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ORIGINAL ARTICLE

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Naphthalene derivatives from *Diospyros kaki*

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Abstract A detailed chemical investigation of the extractives of the blackened heartwood of *Diospyros kaki* was carried out to understand their chemical characteristics and to obtain chemotaxonomic information. Three novel naphthalene derivatives were isolated, i.e., 4-hydroxy-5,6-dimethoxy-2-naphthaldehyde (**1**), 5,6,8-trimethoxy-3-methyl-1-naphthol (**2**), and 4,8-dihydroxy-5-methoxy-2-naphthaldehyde (**3**), in addition to two previously reported 2-naphthaldehydes: 4-hydroxy-5,8-dimethoxy-2-naphthaldehyde (**4**) and 4-hydroxy-5-methoxy-2-naphthaldehyde (**5**) Their structures were identified by 1D and 2D nuclear magnetic resonance spectroscopy, as well as by high-resolution mass spectrometry.

Key words Naphthalene derivatives · *Diospyros* · Ebenaceae · NMR

Introduction

Diospyros is economically the most important genus of Ebenaceae. Most *Diospyros* species are distributed in the tropical to temperate regions of the world. Many of them have black heartwood, which is usually used in high-end furniture, carving, and musical instruments, among others. In addition, their extractives have been studied for their medicinal importance. A unique feature of the *Diospyros* genus is the presence of a large number of naphthalene derivatives. Mallavadhani et al.[1] reported about 300 organic chemicals from more than 130 species of *Diospyros*. Ito et al.[2] isolated some naphthoquinones from six species of the genus *Diospyros* in Japan and investigated their antifungal activities. Ganapaty et al.[3] showed that naphthalene derivatives obtained from *Diospyros assimilis* exhibited moderate inhibition effects against protozoan parasites.

Diospyros kaki yields edible fruits, and it is cultivated mainly in Japan and Korea. Blackened heartwood is rarely found in *Diospyros kaki*. The blackened heartwood is highly valued for ornamental purposes such as making boxes and desks. It usually forms a black-brown-striped pattern that is independent of the annual rings. Physical and mechanical properties such as specific gravity, the equilibrium moisture content, the modulus of rupture, and modulus of elasticity differ between the black and brown parts.[4] Yasue et al.[5] investigated the extractives of the blackened heartwood and isolated 4,5-dihydroxy-8-methoxy-2-naphtaldehyde and related compounds. However, the information obtained from the abovementioned chemical research of the blackened heartwood of *Diospyros kaki* was insufficient. In this study, a detailed chemical investigation of the extractives of the blackened heartwood of *Diospyros kaki* was carried out to understand its chemical characteristics and to obtain chemotaxonomic information. We isolated three novel naphthalene derivatives, **1**, **2**, and **3**, and two known compounds, **4** and **5** (Fig. 1).

Experimental

General experimental procedures

Melting points were measured by using micro melting point apparatus (Yanagimoto Seisakusho, Japan). Silica gel (63–210 µm, Kanto Chemical, Japan) was used for column chromatography. UV spectra were recorded in MeOH on a V-530 UV/VIS spectrophotometer (Nippon Bunko, Japan). Both 1D and 2D nuclear magnetic resonance (NMR) spectra were run on a Bruker AVANCE 400 spectrometer. Mass measurements were performed on an Mstation JMS-700 mass spectrometer (JEOL, Japan).

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Plant material

Wood samples from *Diospyros kaki* were obtained from Kanra-gun, Gunma prefecture, Japan.

Extraction and isolation

The blackened heartwood of *Diospyros kaki* (430.8 g) was extracted three times for 3 days, each time with methanol, at room temperature. The combined methanol solution was evaporated under reduced pressure to give 5.65 g (1.3% by weight of the wood sample) of methanol extract.

The methanol extract was chromatographed on silica gel with ethyl acetate and *n*-hexane (1:2 v/v) to give **3** (372.1 mg), and the fraction contained some minor compounds. The fraction was purified by silica gel chromatography with benzene to obtain **1** (2.5 mg), **4** (33.9 mg), and a mixture of **2** and **5**. The mixture was separated by silica gel chromatography using chloroform and benzene (3:1 v/v) to give compound **2** (4.7 mg) and **5** (1.8 mg).

