, respectively.

Keywords: *Dioscorea opposita*; phenanthrene glycosides; dioscopposide A, B

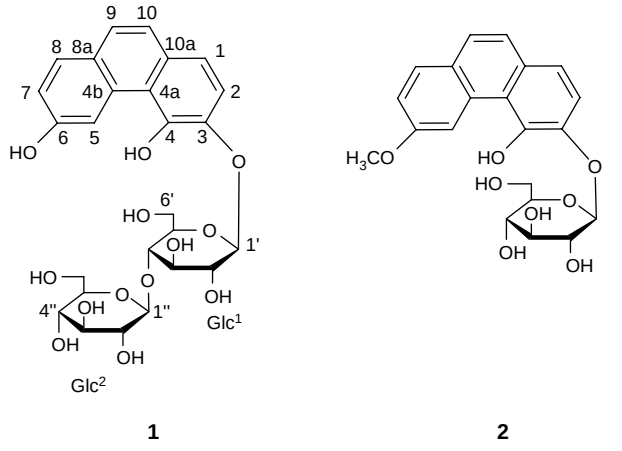
1. Introduction

The *Dioscorea opposita* Thunb. grows wild in China, and the tuber is used as a food. This plant is also included in the Pharmacopoeia of the People's Republic of China and is widely used to treat anorexia, chronic diarrhea, diabetes, seminal emission and excessive leukorrhea [1,2]. Previous investigation on the phytochemistry of this plant leads to isolation of phenanthrene and bibenzyl derivatives, which showed anti-inflammatory, neuroprotective, and radical scavenging activities [3–6]. In the course of a search for biologically active compounds from Chinese medicinal plants, we have investigated the rhizomes of *D. opposita*. Two phenanthrene glycosides were isolated and characterized (**1** and **2**) (Figure 1). This paper describes the isolation and structural elucidation of these compounds and the evaluation of their effects against nitric oxide (NO) production.

2. Results and discussion

Dioscopposide A (**1**) was isolated as an amorphous solid. Its molecular formula was determined as $C_{26}H_{30}O_{13}$ by negative HR-ESI-MS, showing an $[M-H]^-$ peak at *m/z* 549.1603. The IR spectrum exhibited absorptions at 3288, 1730, and 1432 cm^{-1} (aromatic rings). The ^1H NMR spectrum of **1** (Table 1) showed the presence of seven aromatic protons of aglycone (H-1, H-2, H-5, H-7, H-8, H-9, and H-10) and protons of sugar in the range of δ_H 3.63–5.12. The ^1H NMR and ^{13}C NMR (Table 1), combined with distortionless enhancement by polarization transfer (DEPT) and HMQC, spectra of **1** revealed that **1** contained seven aromatic methine carbons (C-1, C-2, C-5, C-7, C-8, C-9, and C-10), seven quaternary aromatic carbons (C-3, C-4, C-4a, C-4b, C-8a, and C-10a), two anomeric carbons (C-1′ and C-1″), two methylene carbons (C-6′ and C-6″), and eight methine carbons (C-2′–C-5′ and

*Corresponding author. Email: zhengyuzhong@gmail.com



1 **2**

Figure 1. The structures of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (500 MHz, in pyridine-d_5).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1	7.02 d (8.0)	115.8	7.01 d (8.0)	115.7
2	7.22 d (8.0)	118.3	7.23 d (8.0)	118.2
3		151.1		151.2
4		152.7		152.6
4a		109.3		109.2
4b		136.7		136.6
5	7.56 d (1.5)	123.9	7.29 d (1.5)	121.5
6		143.7		144.9
7	7.01 dd (7.5, 1.5)	118.8	6.82 dd (7.0, 1.5)	118.2
8	7.95 dd (7.5, 1.0)	118.6	7.94 dd (7.0, 1.0)	117.8
8a		127.6		127.5
9	7.04 dd (7.5, 1.0)	121.3	7.04 dd (7.5, 1.0)	121.2
10	7.41 d (7.5)	111.5	7.41 d (7.5)	111.4
10a		118.6		118.5
Glc[1]				
1′	5.04 d (8.0)	102.8	5.14 d (8.0)	102.6
2′	3.84 dd (9.0, 8.0)	74.2	4.12 dd (9.5, 8.0)	74.3
3′	4.12 dd (9.0, 8.0)	76.6	4.26 dd (9.5, 8.0)	77.5
4′	4.21 m	81.2	4.14 m	70.8
5′	3.63 m	76.7	3.96 m	77.6
6′a	4.25 dd (12.0, 6.0)	61.9	4.31 dd (12.0, 6.0)	61.5
6′b	4.11 dd (12.0, 2.0)		4.22 dd (12.0, 2.0)	
Glc[2]				
1″	5.12 d (8.0)	104.6		
2″	3.98 dd (9.5, 8.0)	74.9		
3″	4.20 dd (9.5, 8.0)	78.3		
4″	4.15 m	71.5		
5″	3.92 m	78.4		
6″a	4.50 dd (13.0, 7.0)	62.3		
6″b	4.20 dd (13.0, 2.0)			
OMe			3H, 3.70 s	55.2

