Neuroprotective and Antioxidant Activity of Compounds from the Aerial Parts of *Dioscorea opposita*

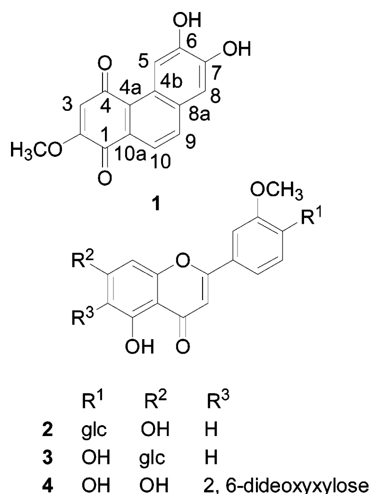
Chao Ma, Wei Wang, Yun-Yun Chen, Rui-Ning Liu, Ru-Feng Wang, and Li-Jun Du*

Laboratory of Pharmaceutical Sciences, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, People's Republic of China

Received January 20, 2005

Bioactivity-guided fractionation of an ethanol extract of the aerial parts of *Dioscorea opposita* afforded a new compound, 6,7-dihydroxy-2-methoxy-1,4-phenanthrenedione (**1**), and four known compounds, chrysoeriol 4′-*O*-*β*-D-glucopyranoside (**2**), chrysoeriol 7-*O*-*β*-D-glucopyranoside (**3**), alternanthin (**4**), and daucosterol. The structure of **1** was established on the basis of the interpretation of its 1D and 2D NMR spectrascopic data. Compounds **1**−**4** exhibited both promising neuroprotective effects and discernible to moderate antioxidant activities in vitro.

Dioscorea opposita Thunb., which belongs to the family Dioscoreaceae, is cultivated in Asia as a foodstuff.[1,2] In the People's Republic of China, this plant is distributed widely in Henan Province, and its rhizomes have been used in traditional clinical practice for the treatment of anorexia, chronic diarrhea, diabetes, problems with seminal emission, and excessive leukorrhea and also to promote immune functions.[3] Previous phytochemical and biological investigations carried out on the rhizomes of *D. opposita* have resulted in the isolation of various constituents such as diosgenin, bibenzyl derivatives, and phenanthrenes and their glycosides.[3] However, no investigation of the phytochemical constituents and bioactivity of the aerial parts of the title plant has yet been carried out. Accordingly, an ethanol extract of the aerial parts of *D. opposita* was discovered to have neuroprotective and antioxidant activities. We report herein the isolation, characterization, and bioactivity of a new compound, 6,7-dihydroxy-2-methoxy-1,4-phenanthrenedione (**1**), together with the biological properties of three known compounds, **2**−**4**, from the aerial parts of *D. opposita*.



Compound **1** was obtained as a red amorphous powder with a melting point of 267−269 °C. The negative HR-FABMS of **1** exhibited a molecular ion peak at *m/z* 269.0459 (calcd for $C_{15}H_9O_5$, 269.0455), so the molecular formula of **1** was calculated as $C_{15}H_{10}O_5$, requiring 11 degrees of unsaturation. The UV spectrum of **1** exhibited aromatic absorptions at 246 and 282 nm. In turn, the IR spectrum of this compound showed hydroxyl absorptions at 3351 cm^{-1}, aromatic bands at 1608, 1570, and 1446 cm^{-1}, C−O stretching bands at 1067 and 1234 cm^{-1}, and carbonyl absorptions at 1634 and 1674 cm^{-1}. The ^{1}H NMR spectroscopic data of **1** indicated that there were five aromatic protons, appearing as three singlets and two *ortho*-coupled doublets. The other proton signals could be assigned as one methoxy group and two hydroxyl groups, which were consistent with the carbon signals observed at δ 56.2, 150.9, and 149.3. The ^{13}C NMR and DEPT spectra of **1** revealed the presence of one methyl, five methines, and nine quaternary carbons. Two of the nine quaternary carbon signals were assigned as carbonyl carbons according to their chemical shifts at δ 180.4 (s) and 188.3 (s). On the basis of the above analysis, compound **1** could be suggested as a phenanthrenedione or an anthrenedione with two hydroxyl groups and a methoxy group. Both the ^{1}H and ^{13}C NMR spectra of this compound were similar to those of the known phenanthrenedione densiflorol B (7-hydroxy-2-methoxy-1,4-phenanthrenedione).[4,5] Therefore, compound **1** was assigned as a phenanthrenedione. Two aromatic proton signals at δ 8.94 (1H, s) and 7.21 (1H, s) in the ^{1}H NMR spectrum of **1** enabled the deduction of one aromatic ring (A ring) with a 1,2,4,5-tetrasubstitution pattern. Another aromatic ring (B ring) was suggested to have a 1,2,3,4-tetrasubstitution pattern from two coupled aromatic proton signals at δ 7.97 (1H, d, J = 8.5 Hz) and 7.80 (1H, d, J = 8.5 Hz). According to the ^{1}H−^{13}C long-range correlation signals between the methoxy group protons and C-2 (Figure 1), the methoxy group could be located at the C-2 position. By comparison with the known compound densiflorol B,[4,5] differences in the ^{13}C NMR spectroscopic data were observed in the C-5 and C-6 signals, which suggested another hydroxyl was located at the C-6 position. The overall structural determination of **1** was based on the detailed analysis of 1D and 2D NMR spectroscopic data including the ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. On the basis of the above evidence, compound **1** was assigned as 6,7-dihydroxy-2-methoxy-1,4-phenanthrenedione.

