Two New Cytotoxic Naphthoquinones from *Didymocarpus hedyotideus*

by **Xi-Bin Xiao***[a]), **Yue-Xing Lin**[b]), **Geng-Bo Xu**[a]), **Xu-Bo Gong**[a]), **Ying Gu**[a]), **Jie-Feng Tong**[a]), and **Jing Yang**[a])

[a]) Department of Hematology, 2nd Affiliated Hospital of Zhejiang University School of Medicine, No. 88 Jiefang Road, Hangzhou 310009, P. R. China (phone: +86-571-87783652, +86-13858015535 (cell); e-mail: xiaoxibincsu@163.com)

[b]) Blood Bank, 2nd Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, P. R. China

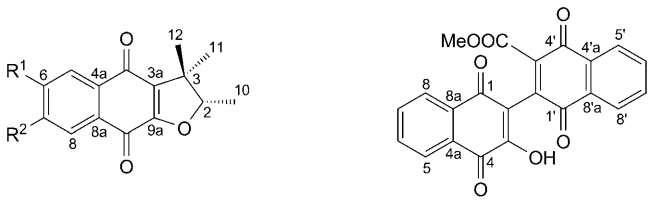
A new naphthoquinone, 6-hydroxy-α-dunnione (**1**) and a new binaphthoquinone, methyl 1,1′,4,4′-tetrahydro-3-hydroxy-1,1′,4,4′-tetraoxo[2,2′-binaphthalene]-3′-carboxylate (**2**), along with ten known compounds, including naphthoquinones, anthraquinones, and phenylethanoid glucosides, were isolated from the roots of *Didymocarpus hedyotideus* CHUN. Their structures were identified by spectroscopic analyses, particularly 1D- and 2D-NMR spectroscopy. The cytotoxic activities of the two new naphthoquinones were also evaluated.

Introduction. – The genus *Didymocarpus* (Gesneriaceae family) consists of *ca.* 120 species of which many are widely distributed in tropical countries. There are few published phytochemical studies of the genus in which phenolic constituents and anthraquinoids were found to be the major secondary metabolites [1].

Didymocarpus hedyotideus CHUN., a species occurring in China, was used to treat eczema, urticaria, psoriasis, bone fracture, and trauma in Chinese folk medicine [2]. Nothing has been reported on the constituents of the plant. Our phytochemical investigation on the 95% EtOH extract of the title plant led to the isolation of 12 compounds, comprising three naphthoquinones, *i.e.*, 6-hydroxy-α-dunnione[1]) (**1**), methyl 1,1′,4,4′-tetrahydro-3-hydroxy-1,1′,4,4′-tetraoxo[2,2′-binaphthalene-3′-carboxylate (**2**), and 7-hydroxy-α-dunnione (**3**) [3], four 9,10-anthraquinones, *i.e.*, 2-(methoxycarbonyl)-9,10-anthraquinone (= methyl 9,10-dihydro-9,10-dioxoanthracene-2-carboxylate) [4], 2-hydroxy-6-methyl-9,10-anthraquinone [5], 2-hydroxy-9,10-anthraquinone [6], and 2,6-dihydroxy-9,10-anthraquinone 2-β-D-glucopyranoside [7], three phenylethanoid glucosides, *i.e.*, plantainoside A [8], calceolarioside [8], and calceolarioside B [8], and two steroids, *i.e.*, β-sitosterol and daucosterol. Except for the two steroids, all these compounds are reported for the first time from this genus. In this article, we report on the isolation and structure elucidation of the two new naphthoquinones **1** and **2** (*Fig.*) and on their cytotoxic activities.

Results and Discussion. – Compound **1** had the molecular formula $C_{15}H_{14}O_4$ as inferred from HR-EI-MS and ^{1}H- and ^{13}C-NMR data. Its UV spectrum exhibited

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



1[1]) R^1 = OH, R^2 = H
3[1]) R^1 = H, R^2 = OH

2[1])

