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C. -I. Chang^a; S. -L. Huan^b; Y. -H. Kuo^b

^a Graduate Institute of Biotechnology, National Pingtung University of Science and Technology, Pingtung, Taiwan ^b Department of Chemistry, National Taiwan University, Taipei, Taiwan

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Two new naphthoquinones from the stem of *Diospyros maritima*

C.-I. CHANG[†], S.-L. HUAN[‡] and Y.-H. KUO^{‡*}

[†]Graduate Institute of Biotechnology, National Pingtung University of Science and Technology,
Pingtung 912, Taiwan

[‡]Department of Chemistry, National Taiwan University, Taipei, 106, Taiwan

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Two new naphthoquinones, 2-ethoxy-8'-hydroxyisodiospyrin (**1**) and 3-ethoxy-8'-hydroxyisodiospyrin (**2**), together with two known naphthoquinones, 6-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone and bisisodiospyrin were isolated from the stems of *Diospyros maritima*. The structures of **1** and **2** were established on the basis of spectroscopic data.

Keywords: *Diospyros maritima*; Ebenaceae; Naphthoquinones

1. Introduction

Of 13 species of *Diospyros* (Ebenacea) growing in Taiwan, several have been studied for their chemical constituent, resulting in the isolation and structure elucidation of various triterpenes, lignans, steroids, benzoquinone, and naphthoquinones. Species investigated include barks and stems of *D. eriantha* Champ [1] and stems of *D. morrisiana* Hance [2]. The stems of *D. maritima* Blume have been used in a folk medicine as traditional treatments for rheumatic disease in Taiwan [3]. In previous studies, many naphthoquinones and triterpenes were isolated from its bark, root, fruits, leaves and twigs by Tezuka *et al.* [4] and Higa *et al.* [5,6]. More recently, we also have reported some new naphthoquinones [7], triterpenes, steroids [8–13], phenolic and aliphatic components from the stems of this plant [14]. The genus *Diospyros* is well-known to produce various naphthoquinone derivatives, some of which exhibit cytotoxic, ichthyotoxic, germination inhibitory, and antimicrobial activities [5,6,9,15]. In continuation of our work on the discovery of potential antitumor constituents from the stems of *D. maritima*, we also have isolated two new naphthoquinones, 2-ethoxy-8'-hydroxyisodiospyrin (**1**) and 3-ethoxy-8'-hydroxyisodiospyrin (**2**), together with two known naphthoquinones, 6-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone (**3**) and bisisodiospyrin (**4**). In this paper,

*Corresponding author. Email: yhkuo@ntu.edu.tw

we report the extraction, isolation, purification, and structural elucidation of two new naphthoquinones.

2. Results and discussion

The EtOH extracts of the stems of *Diospyros maritima* was concentrated to give a residue which was suspended in water and partitioned with *n*-hexane and *n*-BuOH, successively. The combined *n*-BuOH soluble layer was subjected to repeated chromatography using silica gel and further purification by preparative HPLC to furnish four naphthoquinone derivatives, including two new naphthoquinones, 2-ethoxy-8'-hydroxyisodiospyrin (**1**) and 3-ethoxy-8'-hydroxyisodiospyrin (**2**). The identification of the two known compounds, 6-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone (**3**) [16] and bisisodiospyrin (**4**) [4] was performed by comparing their physical and spectral data with those reported. This paper deals with the structural elucidation of the two new naphthoquinones.

Compound **1** (figure 1) was obtained as a dark-red solid. The HREIMS displayed a molecular ion peak at m/z 434.1007, consistent with the molecular formula $C_{24}H_{18}O_8$. The IR spectrum showed the presence of a conjugated ketone (1672 cm^{-1}) and phenyl group (1620 , 1590 , and 1460 cm^{-1}) functionalities. The UV spectrum exhibited absorption peaks at 212, 245, 295, and 436 nm. The ^1H NMR spectrum of **1** (table 1) indicated the presence of two