Four known compounds were identified from their spectral data by comparison with values reported in the

* To whom correspondence should be addressed. Tel and Fax: +86-10-6277-3630. E-mail: lijundu@mail.tsinghua.edu.cn.

10.1021/np050021c CCC: $30.25 © 2005 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/27/2005

Table 1. Effect of Compounds **1**−**4** on Lactic Dehydrogenase (LDH) Leakage, Conjugated Diene (CD) Formation of LDL, and Malondialdehyde (MDA) Amount in Vitro[a]

agent	concentration (μM)	LDH leakage (%)[b]	CD increase (%)[b]	MDA (mmol/mg protein)[b]
control		53.36 ± 2.56	86.49 ± 6.38	1.65 ± 0.12
vitamin C	568		0.17 ± 0.12**	1.35 ± 0.02**
1	370	36.58 ± 8.09	95.09 ± 14.43	1.23 ± 0.12*
	37.0	41.09 ± 6.98*	112.97 ± 6.11	1.43 ± 0.05*
	3.70	38.30 ± 7.29*	67.46 ± 5.46	1.52 ± 0.09
2	217	21.14 ± 3.99**	0.32 ± 0.08**	1.16 ± 0.19**
	21.7	22.74 ± 4.00**	30.16 ± 0.01**	1.43 ± 0.07*
	2.17	25.52 ± 3.95**	51.62 ± 2.07*	1.46 ± 0.05
3	217	21.99 ± 3.67**	11.52 ± 1.02**	0.95 ± 0.05**
	21.7	22.51 ± 3.19**	66.21 ± 3.16*	1.19 ± 0.07**
	2.17	27.35 ± 2.88**	62.24 ± 17.09	1.37 ± 0.12*
4	233	25.08 ± 5.28**	70.27 ± 3.96	1.40 ± 0.05*
	23.3	28.29 ± 4.32**	96.06 ± 3.27	1.34 ± 0.12*
	2.33	38.12 ± 6.09*	129.42 ± 9.87	1.57 ± 0.11

[a] For protocols used, see Experimental Section. [b] Values are the mean ± SD of three assays (*$P < 0.05$, **$P < 0.01$, versus control).

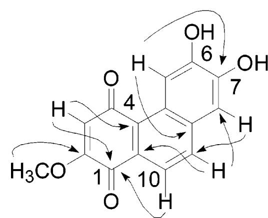


Figure 1. Key HMBC correlations for **1**.

literature as chrysoeriol 4′-*O*-β-D-glucopyranoside (**2**),[6] chrysoeriol 7-*O*-β-D-glucopyranoside (**3**),[7] alternanthin (**4**),[8] and daucosterol.[9]

The neuroprotective activities in vitro of compounds **1**−**4** were examined by an assay to evaluate decreasing lactic dehydrogenase (LDH) leakage from neocortical cell cultures with oxygen and glucose deprivation (OGD) (Table 1). Compounds **2** and **3** exhibited neuron-protective activity in a dose-dependent manner, while compounds **1** and **4** showed discernible neuron-protective activity. The antioxidant activities of compounds **1**−**4** were estimated by measurement of low-density lipoprotein (LDL) susceptibility to oxidation and determination of malondialdehyde (MDA) levels in the rat brain in vitro. The data collected (Table 1) indicated that both new compound **1** and known compounds **2**−**4** exhibited moderate to significant antioxidant bioactivity.

