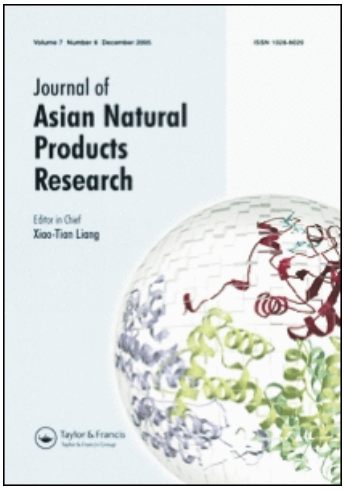

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ORIGINAL ARTICLE

Phenanthrene derivatives from the stems and leaves of *Dioscorea nipponica* Makino

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From the $CHCl_3$-soluble portion of the 70% EtOH extract of the stems and leaves of *Dioscorea nipponica* Makino, two new phenanthrenes, 7-hydroxy-2,3,5-trimethoxy-9,10-dihydrophenanthrene (**1**) and 2,2′,7,7′-tetrahydroxy-4,4′,6,6′-tetramethoxy-1,1′-biphenanthrenes (**2**), as well as three known phenanthrenes, 6-methoxycoelonin (**3**), 4,7-dihydroxy-2,3,6-trimethoxyphenanthrene (**4**), and 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene (**5**), were isolated. The structures were determined by means of HR-MS, ^{1}H NMR, ^{13}C NMR, and HMBC experiments.

Keywords: *Dioscorea nipponica* Makino; dihydrophenanthrene; biphenanthrenes

1. Introduction

Dioscorea nipponica Makino is a plant of the family Dioscorea, which is distributed mainly in northeast and north China. Its rhizome has long been used as a folk herb. The saponins extracted from the rhizome are raw materials in synthesizing steroid hormones [1–3], and the anticancer activities of dioscin *in vitro* were confirmed [4–9]. Therefore, *D. nipponica* is cultivated in a large scale in the northeast of China, and thus the resource of aerial parts of *D. nipponica* is very abundant. We noticed that no research on the aerial parts of *D. nipponica* has been reported to date. To utilize this kind of medical plant sufficiently, we studied the chemical constituents and pharmacological activities of the aerial parts of *D. nipponica*. In our previous studies, flavones, dihydrophenanthrenes, and other aromatic compounds were isolated from the stems and leaves of *D. nipponica* [10,11]. We now deal with the structural elucidation of two new and three known phenanthrene derivatives.

2. Results and discussion

Compounds **1**–**5** were isolated after repeated silica gel column chromatography of the $CHCl_3$-soluble portion of the 70% EtOH extract prepared from the dried stems and leaves of *D. nipponica*, and final purification was achieved by recrystallization. The structures were determined by means of HR-MS, ^{1}H NMR, ^{13}C NMR, and HMBC experiments. The two new phenanthrenes were 7-hydroxy-2,3,5-trimethoxy-9,10-dihydrophenanthrene (**1**) and 2,2′,7,7′-tetrahydroxy-4,4′,6,6′-tetramethoxy-1,1′-biphenanthrenes (**2**), while the three known phenanthrenes were

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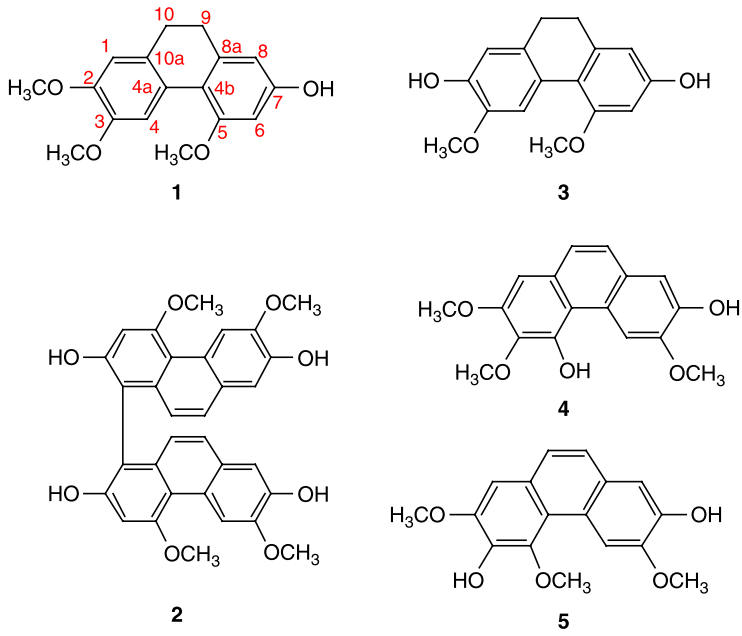

Figure 1. Structures of compounds **1**–**5**.