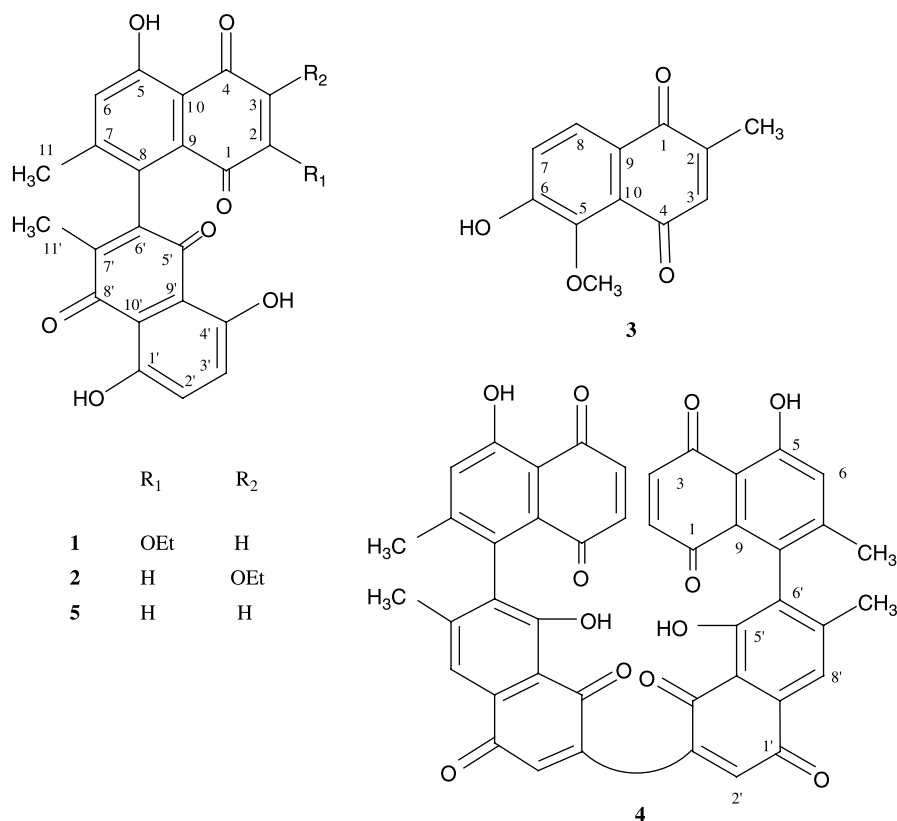
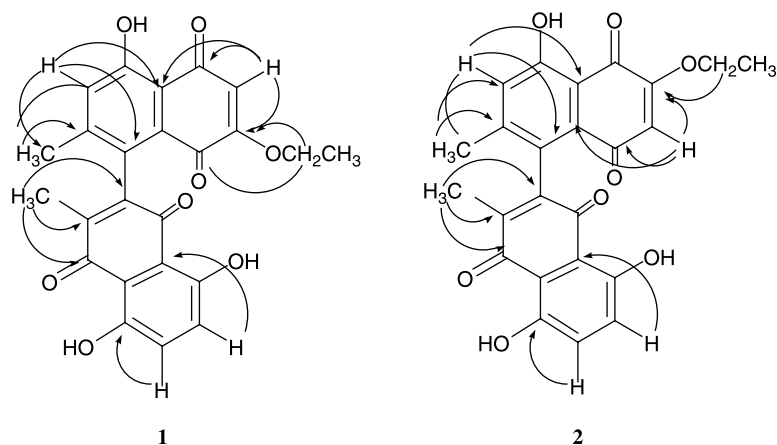


Figure 1. Structures of compounds 1–5.

Table 1. ^{13}C and ^1H -NMR spectral data for compounds **1**, **2**, and **5** (300 MHz, 75 MHz, In CDCl_3).

No	1		2		5	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		179.8		184.8		184.8
2		159.6	5.90	110.9	6.75 d (10.3)	139.7
3	6.06	109.3		158.7	6.92 d (10.3)	138.0
4		190.6		184.5		190.1
5		161.6		162.5		162.1
6	7.24	126.4	7.21	124.9	7.30	125.7
7		145.5		147.4		145.8
8		127.5		126.7		127.0
9		128.4		129.4		129.1
10		113.1		113.5		114.0
11	2.15	20.4	2.16	20.6	2.17	20.6
2-OCH ₂ CH ₃	4.01 q (6.7)	65.9				
2-OCH ₂ CH ₃	1.44 t (6.7)	13.9				
3-OCH ₂ CH ₃			4.03 q (6.9)	65.6		
3-OCH ₂ CH ₃			1.49 t (6.9)	13.9		
5-OH	12.67		12.19		12.32	
1'		186.8		187.0		185.2
2'		147.4		147.8		147.2
3'		142.9		142.4		143.0
4'		185.4		185.6		186.6
5'		158.7		158.5		158.8
6'	7.24	129.5	7.26	129.4	7.30	129.6
7'	7.24	129.5	7.26	129.4	7.30	129.6
8'		158.7		158.4		158.9
9'		112.2		112.2		112.1
10'		112.2		112.2		112.1
11'	1.83	13.4	1.83	13.3	1.83	13.4
5'-OH	12.63		12.65		12.64	
8'-OH	12.30		12.31		12.29	