Figure. *Compounds* **1**–**3**, *isolated from* Didymocarpus hedyotideus

characteristic absorption peaks of the conjugated C=O moiety at 266, 308, 361, and 385 nm. The IR spectrum revealed absorption bands for OH (3354 cm^{-1}) and C=O groups (1680 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table 1*) showed signals of two conjugated C=O groups (δ(C) 181.9 (C(4)) and 179.9 (C(9))), eight aromatic C-atoms, a quaternary C-atom (δ(C) 44.7 (C(3))), an oxygenated tertiary C-atom (δ(C) 90.4 (C(2))), and three Me groups (δ(C) 25.4 (C(11)), 20.3 (C(12)), and 14.0 (C(10))). These data suggested that **1** is an α-dunnione derivative [3]. The ^{1}H-NMR spectrum of **1** showed three H-atoms arising from an aromatic moiety at δ(H) 7.62 (*d*, J = 8.4 Hz, H–C(8)), 7.01 (*d*, J = 2.4 Hz, H–C(5)), and 6.77 (*dd*, J = 8.4, 2.4 Hz, H–C(7)). This ^{1}H-NMR pattern indicated the location of the OH group at C(7) or C(6). The NMR data of **1** were compared with those of the known compound 7-hydroxy-α-dunnione (**3**) which was also isolated during the investigation. The three aromatic H-atoms of **1** were shifted towards high field in the ^{1}H-NMR spectrum compared with those of **3**; these results were consistent with the literature [9]. The ^{13}C-NMR spectrum gave further evidence of a difference in structure between the two compounds (*Table 1*). In the HMBC spectrum of **1**, the observed cross-peaks H–C(5)/C(4), C(7), and C(8a),

Table 1. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp.; (D_6)DMSO) *of* **1**[1]) *and* **3**[1]). δ in ppm, J in Hz.

	1			**3**	
	δ(C)	δ(H)	HMBC	δ(C)	δ(H)
H–C(2)	90.4 (*d*)	4.49 (*q*, J = 6.9)	C(11), C(12)	90.6 (*d*)	4.51 (*q*, J = 6.9)
C(3)	44.7 (*s*)			44.6 (*s*)	
C(3a)	130.1 (*s*)			129.8 (*s*)	
C(4)	181.9 (*s*)			181.7 (*s*)	
C(4a)	133.0 (*s*)			123.8 (*s*)	
H–C(5)	116.1 (*d*)	7.01 (*d*, J = 2.4)	C(4), C(7), C(8a)	128.2 (*d*)	7.78 (*d*, J = 8.4)
C(6) or H–C(6)	155.3 (s)			120.6 (*d*)	7.13 (*dd*, J = 2.4, 8.4)
H–C(7) or C(7)	121.8 (*d*)	6.77 (*dd*, J = 2.4, 8.4)	C(5), C(8a)	162.0 (*s*)	
H–C(8)	128.1 (*d*)	7.62 (*d*, J = 8.4)	C(4a), C(6), C(9)	111.8 (*d*)	7.22 (*d*, J = 2.4)
C(8a)	124.1 (*s*)			134.0 (*s*)	
C(9)	179.9 (*s*)			177.4 (*s*)	
C(9a)	154.9 (*s*)			158.5 (*s*)	
Me(10)	14.0 (*q*)	1.33 (*d*, J = 6.9)	C(2), C(3)	13.9 (*q*)	1.35 (*d*, J = 6.9)
Me(11)	25.4 (*q*)	1.36 (*s*)	C(2), C(3), C(3a), C(12)	25.4 (*q*)	1.38 (*s*)
Me(12)	20.3 (*q*)	1.16 (*s*)	C(2), C(3), C(3a), C(11)	20.3 (*q*)	1.16 (*s*)

H–C(7)/C(5) and C(8a), and H–C(8)/C(4a), C(6), and C(9) also suggested that the OH group was definitely located at C(6). The correlations Me(10)/C(2) and C(3), Me(11)/C(2), C(3), C(3a), and C(12), and Me(12)/C(2), C(3), C(3a), and C(11) in the HMBC spectrum (*Table 1*) also confirmed the structure of the 2,3,3-trimethylnaphtho[2,3-*b*]furan moiety. The specific optical rotation value of **1** was -98 which suggested the absolute configuration (2*S*) of **1** according to the X-ray diffraction analysis of the (4-bromophenyl)hydrazone derivative of (2*S*)-α-dunnione in [10]. Compound **1** was thus identified to be (2*S*)-6-hydroxy-α-dunnione[1]).

Compound **2** had the molecular formula $C_{22}H_{12}O_7$ according to the HR-EI-MS (*m*/*z* 388.0579). The UV spectrum of **2** exhibited absorption maxima at 270, 308, and 385 nm, suggesting a naphthoquinone derivative [11]. The signal at δ(H) 3.63 (*s*, 3 H) in the ^{1}H-NMR spectrum and those at δ(C) 164.9 and 52.0 in the ^{13}C-NMR spectrum suggested the presence of a COOMe group (*Table 2*). The remaining signals in the ^{1}H-NMR spectrum were from eight H-atoms, classified into two pairs of four H-atoms, which were coupled to each other suggesting the presence of two *AA′BB′* spin systems of four aromatic H-atoms each. ROESY, HSQC, and HMBC experiments were made due to the overlapped signals. The first *AA′BB′* spin system of four aromatic H-atoms at δ(H) 7.62 (*dt*, *J* = 1.2, 7.8 Hz, H–C(6)), 7.72 (*dt*, *J* = 1.2, 7.8 Hz, H–C(7)), and 7.85–7.90 (*m*, H–C(5), H–C(8)), along with the second *AA′BB′* spin system of four aromatic H-atoms at δ(H) 8.03 (*dd*, *J* = 1.2, 7.8 Hz, H–C(5′)), 7.95 (*dd*, *J* = 1.2, 7.8 Hz, H–C(8′)),