6-methoxycoelonin (**3**), 4,7-dihydroxy-2,3,6-trimethoxyphenanthrene (**4**), and 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene (**5**) (Figure 1).

Compound **1** was obtained as a brown amorphous powder with mp 179–181°C. A fresh green spot was visualized on a silica gel plate after spraying with 10% H_2SO_4 (ethanol solution), and then heating at 105°C. It exhibited a molecular ion at *m/z* 285.1146 $[M-H]^-$, and based on this the molecular formula was deduced as $C_{17}H_{17}O_4$ with nine degrees of unsaturation. Also, compound **1** had UV absorptions at 266, 300, 313, 337, 353, and 372 nm, which is indicative of an aromatic system. The IR spectrum showed hydroxyl absorption at 3419 cm^{-1}.

The ^1H NMR spectrum of **1** showed two aromatic singlets [δ 7.78 (1H, s) and 6.69 (1H, s)], two meta-coupled aromatic proton signals [δ 6.31 (1H, d, $J = 2.0$ Hz) and 6.22 (1H, d, $J = 2.0$ Hz)], three methoxyl signals [δ 3.75 (3H, s), 3.74 (3H, s), and 3.73 (3H, s)], and a four-proton singlet [δ 2.55]. The most deshielded resonance at δ 7.78 corresponds to H-4 or H-5 of a 9,10-dihydrophenanthrene derivative [12]. Therefore, this compound also had characteristics of the 9,10-dihydrophenanthrene derivative. If this signal is assigned to H-4, H-5 must contain an oxygen substituent. The appearance of the sharp singlet implies that C-2 and C-3 of **1** must contain oxygen functions [13]. The HMBC experiment (Table 1) was used to elucidate the structure of **1**.

The protons at δ 6.69 (H-1) and 7.78 (H-4) ought to be placed in the *para* positions of the same ring since both of them showed correlations with oxygenated aromatic carbons at δ 148.1 (C-2) and 148.2 (C-3). Also, C-2 and C-3 must bear a methoxyl, respectively, since the methoxyl protons at δ 3.74 and 3.73 showed HMBC correlations with them. The remaining methoxyl proton signal at δ 3.75 showed correlation with C-5. The HMBC correlations of H-1 with C-4a and H-4 with C-10a led to the identification of the other two carbons C-4a and C-10a of this phenyl

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125.8 MHz) spectral data and HMBC correlations of **1** (CD_3OD).

No.	^{13}C NMR (DEPTQSP) (δ)	^{1}H NMR (δ, J in Hz)	HMBC	
			^{2}J	^{3}J
1	112.5	6.69 (1H, s)	C-2,10a	C-3,4a,10
2	148.1 (C)			
3	148.2 (C)			
4	114.0	7.78 (1H, s)	C-3,4a	C-2,4b,10a
4a	127.4 (C)			
4b	116.5 (C)			
5	159.2 (C)			
6	99.4	6.31 (1H, d, $J = 2.0$ Hz)	C-5,7	C-4b,8
7	158.0 (C)			
8	108.6	6.22 (1H, d, $J = 2.0$ Hz)	C-7	C-4b,6,9
8a	142.2 (C)			
9	32.0	2.55 (2H, s)	C-8a,10	C-4b,8,10a
10	30.4	2.55 (2H, s)	C-9,10a	C-1,4a,8a
10a	132.1 (C)			
2-OCH_3	56.5	3.74 (3H, s)		C-2
3-OCH_3	56.8	3.73 (3H, s)		C-3
5-OCH_3	56.1	3.75 (3H, s)		C-5

ring. The meta-coupled aromatic protons at δ 6.31 and 6.22 were assigned to be H-6 and H-8, since the proton at δ 6.31 showed HMBC correlations with C-4b and C-8, and the proton at δ 6.22 showed HMBC correlations with C-4b, C-6, and C-9. The group between the two meta-coupled protons (H-6 and H-8) seemed to be a hydroxyl from the molecular formula. Thus, the above spectral data established the structure of **1** as 7-hydroxy-2,3,5-trimethoxy-9,10-dihydrophenanthrene.