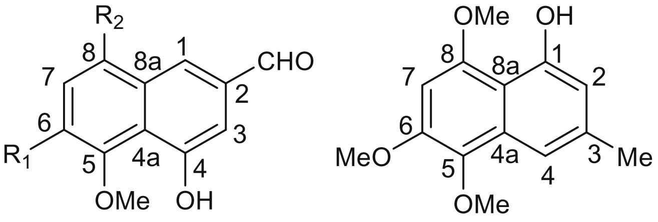
4-Hydroxy-5,6-dimethoxy-2-naphthaldehyde (**1**) – pale orange needles from chloroform; mp 132°–133°C; UV λ_{max} nm (log ε): 224 (4.19), 264 (4.30), 320 (3.71), 370 (3.81); high-resolution electron impact mass spectrometry (HR-EIMS) m/z 232.0768 [M^+], calculated for $C_{13}H_{12}O_4$, 232.0736; ^1H and ^{13}C NMR ($CDCl_3$) spectra, see Table 1.

5,6,8-trimethoxy-3-methyl-1-naphthol (**2**) – colorless needles from chloroform; mp 87°–89°C; UV λ_{max} nm (log ε): 235 (4.65), 312 (3.82), 338 (3.73); HR-EIMS m/z 248.1042 [M^+], calculated for $C_{14}H_{16}O_4$, 248.1049; ^1H and ^{13}C NMR ($CDCl_3$) spectra, see Table 1.

4,8-dihydroxy-5-methoxy-2-naphthaldehyde **3** – pale green needles from chloroform; mp 204°–205°C; UV λ_{max} nm (log ε): 221 (4.42), 257 (4.47), 321 (3.77); elementary analysis C 65.8%, H 4.7%; HR-EIMS m/z 218.0570 [M^+], calculated for $C_{12}H_{10}O_4$, 218.0579; ^1H and ^{13}C NMR ($(CD_3)_2CO$) spectra, see Table 1.

4-hydroxy-5,8-dimethoxy-2-naphthaldehyde (**4**) – pale green needles from chloroform; mp 194°–195°C; HR-EIMS m/z 232.0702 [M^+], calculated for $C_{13}H_{12}O_4$, 232.0736; ^1H and ^{13}C NMR ($CDCl_3$) spectra, see Table 2.

4-hydroxy-5-methoxy-2-naphthaldehyde (**5**) – HR-EIMS m/z 248.1042 [M^+], calculated for $C_{14}H_{16}O_4$, 248.1049; ^1H and ^{13}C NMR ($CDCl_3$) spectra, see Table 2.



1 R_1=OMe, R_2=H

2

3 R_1=H, R_2=OH

4 R_1=H, R_2=OMe

5 R_1=H, R_2=H

Fig. 1. Naphthalene derivatives **1**–**5**, isolated from *Diospyros kaki*

Results and discussion

Compound **1** was a minor extractive of the blackened heartwood of *Diospyros kaki*. In this study, only 2.3 mg of **1** was obtained from 430 g of wood. **1** was obtained as pale orange

Table 1. ^1H and ^{13}C NMR spectral data for naphthalene derivatives **1**–**3**[a] (δ_H and δ_C in ppm, J in Hz)

Position	1			2			3		
	δ_H	δ_C	Observed HMBC	δ_H	δ_C	Observed HMBC	δ_H	δ_C	Observed HMBC
1	7.78 *d* (1.2)	126.1	CHO, C-3, C-4a, C-8		154.3		8.26 *d* (1.5)	120.0	CHO, C-3, C-4a, C-8
2		134.1		6.64 *s*	110.7	CH_3, C-1, C-4, C-8a		135.1	
3	7.27 *d* (1.2)	106.1	CHO, C-1, C-4, C-4a		137.3		7.16 *d* (1.5)	105.5	CHO, C-1, C-4, C-4a
4		154.3		7.35 *d* (0.8)	111.6	CH_3, C-2, C-8a, C-5		155.6	
4a		121.5			131.7			118.5	
5		142.9			137.3			149.2	
6		145.0			148.1		7.03 *d* (8.4)	108.5	C-4a, C-5, C-8
7	7.35 *d* (8.8)	115.4	C-5, C-8a	6.55 *s*	94.4	C-5, C-6, C-8, C-8a	6.95 *d* (8.6)	108.9	C-5, C-8, C-8a
8	7.76 *d* (9.2)	127.2	C-1, C-4a, C-6,		153.0			148.8	
8a		130.1			109.5			127.0	
1-OH				9.14 *s*		C-1, C-2, C-8a			
2-CHO	10.00 *s*	191.8	C-2, C-3				10.08 *s*	191.8	C-2, C-3
3-CH_3				2.45 *s*	22.0	C-2, C-3, C-4			
4-OH	9.73 *s*		C-3, C-4, C-4a				9.66 *s*		C-3, C-4, C-4a
5-OMe	4.10 *s*	62.2	C-5	3.91 *s*	60.9	C-5	4.09 *s*	56.3	C-5
6-OMe	4.03 *s*	56.7	C-6	4.00 *s*	57.3	C-6			
8-OH							9.12 *s*		
8-OMe				4.06 *s*	56.3	C-8			