Table 2. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp.; (D_6)DMSO) *of* **2**[1]). δ in ppm, *J* in Hz.

	δ(C)	δ(H)	HMBC	ROESY
C(1)	185.0 (*s*)			
C(2)	112.1 (*s*)			
C(3)	148.9 (*s*)			
C(4)	177.6 (*s*)			
C(4a)	131.3 (*s*)			
H–C(5)	125.5 (*d*)	7.85–7.90 (*m*)	C(4)	H–C(6)
H–C(6)	131.0 (*d*)	7.62 (*dt*, *J* = 1.2, 7.8)	C(4a), C(5), C(8)	H–C(5), H–C(7)
H–C(7)	134.1[a]) (*d*)	7.72 (*dt*, *J* = 1.2, 7.8	C(8a), C(5), C(8)	H–C(6), H–C(8)
H–C(8)	125.5 (*d*)	7.85–7.90 (*m*)	C(1)	H–C(7)
C(8a)	135.5 (*s*)			
C(1′)	181.1 (*s*)			
C(2′)	169.7 (*s*)			
C(3′)	136.6 (*s*)			
C(4′)	184.1 (*s*)			
C(4′a)	133.1 (*s*)			
H–C(5′)	126.2 (*d*)	8.03 (*dd*, *J* = 1.2, 7.8)	C(4′)	H–C(6′)
H–C(6′)	134.0[a]) (*d*)	7.85–7.90 (*m*)	C(4′a)	H–C(5′), H–C(7′)
H–C(7′)	134.1[a]) (*d*)	7.85–7.90 (*m*)	C(8′a)	H–C(6′), H–C(8′)
H–C(8′)	125.8 (*d*)	7.95 (*dd*, *J* = 1.2, 7.8)	C(1′)	H–C(7′)
C(8′a)	131.2 (*s*)			
*C*OOMe	164.9 (*s*)			
COO*Me*	52.0 (*q*)	3.63 (*s*)	*C*OOMe	

[a]) Maybe interchanged.

and 7.85–7.90 (*m*, H–C(6′), H–C(7′)) were elucidated by analysis of the ROESY spectrum. These results indicated that **2** contained two naphthoquinone units each possessing an unsubstituted ring moiety and a link between the two naphthoquinone units at positions C(2) and C(2′). In the HMBC spectrum of **2**, the observed cross-peaks δ(H) 7.85–7.90 (*m*, H–C(5), H–C(8))/δ(C) 185.0 (C(1)) and 177.6 (C(4)), δ(H) 7.95 (*dd*, H–C(8′))/δ(C) 181.1 (C(1′)), and δ(H) 8.03 (*dd*, H–C(5′))/δ(C) 184.1 (C(4′)) suggested that the C=O signals at δ(C) 185.0 (C(1)) and 177.6 (C(4))) had to be attributed to the first naphthoquinone carrying the OH group at C(3), according to [12], while the other two C=O signals at δ(C) 181.1 (C(1′)) and 184.1 (C(4′)) arose from the second naphthoquinone carrying the COOMe group at C(3′). All the HMBCs and ROESY correlations are listed in *Table 2*. Therefore, compound **2** was characterized as methyl 1,1′,4,4′-tetrahydro-3-hydroxy-1,1′,4,4′-tetraoxo[2,2′-binaphthalene]-3′-carboxylate[1]). Although this kind of binaphthoquinone derivatives arose much interests due to their multiple bioactivities [12][13], no complete NMR assignments were achieved because of severe signal overlapping.

The cytotoxic activities of the compounds **1** and **2** were evaluated. The results are listed in *Table 3*. Compound **1** demonstrated inhibitory activity against KB and *LoVo* cell lines with IC_{50} values of less than 10 μM, and compound **2** showed cytotoxicity against the K562, HepG2, KB, and *LoVo* cell lines with IC_{50} values of 12.6, 8.9, 2.6, and 3.8 μM, respectively.