The neuroprotective and antioxidant activities reported herein for the aerial parts of *D. opposita* are not supported by practices in Chinese traditional medicine. However, the present results may lead to the fuller utilization of *D. opposita* as a medicinal plant in the future.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4A micromelting point apparatus and were uncorrected. UV spectra were run on a Varian Cary Eclipse 300 spectrometer using methanol as solvent. IR spectra were measured on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H and ^{13}C NMR experiments were performed on a Bruker DRX 500 NMR spectrometer using DMSO-d_6 as internal standard. The HRFABMS were run with a Bruker APEX II mass spectrometer. Column chromatography was carried out with silica gel (100−300 mesh) (Tsingtao Marine Chemistry Co., Ltd.) and Sephadex LH-20 (18−110 μm) (Pharmacia Co., Ltd.). Laboratory animals were obtained from the Laboratory Animal Institute, Chinese Academy of Medical Science, Beijing, People's Republic of China.

Plant Material. The plant material was collected in September 2002 from Jiaozuo City, Henan Province, People's Republic of China. One of the authors (L.J.D.) identified this as the leaves and stems of *Dioscorea opposita* Thunb., and a voucher specimen (No. 031009) has been deposited in the herbarium of the Laboratory of Pharmaceutical Science, Department of Biological Sciences and Biotechnology, Tsinghua University.

Extraction and Isolation. The dried leaves and stems (4 kg) were extracted with 95% ethanol under reflux. After concentration in vacuo, the ethanol crude extract (115 g) was suspended in water and partitioned successively with petroleum ether, ethyl acetate (EtOAc), and *n*-butanol. The EtOAc-soluble part was subjected to silica gel column chromatography using $CHCl_3$−MeOH mixtures (95:5 → 3:1) as eluents to afford 40 fractions (Fr1−Fr40). Fr23 was chromatographed on a silica gel column eluted with a $CHCl_3$−MeOH mixture (15:1) to give compound **1** (46 mg). Fr9−Fr12 were subjected to silica gel column chromatography using gradient $CHCl_3$−MeOH mixtures (20:1 → 5:1) as eluents to give 30 fractions (G1−G30). From this, G12−G15 were chromatographed on a Sephadex LH-20 column with methanol as eluent, and compounds **2** (20 mg) and **4** (25 mg) were purified. Fr13 was treated by silica gel and Sephadex LH-20 column chromatography, successively, to yield compound **3** (20 mg). Daucosterol (300 mg) precipitated from the solution of Fr19−Fr22 in methanol.

6,7-Dihydroxy-2-methoxy-1,4-phenanthrenedione (1): red amorphous powder, mp 267−269 °C; UV (MeOH) λ_{max} (log ϵ) 433 (2.98), 282 (3.24), 246 (3.32) nm; IR (KBr) ν_{max} 3351, 2930, 1674, 1634, 1608, 1570, 1540, 1479, 1446, 1397, 1234, 1199, 1067, 1010, 943, 885, 848, 796, 753, 687 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MH$_Z$) δ 6.22 (1H, s, H-3), 8.94 (1H, s, H-5), 7.21 (1H, s, H-8), 7.97 (1H, d, J = 8.5 Hz, H-9), 7.80 (1H, d, J = 8.5 Hz, H-10), 3.84 (3H, s, CH_3-2); ^{13}C NMR (DMSO-d_6, 125 MH$_Z$) δ 188.3 (C, C-4), 180.4 (C, C-1), 158.2 (C, C-2), 150.9 (C, C-6), 149.3 (C, C-7), 133.8 (C, C-8a), 131.6 (CH, C-9), 128.5 (C, C-10a), 125.1 (C, C-4b), 124.3 (C, C-4a), 118.9 (CH, C-10), 112.2 (CH, C-3), 110.3 (CH, C-8), 109.6 (CH, C-5), 56.2 (CH_3, COCH_3); HRFABMS (negative) m/z 269.0459 $[M - H]^-$ (calcd for $C_{15}H_9O_5$, 269.0455).