[a] Assignments were confirmed by 2D NMR experiments (NOESY, ^1H-^1H COSY, HMQC)

HMBC, Heteronuclear multiple bond correlation; NOESY, nuclear Overhauser effect spectroscopy; HMQC, heteronuclear multiple quantum coherence

Table 2. ^{1}H and ^{13}C NMR spectral data for naphthalene derivatives **4** and **5**^{a} (δ_H and δ_C in ppm, J in Hz)

Position	4		5	
	δ_H	δ_C	δ_H	δ_C
1	8.23 *d* (0.8)	120.1	7.84 *d* (1.6)	124.9
2		135.3		135.8
3	7.32 *s*	107.2	7.32 *d* (1.6)	106.8
4		155.4		155.6
4a		118.7		118.2
5		149.8		156.1
6	6.74 *d* (8.4)	107.2	6.99 *d* (8.0)	107.1
7	6.86 *d* (8.8)	104.3	7.46 *t* (8.0, 8.0)	127.0
8		151.3	7.61 *d* (8.0)	123.4
8a		128.4		136.1
2-CHO	10.05 *s*	192.3	10.08 *s*	192.2
2-CH_3				
4-OH	9.55 *s*		9.46 *s*	
5-OH				
5-OMe	4.04 *s*	56.6	4.12 *s*	56.4
6-OMe				
7-OMe				
8-OMe	3.98 *s*	55.8		

^{a}Assignments were confirmed by 2D NMR experiments (NOESY, ^{1}H-^{1}H COSY, HMQC, HMBC)

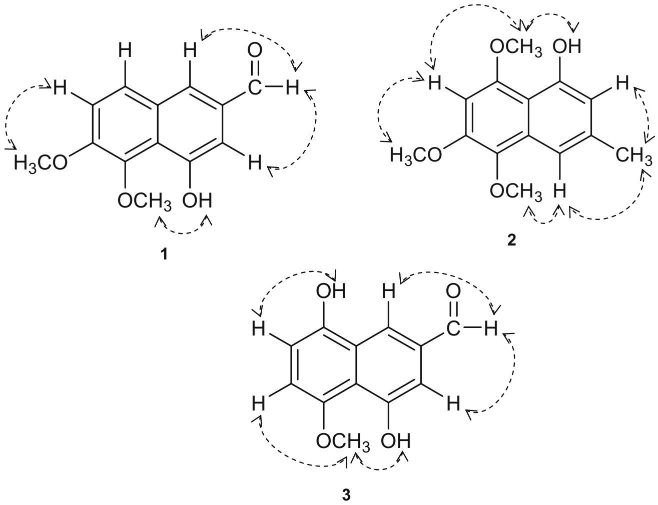


Fig. 2. NOESY interactions of **1**–**3**

needles, mp 132°–133°C. The HR-EI mass spectrum of **1** showed a molecular ion at m/z 232.0768, corresponding to the molecular formula $C_{13}H_{12}O_4$. ^{1}H NMR spectral data revealed a naphthaldehyde structure with two methoxy groups (δ_H 4.03 and 4.10) and one hydroxyl group (δ_H 9.73). Signals with a small coupling constant (J = 1.2 Hz) were observed at δ_H 7.27 and δ_H 7.78, corresponding to the *meta*-coupled protons. In addition, two signals with a coupling constant of 8.8 Hz were observed at δ_H 7.35 and δ_H 7.76. This result indicated the positional relationship between the protons at the *ortho* position. The ^{1}H NMR spectrum of **1** was similar to that of 4,5,6-trimethoxy-2-naphthaldehyde.[6] A heteronuclear multiple bond correlation (HMBC) experiment showed that H-7 (δ_H 7.35) interacted with C-5 (δ_C 142.9) and C-8a (δ_C 130.1). The signal corresponding to H-8 (δ_H 7.76) showed a correlation with that corresponding to C-1 (δ_C 126.1), C-4a (δ_C 121.5), and C-6 (δ_C 145.0). The proton at the hydroxyl group (δ_H 9.73) interacted with C-4 (δ_C 154.3), C-3 (δ_C 106.1), and C-4a. The nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum showed that the signal corresponding to the aldehyde proton interacted with H-1(δ_H 7.78) and H-3 (δ_H 7.78), that corresponding to the hydroxyl proton interacted with the protons of 5-O-Me (δ_H 4.10), and that corresponding to H-7 interacted with the protons of 6-OMe (δ_H 4.03) (Fig. 2). From these NMR data, **1** was characterized as a new naphthalene derivative, 4-hydroxy-5,6-dimethoxy-2-naphthaldehyde. The NMR spectroscopic data are summarized in Table 1.