C-2″–C-5″). A detailed analysis of the $^{1}H-^{1}H$ correlated spectroscopy (COSY) spectrum of **1** allowed to elucidate the following three pairs of *ortho*-coupled aromatic protons at δ_H 7.02 (H-1) and 7.22 (H-2), 7.01 (H-7) and 7.95 (H-8), and 7.04 (H-9) and 7.41 (H-10). In the HMBC spectrum, H-1 showed correlations with two aromatic carbons C-3 and C-10, and H-2 correlated with C-3 and C-4, respectively. H-5, an isolated proton, showed HMBC correlations with three quaternary carbons C-4a, C-4b, and C-8a. Cross-peaks were equally observed between H-7 and C-6 and C-8a. Additional HMBC correlations were observed between H-8 and C-4b and C-9, between H-9 and C-4b and C-10a, and between H-10 and C-8a and C-10a (Figure 2). All these data suggested the presence of a 3,4,6-trisubstitued phenanthrene [4]. The ^{1}H and ^{13}C NMR data, as well as acid hydrolysis and GC comparison with an authentic sample, indicated the presence of two D-glucose moieties. The β-configuration of the glycosidic linkage was determined from the coupling constant of the anomeric proton. The connections of the Glc^{1}-C-1′/aglycone-C-3 and Glc^{2}-C-1″/Glc^{1}-C-4′ were revealed by HMBC correlations H-1′/C-3 and H-1″/C-4′. The proton and carbon signals of **1** were assigned using

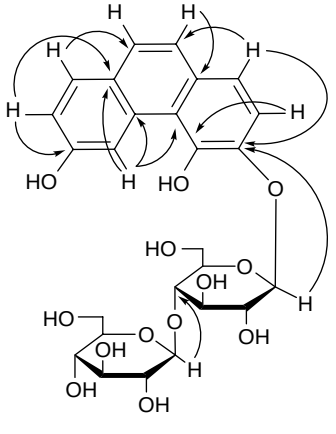


Figure 2. Selected HMBC correlations of compound **1**.

^{1}H, ^{13}C, $^{1}H-^{1}H$ COSY, 1D TOCSY, HSQC, HMBC, and NOESY NMR experiments. From these results, the structure of **1** was determined as shown in Figure 1 and named as dioscopposide A.

Dioscopposide B (**2**) was isolated as an amorphous solid. Its molecular formula was determined as $C_{21}H_{22}O_8$ by negative HR-ESI-MS, showing an $[M-H]^-$ peak at *m/z* 401.1233. Comparison of the NMR data for **1** and **2** revealed that the compounds are similar except for the absence of signals for Glc^{2} connected to Glc^{1}–C-4′ in **1**. This was confirmed by the C-4′ appeared comparatively upfield at δ 70.8. In addition, the connection of a methoxyl group (δ_H 3.70 and δ_C 55.2) and C-6 was confirmed by HMBC correlation of δ_H OCH_3-3.70/C-6. Thus, compound **2** was determined as shown in Figure 1 and named as dioscopposide B.

Compounds **1** and **2** were evaluated for their inhibitory activity against NO production. Aminoguanidine was used as a positive control. The IC_{50} values of **1**, **2**, and aminoguanidine were 5.8, 7.2 and 15.3 μM, respectively.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco DIP-360 digital polarimeter (Jasco Corporation, Tokyo, Japan). UV spectra were obtained on a TU-1901 spectrometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China). IR spectra were detected by a Nexus-470 FT-IR (Nicolet) spectrometer (Thermo Electron, Madison, WI, USA). NMR spectra were recorded on a Bruker Inova 500 spectrometer (Varian, Palo Alto, CA, USA) with tetramethylsilane as an internal standard. HR-ESI-MS data were measured using a Q-TOF micro mass spectrometer (Waters, Millford, MA, USA). GC was obtained on an Agilent 6890N gas chromatography (Agilent Technologies, CA, USA). Column chromatography was carried out on D101

porous polymer resin (Tianjin Chemical Industry Co., Ltd, Tianjin, China), silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), and C_{18} silica gel (150–200 mesh, Merck, Darmstadt, Germany). HPLC was performed on Waters 600 Controller (Waters, Milford, MA, USA) by using an Agilent Eclipse XDB C_{18} semi-preparative column (250 mm × 9.4 mm, i.d. 5 μm, Agilent Technologies, Inc., CA, USA).

3.2 Plant material

The rhizomes of *D. opposita* were collected from Wenxian, Henan Province, China, in July 2012, and were identified by Dr Ken Yuzhong Zheng, Hanshan Normal University. A voucher specimen (No. 201204) has been deposited in the herbarium of Department of Biology of Hanshan Normal University.