Determination of Lactic Dehydrogenase (LDH) Release in Neocortical Cell Cultures with Oxygen and Glucose Deprivation (OGD). Neocortical cell cultures were prepared by a published method.[10] The cortical cells were exposed to OGD as previously described in the literature.[11] Compounds **1**−**4** (for each, three different final concentrations were used, see Table 1) were added to the OGD group, and the same volume of non-glucose Earle's buffer solution was added to the control group. After 95% N_2/5% CO_2 was blown through the chamber for 10 min to remove O_2, the chamber with the OGD cells was sealed and moved into the cell incubator at 37 °C for 25 min. The cultures were removed from the chamber and replaced into the cell incubator under normal conditions for 30 min. The OGD treatment was repeated twice, and the cells were kept in the cell incubator at 37 °C for 6 h after the last treatment. The control cells were kept in the cell incubator throughout. The culture medium was collected as an extracellular solution. The intracellular solution was obtained through repeated refrigeration (−20 °C) and melting (4 °C) of cells added with non-glucose Earle's solution. LDH

activity was carried out by a method using a LDH kit (Beijing Zhongsheng biochemical reagent company, batch number: 040624). The LDH leakage was reflected by the following formula: LDH $\text{activity}_{\text{extracell}}$/(LDH $\text{activity}_{\text{extracell}}$ + LDH $\text{activity}_{\text{intracell}}$) × 100%.

Measurement of Low-Density Lipoprotein (LDL) Susceptibility to Oxidation and Determination of Malondialdehyde (MDA) Levels. The measurement of LDL from the plasma of New Zealand White rabbits and the MDA levels in the brains of rats determined through the thiobarbituric acid (TBA) method were performed as published previously.[12] Each compound (**1**−**4**) was evaluated at three different final concentrations, and freshly prepared vitamin C was used as a positive control.

Acknowledgment. The study was supported by grants from Beijing Science and Technology Program (No. Z0004105040311) and the Science and Technology Grant, Tsinghua University (No. 03FD28).

References and Notes

(1) Ireland, C. R.; Schwabe, W. W.; Coursey, D. G. *Phytochemistry* **1981**, *20*, 1569−1571.
(2) Tomoda, M.; Ishikawa, K.; Yokoi, M. *Chem. Pharm. Bull*. **1981**, *29*, 3256−3261.
(3) Sautour, M.; Mitaine-Offer, A.-C.; Miyamoto, T.; Wagner, H.; Lcaille-Dubois, M.-A. *Chem. Pharm. Bull.* **2004**, *52*, 1235−1237.
(4) Cheng, Q. F.; Zhao, W. M.; Qin, G. W. *Chin. Chem. Lett.* **2000**, *11*, 705−706.
(5) Cheng, Q. F.; Wang, W.; Wang, Y. P.; Qin, G. W.; Zhao, W. M. *Phytochemistry* **2001**, *57*, 1255−1258.
(6) Lu, Y. R.; Sun, Y.; Foo, L. Y.; McNabb, W. C.; Molan, A. L. *Phytochemistry* **2002**, *55*, 67−75.
(7) Tang, Y. P.; Zheng, W. P.; Lou, F. C.; Li, Y. F.; Wang, J. H. *J. Chin. Pharm. Sci.* **2000**, *9*, 119−121.
(8) Zhou, B. N.; Blasko, G.; Cordell G. A. *Phytochemistry* **1988**, *27*, 3633−3636.
(9) Sang, S.; Kikuzaki, H.; Lapsley, K.; Rosen, R. T.; Nakatani, N.; Ho, C. T. *J. Agric. Food. Chem.* **2002**, *50*, 4709−4712.
(10) Hartley, D. M.; Kurth, M. C.; Bjerkness, L.; Weiss, J. H.; Choi, D. W. *J. Neurosci.* **1993**, *13*, 1993−2000.
(11) Scorziello, A.; Pellegrini, C.; Forte, L.; Tortiglione, A.; Gioielli, A.; Iossa, S.; Amoroso, S.; Tufano, R.; Renzo, G. D.; Annunziato, L. *J. Neurosci. Res.* **2001**, *63*, 20−26.
(12) Wang, R. F.; Xie, W. D.; Zhang, Zh.; Xing, D. M.; Ding, Y.; Wang, W.; Ma, Ch.; Du, L. J. *J. Nat. Prod.* **2004**, *67*, 2096−2098.

NP050021C