Compound **2** was obtained as colorless needles, mp 87°–89°C. This compound was also a minor extractive (4.7 mg in 430 g of blackened heartwood of *Diospyros kaki*). The HR-EI mass spectrum of **2** showed a molecular ion at m/z 248.1042, which implied that the molecular formula was $C_{14}H_{16}O_4$. ^{1}H NMR and correlated spectroscopy (COSY) spectra showed signals corresponding to the *meta*-coupled protons at δ_H 7.35 and δ_H 6.64 and one signal corresponding to the protons at δ_H 6.55 that was independent of the signals corresponding to other protons; ^{1}H NMR and ^{13}C NMR spectra showed that **2** had one methyl group, three methoxy groups, and one hydroxyl group, but no aldehyde group. An HMBC experiment showed that the methyl protons interacted with C-2 (δ_C 110.7), C-3 (δ_C 137.3), and C-4 (δ_C 111.6), and the hydroxyl proton (δ_H 9.14) correlated with C-1 (δ_C 154.3), C-2, and C-8a (δ_C 109.5). Further, H-7 (δ_H 6.55) interacted with C-5 (δ_C 137.3), C-6 (δ_C 148.1), C-8 (δ_C 153.0), and C-8a. A NOESY experiment revealed that the proton at the hydroxyl group interacted with the protons of 8-OMe (δ_H 4.06). The signal corresponding to H-7 showed a correlation with the protons of 6-OMe (δ_H 4.00) and 8-OMe. The protons of 5-OMe (δ_H 3.91) interacted with H-4 (δ_H 7.35) (Fig. 2). From the NMR data, **2** was characterized as 5,6,8-trimethoxy-3-methyl-1-naphthol. The NMR spectroscopic data are summarized in Table 1. This compound has been synthesized previously;[7] however, this study is the first reported occurrence of this compound in plants.

Compound **3** was a major extractive of the blackened heartwood of *Diospyros kaki*: 372.1 mg of **3** was obtained from 430 g of the heartwood as pale green needles, mp 204°–205°C. The result of an elementary analysis (C, 65.8%; H, 4.7%) and an HR-EI mass spectrum (m/z 218.0570) showed that the molecular formula was $C_{12}H_{10}O_4$. ^{1}H NMR spectral data suggested a naphthaldehyde structure with one methoxy group (δ_H 4.09) and two hydroxyl groups (δ_H 9.12 and δ_H 9.66). One of the hydroxyl protons showed a sharp signal, as shown in **1**, **2**, **4**, and **5**, and the other hydroxyl group showed a broad signal. Signals with a small coupling constant (J = 1.5 Hz) were observed at δ_H 7.16 and δ_H 8.26, suggesting the positional relationship between these protons at the *meta* position. In addition, two signals with a coupling constant of 8.4–8.6 Hz were observed at δ_H 6.95 and δ_H 7.03. This result indicated the positional relationship between these protons at the *ortho* position. The structure of **3** was studied by performing NOESY and HMBC experiments. The NOESY spectrum showed interactions between the aldehyde proton and *meta*-coupled protons, H-1 (δ_H 8.26) and H-3 (δ_H 7.16). The signal corresponding to methoxy protons interacted with the 4-OH hydroxyl proton (δ_H 9.66) and with H-6 (δ_H 7.03). H-7 (δ_H 6.95) located at the *ortho* position of H-6 interacted with the signal of the hydroxyl proton (δ_H 9.12), which was attributed to 8-OH (Fig. 2). The HMBC experiment results indicated that H-1 (δ_H 8.26) and H-3 (δ_H 7.16) were correlated with the aldehyde carbon (δ_C 191.8). The signal at H-1 also showed correlations with C-3 (δ_C 105.5), C-4a (δ_C 118.5), and C-8 (δ_C 148.8). H-3 interacted with C-1 (δ_C 120.0), C-4 (δ_C 155.6), and C-4a. The hydroxyl proton at 4-OH showed a correlation with C-3, C-4, and C-4a. H-6 was correlated with C-4a, C-5 (δ_C 149.2), and C-8. H-7 showed interactions with C-5, C-8, and C-8a. **3** was, therefore, characterized as a new naphthalene derivative, 4,8-dihydroxy-5-methoxy-2-naphthaldehyde. The NMR spectroscopic data are shown in Table 1.