3.3 Extraction and isolation

The air-dried powdered rhizomes of *D. opposita* (15 kg) were successively extracted with 75% EtOH under reflux. The resultant extract was combined and dried under reduced pressure to give concentrated extractives. The latter was subsequently suspended in water and partitioned successively with petroleum ether, EtOAc, and *n*-butanol. The *n*-butanol part (322 g) was subjected to column chromatography by a combination of D101 macroporous resin, eluted gradiently with 10% EtOH and 60% EtOH, successively. The fraction eluted with 60% EtOH (120 g) was subjected to repeated ODS column chromatography [MeOH:H_2O (5:95–95:5)] to afford four subfractions. Subfraction 3 (12.5 g) was separated by semi-preparative HPLC (MeOH:H_2O 20:80, 2.0 ml/min) to yield **1** (33 mg) and **2** (36 mg).

3.3.1 Dioscopposide A (1)

Yellow amorphous solid; $[\alpha]_D^{20}$ −22.6 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 222, 239, 341 nm; IR (KBr) ν_{max} (cm^{-1}): 3288, 1730, and 1432. ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) spectral data, see Table 1. HR-ESI-MS: *m/z* 549.1603 $[M-H]^-$ (calcd for $C_{26}H_{29}O_{13}$, 549.1608).

3.3.2 Dioscopposide B (2)

Yellow amorphous solid; $[\alpha]_D^{20}$ −16.6 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 222, 239, 341 nm; IR (KBr) ν_{max} (cm^{-1}): 3328, 2832, 1690, and 1441. ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) data, see Table 1. HR-ESI-MS: *m/z* 401.1233 $[M-H]^-$ (calcd for $C_{21}H_{21}O_8$, 401.1236).

3.4 Acid hydrolysis

Compounds **1** and **2** (5 mg, respectively) were hydrolyzed separately with 2 M HCl (0.5 ml) for 10 h at 95°C. After filtration of the reaction mixture, the filtrate was evaporated under vacuum. After addition of H_2O, the acidic solution was evaporated again to remove HCl. This procedure was repeated until a neutral solution was obtained, which was finally evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (1 ml), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60°C for 2 h and evaporated under N_2 stream and dried *in vacuo*. The residue was trimethylsilylated with *N*-trimethylsilylimidazole (0.2 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (2 ml each), and the *n*-hexane extract was analyzed by GC under the following conditions: capillary column, DB-5 (30 m × 0.25 mm × 0.25 mm); detection, FID; detector temperature, 280°C; injection temperature, 250°C; initial temperature was maintained at 100°C for 2 min and then raised to 280°C at the rate of 10°C/min, and final temperature was maintained for 5 min; carrier, N_2 gas. The peak of the hydrolysate of **1** and **2** was detected at

24.94 min (D-glucose) by comparison with authentic sample [7].

3.5 NO production from lipopolysaccharide-stimulated macrophages

Inhibitory effect on the NO production by mouse macrophages (RAW 264.7) was evaluated using the method reported previously [8]. Briefly, thioglycolate-induced peritoneal exudate cells (5200 cells/well) were collected from the peritoneal cavities of male ddY mice and were suspended in 100 ml of RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin G (100 units/ml), and streptomycin sulfate (100 mg/ml), and were precultured in 96-well microplates at 37°C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing with phosphate buffer solution, and the adherent cells were cultured in 200 ml of fresh medium containing 10 mg/ml lipopolysaccharide (LPS) and various concentrations of each test compound for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent.

Acknowledgments

This research was financially supported by grants from Natural Science Foundation of China (No. 81202907) and Natural Science Foundation of Guangdong Province (No. S2012040008079).

References

[1] Editorial Committee of Chinese Pharmacopeia, *Chinese Pharmacopoeia*, 2010 ed., (Chemical & Industry Press, Beijing, 2010), p. 27.
[2] M. Tomoda, K. Ishikawa, and M. Yokoi, *Chem. Pharm. Bull.* **3256**, 29 (1981).
[3] W. Tang and G. Eisenbrandt, *Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine* (Springer-Verlag, Berlin, 1992), Vol. 281, p. 26.
[4] M. Sautuor, A.C. Mitaine-Offer, T. Miyamoto, H. Wagner, and M.A. Lacaille-Dubios, *Chem. Pharm. Bull.* **52**, 1235 (2004).
[5] M. Ito, K. Matsuzaki, J. Wang, A. Daikonya, N.L. Wang, X.S. Yao, and S. Kitanaka, *Chem. Pharm. Bull.* **628**, 58 (2010).
[6] C. Ma, W. Wang, Y.Y. Chen, R.N. Liu, R.F. Wang, and L.J. Du, *J. Nat. Prod.* **1579**, 72 (2009).
[7] Y.L. Feng, H.R. Li, L.Z. Xu, and S.L. Yang, *J. Asian Nat. Prod. Res.* **9**, 505 (2007).
[8] H. Matsuda, T. Morikawa, S. Ando, H. Oominami, T. Murakami, I. Kimura, and M. Yoshikawa, *Bioorg. Med. Chem.* **12**, 3037 (2004).