Table 3. *Cytotoxic Activities of Compounds* **1** *and* **2** *against Four Tumor Cell Lines*

	IC_{50} [μM] ± s.d.			
	K562	HepG2	KB	*LoVo*
1	NE[a])	NE[a])	5.8 ± 0.5	8.9 ± 0.8
2	12.6 ± 1.1	8.9 ± 0.7	2.6 ± 0.2	3.8 ± 0.3
Taxotere[b])	–[c])	–[c])	$1.1 \cdot 10^{-3} \pm 5.9 \cdot 10^{-5}$	$2.1 \cdot 10^{-3} \pm 3.9 \cdot 10^{-5}$
Adriamycin[b])	0.09 ± 0.01	$0.07 \pm 6.7 \cdot 10^{-3}$	–[c])	–[c])

[a]) NE = $IC_{50} > 100$ μM. [b]) Positive control substances. [c]) – = No cytotoxic activity ($IC_{50} > 400$ μM).

We thank the *State Administration of TCM of Zhejiang of China* (No. 2008YA020) for financial support.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 and 200–300 mesh; *Qingdao Haiyang Chemical Group Co.*), *Sephadex LH-20* (*Pharmacia Fine Chemical Co.*), and *RP-18* (0.015–0.040 mm; *Merck*). Thin-layer chromatography (TLC): SiO_2 GF_{254} (*Qingdao Marine Chemical Factory*) and *RP-18* F_{254} plates (*Merck*); detection under UV light and visualization by spraying with 10% H_2SO_4 in EtOH (*v*/*v*), followed by heating. Optical rotations: *Perkin-Elmer-M341* polarimeter. UV Spectra: *Shimadzu-UV-2550* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Magna-750* FT-IR spectrophotometer; KBr discs; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian-Mercury* NMR spectrometer; at 500 (^{1}H) and 125 Hz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. LR- and HR-EI-MS: *Finnigan*/*MAT-95* spectrometer; in *m*/*z* (rel. %).

Plant Material. The roots of *Didymocarpus hedyotideus* CHUN. were collected in June 2007, in Wuming County, Guangxi Province, P. R. China, and identified by Prof. *Yu Zhao* at the Department of

Pharmacy of the Zhejiang University. A voucher specimen (No. 2007618) was deposited with the Herbarium of the College of Agriculture and Biotechnology.

Extraction and Isolation. Powdered and air-dried roots of *Didymocarpus hedyotideus* (0.6 kg) were extracted by refluxing three times (3×1.5 h) with 95% EtOH (3×4 l). The obtained extract was concentrated and the residue (95 g) partitioned successively with AcOEt (3×1 l) and BuOH (4×1 l) to afford the AcOEt fraction (19.5 g) and the BuOH fraction (31.5 g). The AcOEt fraction (19.5 g) was subjected to CC (SiO_2, step gradient $CHCl_3$/MeOH $40:1 \rightarrow 5:1$): *Fractions 1–6*. *Fr. 2* (1.20 g) was subjected to CC (SiO_2, petroleum ether/acetone $10:1 \rightarrow 2:1$) and gave four fractions which were further separated by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): β-sitosterol (8 mg), 2-(methoxycarbonyl)-9,10-anthraquinone (8 mg), 2-hydroxy-6-methyl-9,10-anthraquinone (7 mg), and 2-hydroxy-9,10-anthraquinone (7 mg), resp. *Fr. 3* (0.45 g) was subjected to CC (*Sephadex LH-20*, MeOH): **2** (23 mg) and 2,6-dihydroxyanthraquinone 2-β-D-glucopyranoside (13 mg). *Fr. 4* (0.37 g) was first subjected to CC (SiO_2, $CHCl_3$/MeOH $20:1 \rightarrow 9:1$; then *Sephadex LH-20*, MeOH) and then to prep. TLC ($CHCl_3$/acetone 9:1): **1** (12 mg, R_f 0.55) and **3** (8 mg, R_f 0.45). Isolation of *Fr. 1* was not pursued for its weak polarity, and *Frs. 5* and *6* contained minor quantities. The BuOH fraction (31.5 g) was subjected to macroporous resin and eluted with H_2O (abundant) and 95% EtOH. The 95% EtOH fraction (10.5 g) was resubjected to CC (*RP-C_{18}*, MeOH/H_2O 3:7; then *Sephadex LH-20*, MeOH/H_2O 1:1): plantainoside A (15 mg), calceolarioside A (8 mg), calceolarioside B (10 mg), and daucosterol (17 mg) in this order. The known compounds were determined by comparison of their spectroscopic data with literature values.