Compound **4** was obtained as pale green needles, mp 194°–195°C. The HR-EI mass spectrum of **4** showed a molecular ion at m/z 232.0702. This implied that the molecular formula was $C_{13}H_{12}O_4$, which was the same as that of **1**. The ^{1}H NMR spectral data was similar to that of **3** except for the presence of signals corresponding to two methoxy groups and one hydroxyl group. This suggested that the structure of **4** was formed because of the exchange of one hydroxyl group for one methoxy group of **3**. A NOESY experiment of **4** showed interactions between H-6 (δ_H 6.74) and methoxy protons of 5-OMe (δ_H 4.04) and between H-7 (δ_H 6.86) and methoxy protons of 8-O-Me (δ_H 3.98). An HMBC experiment revealed that H-6 interacted with C-4a (δ_C 118.7), C-5 (δ_C 149.8), and C-8 (δ_C 151.3). The signal at H-7 showed correlations with C-5, C-8, and C-8a (δ_C 128.4). The NMR spectroscopic data are summarized in Table 2. Thus, **4** was characterized as 4-hydroxy-5,8-dimethoxy-2-naphthaldehyde. This compound has been obtained from *Diospyros mollis*.[8]

A small amount (1.8 mg) of Compound **5** was obtained from 430 g of wood. The HR-EI mass spectrum of **5** showed a molecular ion at m/z 202.0659, indicating that the molecular formula was $C_{12}H_{10}O_3$. ^{1}H and ^{13}C NMR spectroscopic data suggested that **5** had one aldehyde group, one hydroxyl group, and one methoxy group. A NOESY experiment showed that the signal corresponding to the hydroxyl proton (δ_H 9.46) interacted with methoxy protons (δ_H 4.12), and the aldehyde proton (δ_H 10.08) showed a correlation with *meta*-coupled protons, H-1 (δ_H 7.84) and H-3 (δ_H 7.32). H-3 was correlated with the hydroxyl proton (δ_H 9.46); furthermore, H-1 interacted with H-8 (δ_H 7.61). An HMBC experiment showed that H-3 interacted with the aldehyde carbon (δ_C 192.2), C-1 (δ_C 124.9), and C-4a (δ_C 118.2). The signal corresponding to H-6 (δ_H 6.99) was correlated with C-4a and C-8 (δ_C 123.4). H-7 showed a correlation with C-5 (δ_C 156.1) and C-8a (δ_C 136.1). H-8 interacted with C-1, C-4a, and C-6 (δ_C 107.1). As a result, **5** was characterized as 4-hydroxy-5-methoxy-2-naphthaldehyde. The NMR spectroscopic data are summarized in Table 2. This structure was also confirmed by COSY and heteronuclear multiple quantum coherence (HMQC). This compound has been obtained from *Diospyros melanoxylon*[9] and *Diospyros assimilis*.[3]

Diospyros species characteristically contain naphthalene derivatives; however, the novel naphthalene derivatives, compounds **1**, **2**, and **3**, were characteristic components of the blackened heartwood of *Diospyros kaki*. These compounds could be indicators to help in the identification of *Diospyros kaki*.

The compounds isolated in this study were expected to exhibit high bioactivities because of the presence of a phenolic hydroxyl group. In particular, **3** contained two hydroxyl groups, which gave it the characteristics of an excellent biologically active agent. Noda et al.[4] investigated the difference between the biodegradation properties of the sapwood and blackened heartwood of *Diospyros kaki* and reported that the blackened portion was more resistant to fungal and termite attacks. According to the GC-MS analysis, **3** was not detected in the sapwood of *Diospyros kaki* (data not shown). Thus, the resistance to fungal and termite attacks was thought to be causally related to **3** as well as to other naphthalene derivatives.

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