tertiary methyl singlets [δ 1.83, 2.15 (3H each, s)], an ethoxy group [δ 1.44 (3H, t, $J = 6.7$ Hz), δ 4.01 (2H, q, $J = 6.7$ Hz)], a quinoid proton [δ 6.06 (1H, s)], three aromatic protons [δ 7.24 (3H, s)], and three strong hydrogen-bonded phenolic protons [δ 12.30, 12.63, 12.67 (1H each, s)]. The ^{13}C NMR and DEPT spectra (table 1) exhibited 24 carbon signals

Figure 2. Key HMBC correlations of **1** and **2**.

for three CH₃, one oxymethene, 20 carbons from two naphthoquinone units. The spectral characteristics were similar to those of 8'-hydroxyisodiospyrin (**5**) (table 1) we reported previously [9], the difference is an additional ethoxyl group in compound **1**. A long-range correlation between H-3 (δ 6.06) and C-10 (δ 113.1) and C-4 (δ 190.6) in the HMBC spectrum of **1** (figure 2) together with a H-3 and 5-OH signals at δ 6.06 and 12.67, respectively, suggested that the ethoxy group should be attached to C-2 [7]. Full characterization of the structure was accomplished by examination of the COSY, HMQC, HMBC, and NOESY spectral data. Hence, compound **1** was established as 2-ethoxy-8'-hydroxyisodiospyrin.

Compound **2** also obtained as a dark-red solid and showed a molecular ion at m/z 434.1008 in the HR-EIMS spectrum, analyzing for the molecular formula C₂₄H₁₈O₈ and was seen to be an isomer of **1**. The IR spectrum exhibited a conjugated ketone (1665 cm⁻¹) and aromatic functionalities (1616, 1590 cm⁻¹) and the UV spectrum showed absorption bands at 204, 288 and 435 nm. The pattern of the proton signals in the ¹H NMR spectrum (table 1) was similar to those of compound **1**, including two tertiary methyl singlets [δ 1.83, 2.16 (3H each, s)], an ethoxy group [δ 1.49 (3H, t, $J = 6.9$ Hz), δ 4.03 (2H, q, $J = 6.9$ Hz)], a quinoid proton [δ 5.90 (1H, s)], three aromatic protons [δ 7.21 (1H, s) and δ 7.26 (2H, s)], and three hydrogen-bonded hydroxyls [δ 12.19, 12.31, 12.65 (1H each, s)]. The ¹³C NMR and DEPT spectra (table 1) exhibited 24 carbon signals for three CH₃, one oxymethene, 20 carbons from two naphthoquinone units. In the HMBC spectrum of **2** (figure 2), mutual correlations between H-2 (δ 5.90) and C-9 (δ 128.4) and C-4 (δ 184.5) suggested that the ethoxyl group should be attached to C-3 [7]. Full characterization of the structure was accomplished by examination of the COSY, HMQC, HMBC and NOESY spectral data. Thus, compound **2** was established as 3-ethoxy-8'-hydroxyisodiospyrin.

3. Experimental

3.1 General experimental procedures

Melting points were determined with a Yanagimoto (MP500D) micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ¹H and ¹³C NMR spectra were obtained on a Bruker AM-300 spectrometer, with 2D NMR spectra run on a Bruker DMX-300 spectrometer, using CDCl₃ as solvent. The resonances of residual CDCl₃ at δ _H 7.24 and of at δ _C 77.0 were used as internal references for the ¹H-NMR and ¹³C-NMR spectra, respectively. EI-MS, FAB-MS, HREI-MS, UV, and specific rotations were recorded on a JEOL JMS-HX 300, a JOEL JMS-HX 110, a JOEL SX-102A, a Hitachi S-3210 spectrometer, and a JASCO DIP-1000 digital polarimeter, respectively. Extracts were chromatographed on silica-gel (Merck 9385, 70–230 mesh).