(2S)-6-Hydroxy-α-dunnione (*=(2S)-2,3-Dihydro-6-hydroxy-2,3,3-trimethylnaphtho[2,3-b]furan-4,9-dione*; **1**): Red amorphous powder. $[\alpha]_D^{23} = -98$ ($c = 0.10$, MeOH). UV (MeOH): 266 (4.2), 308 (4.0), 361 (3.4), 385 (2.5). IR: 3354, 2954, 1680, 1600, 1550. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 258 (50, M^+), 243 (100), 215 (10). HR-EI-MS: 258.0890 (M^+, $C_{15}H_{14}O_4^+$; calc. 258.0892).

1,1′,4,4′-Tetrahydro-3′-hydroxy-1,1′,4,4′-tetraoxo[2,2′-binaphthalene]-3-carboxylic Acid Methyl Ester (**2**): Red amorphous powder. $[\alpha]_D^{23} = -17$ ($c = 0.09$, MeOH). UV (MeOH): 270 (2.9), 308 (4.8), 385 (4.5). IR: 3337, 2928, 1725, 1642, 1592, 1459, 1337, 1275, 1143. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 388 (12, M^+), 76 (20), 272 (20), 300 (36), 330 (13), 356 (100). HR-EI-MS: 388.0579 (M^+, $C_{22}H_{12}O_7^+$; calc. 388.0583).

Cell Cultures. Human HepG2 (hepatocellular carcinoma), K562 (human leukemia), KB (cervix carcinoma), and *LoVo* (colon adenocarcinoma) cell lines were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cells were maintained in RPMI1640 medium with 10% FBS (fetal bovine serum). In each case, 100 U/ml of penicillin and 100 U/ml of streptomycin were added.

Cytotoxicity Assay. Cells were cultured in 96-well microtiter plates for the assay. After incubation for 24 h, and treatment with 10^{-2} to 10^2 μM of the test compounds for 72 h, growth inhibition of the cancer cells was evaluated by the SRB method (adherent cells: HepG2, KB and *LoVo*) or WST-1 method (suspended cell: K562) as described in [14][15]. The activity is shown as IC_{50} value (*Table 3*). Results are expressed as the mean value of triplicate data points. Adriamycin and taxotere were used as pos. controls.

REFERENCES

[1] A. Segawa, Y. Miyaichi, T. Tomimori, F. Kiuchi, T. Ohta, *Chem. Pharm. Bull.* **1999**, *47*,1404.
[2] Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 'Chinese Materia Medica', Shanghai Science & Technology Press, Shanghai, 1999, Vol. 7, p. 494.
[3] X.-H. Cai, X.-D. Luo, J. Zhou, X.-J. Hao, *J. Nat. Prod.* **2005**, *68*, 797.
[4] J. Helaja, A. Y.Tauber, I. Kilpeläinen, P. H. Hynninen, *Magn. Reson. Chem.* **1997**, *35*, 619.
[5] Y. Kawasaki, Y. Goda, K. Yoshihira, *Chem. Pharm. Bull.* **1992**, *40*, 1504.
[6] K. Danielsen, *Magn. Reson. Chem.* **1995**, *33*, 823.
[7] M. S. Akhtar, M. M. Ali, S. R. Mir, O. Singh, *Indian J. Chem., Sect. B* **2006**, *45*, 1945.
[8] S. Damtoft, S. R. Jensen, *Phytochemistry* **1994**, *37*, 441.
[9] S. Gafner, J.-L. Wolfender, M. Nianga, H. Stoeckli-Evans, K. Hostettmann, *Phytochemistry* **1996**, *42*, 1315.

[10] R. G. Cooke, E. L. Ghisalberti, B. L. Johnson, C. L. Raston, B. W. Skelton, A. H. White, *Aust. J. Chem.* **2006**, *59*, 925.
[11] R. Gormann, M. Kaloga, X.-C. Li, D. Ferreira, D. Bergenthal, H. Kolodziej, *Phytochemistry* **2003**, *64*, 583.
[12] A. Emadi, J. S. Harwood, S. Kohanim, K. W. Stagliano, *Org. Lett.* **2002**, *4*, 521.
[13] K. W. Stagliano, A. Emadi, Z. Lu, H. C. Malinakova, B. Twenter, M. Yu, L. E. Holland, A. M. Rom, J. S. Harwood, R. Amin, A. A. Johnson, Y. Pommier, *Bioorg. Med. Chem.* **2006**, *14*, 5651.
[14] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
[15] Y. Zhou, W. Zhu, Q. X. Zhang, *J. Trop. Med.* **2005**, *5*, 580.

Received May 15, 2010