3.2 Plant material

The stems of *Diospyros maritima* Blume were collected in Lin-Ko, Taiwan, in 1993. The plant material was identified by Mr Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University, and a voucher specimen has been deposited at the National Research Institute of Chinese Medicine, Taipei, Taiwan.

3.3 Extration and isolation

The stems of *D. maritima* (16 kg) were extracted with EtOH (160 L) at 60°C three times (10 h for each time). The EtOH extract was evaporated *in vacuo*, yielding a black residue, which was suspended in H₂O (12 L), and this phase was then partitioned ($\times 5$) with 1 L of *n*-hexane. The aqueous layer were partitioned ($\times 4$) again with 1 L of *n*-BuOH. The combined *n*-BuOH extracts (180 g) was chromatographed on Si gel and HPLC repeatedly. Four components, 2-ethoxy-8'-hydroxyisodiospyrin (**1**) (3.0 mg) and 3-ethoxy-8'-hydroxyisodiospyrin (**2**) (3.8 mg), 6-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone (**3**) (5.1 mg) and bisisodiospyrin (**4**) (16.5 mg) were obtained.

3.3.1 2-Ethoxy-8'-hydroxyisodiospyrin (1). Dark-red solid; $[\alpha]_D^{25} +179$ (*c* 0.4, CHCl₃); UV (log ϵ) (MeOH) λ_{\max} 212 (4.6), 245 (4.3, sh), 295 (4.2), 436 (3.9) nm; IR (KBr) 3443, 1672, 1620, 1590, 1460, 1300, 1016 cm⁻¹; ¹H and ¹³C NMR: see table 1; EI-MS (70 eV) *m/z* (rel. int.) 434 (M⁺, 10), 419 (5), 391 (6), 363 (2), 318 (2), 222 (37), 204 (23), 149 (50); HREIMS *m/z* 434.1007 [M]⁺ (calcd for C₂₄H₁₈O₈ 434.1001).

3.3.2 3-Ethoxy-8'-hydroxyisodiospyrin (2). Dark-red solid; $[\alpha]_D^{25} +162$ (*c* 0.5, CHCl₃); UV (log ϵ) (MeOH) λ_{\max} 288 (4.3, sh), 413 (3.6), 435 (3.6) nm; IR (KBr) 3400, 1665, 1616, 1590, 1382, 1043 cm⁻¹; ¹H NMR and ¹³C NMR: see table 1; EI-MS (70 eV) *m/z* (rel. int.) 434 (M⁺, 100), 419 (43), 391 (39), 363 (35); HREI-MS *m/z* 434.1008 [M]⁺ (calcd for C₂₄H₁₈O₈, 434.1001).

3.3.3 6-Hydroxy-5-methoxyl-2-methyl-1,4-naphthoquinone (3). Yellow needle; mp 142–144°C; IR (dry film) ν_{\max} 3330, 1665, 1652 cm⁻¹; ¹H NMR: δ 2.15 (3H, d, *J* = 1.6 Hz, 2-Me), 3.94 (3H, s, 5-OCH₃), 6.56 (1H, br s, 6-OH), 6.70 (1H, d, *J* = 1.6 Hz, H-3), 7.25 (1H, d, *J* = 8.3 Hz, H-7), 7.88 (1H, d, *J* = 8.3 Hz, H-8); EI-MS (70 eV) *m/z* 218 [M]⁺ (100), 200 (20), 172 (45), 171 (35).

3.3.4 Bisisodiospyrin (4). Orange solid; $[\alpha]_D^{25} -660$ (*c* 0.7, CHCl₃); IR (dry film) ν_{\max} 3360, 1658, 1639, 1268 cm⁻¹; ¹H NMR: δ 2.01, 2.03 (each 3H, s, 7, 7'-Me), 6.89, 6.71 (each 1H, d, *J* = 10.2 Hz, H-2, 3), 7.00, 7.28, 7.65 (each 1H, s, H-2', 6, 8'), 11.90, 12.40 (each 1H, s, 5, 5'-OH); FAB-MS (70 eV) *m/z* 747 [M + H]⁺ (10), 307 (100), 259 (85).

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