Compound **2** was obtained as a light brown amorphous powder. The TLC test was carried out on silica gel, blue fluorescence was visualized, and a yellow-green spot was shown on a silica gel plate after spraying with 10% H_2SO_4 (ethanol solution), and then heating at 105°C. It exhibited a molecular ion $[M-H]^-$ at *m/z* 537.1535, suggesting the molecular formula of $C_{32}H_{26}O_8$ with 20 degrees of unsaturation. Compound **2** also had UV absorptions at 218, 278, and 302 nm, which is indicative of an aromatic system. The IR spectrum showed hydroxyl absorption at 3394 cm^{-1}.

The two broad singlets at δ 9.29 and 9.01 shown in the ^{1}H NMR spectrum were proton signals of phenolic hydroxyl groups. The proton at δ 9.08 shown as the sharp signal was aromatic proton H-4 or H-5 of a phenanthrene. The corresponding protons of phenathrene usually resonate at δ 9.50, and the relatively upfield shifts of this signal by about 0.5 ppm were attributed to the presence of electron-donating groups (OH or OMe) at *ortho* and *para* to this proton [12]. The *ortho*-coupled aromatic protons at δ 6.79 (1H, d, $J = 9.0$ Hz) and 7.26 (1H, d, $J = 9.0$ Hz) were H-9 and H-10 of phenanthrene. The ^{1}H NMR spectrum also showed signals for two aromatic methoxyl protons at δ 4.14 (3H, s, $-OCH_3$) and 3.98 (3H, s, $-OCH_3$).

The ^{13}C NMR (DEPTQSP) spectrum showed 14 aromatic carbon signals, constituted to a tricyclic aromatic system, and two methoxyl carbon signals at δ 55.5 and 55.9. Thus, the 16 carbon signals, in combination with the molecular formula $C_{32}H_{26}O_8$, indicated that **2** consisted of two monomeric structures of equal elemental composition ($C_{16}H_{13}O_4$). The signal at δ

126.4, obviously lower than the others, was due to the overlap of a substituted aromatic carbon signal and an unsubstituted aromatic carbon signal, according to the DEPT spectrum. The HMBC experiment (Table 2) was again useful in determining the structure of **2**.

Starting from the deshielded signal at δ 9.08 (H-5), HMBC correlations were observed from H-5 to the carbons at δ 147.9 (C-6), 145.1 (C-7), 126.4 (C-8a), 124.2 (C-4b), and 114.2 (C-4a). The proton at δ 7.08 (1H, s) showed correlations with the carbons at δ 147.9 (C-6), 145.1 (C-7), 126.4 (C-9), and 124.2 (C-4b), and thus it was assigned to be H-8. The unassigned aromatic proton signal at δ 6.97(1H, s) showed HMBC correlations with the carbons at δ 157.9 (C-4), 153.4 (C-2), 124.2 (C-4b), 114.2 (C-4a), and 111.2 (C-1), thus it was assigned to be H-3. Methoxyl protons at δ 4.14 (3H, s) showed correlation with C-4, and hydroxyl proton at δ 9.01 showed correlations with C-2, C-1, and C-3. Therefore, the substitution pattern of one aromatic ring was evident. The other methoxyl protons at δ 3.98 (3H, s) showed correlation with C-6, and the other hydroxyl proton at δ 9.29 showed correlations with C-7, C-6, and C-8. Therefore, the substitution pattern of another aromatic ring was elucidated. As the above evidence, the two phenanthrene moieties were connected at C-1 and C-1′. Thus, the structure of **2** was elucidated to be 2,2′,7,7′-tetrahydroxy-4,4′,6,6′-tetramethoxy-1,1′-biphenanthrenes.

The structures of compounds **3**–**5** were confirmed by ^{1}H NMR, ^{13}C NMR, and HMBC spectra. The spectral data of **3**–**5** are consistent with those reported in the literature [14,15].

3. Experimental

3.1 General experimental procedures

The melting points were determined on a WRS-1B digital melting point apparatus (Shanghai, China) and are uncorrected.

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125.8 MHz) spectral data and HMBC correlations of **2** (DMSO-d_6).

No.	^{13}C NMR (DEPTQSP) (δ)	^{1}H NMR (δ, J in Hz)	HMBC	
			^{2}J	^{3}J
1,1′	111.2			
2,2′	153.4			
3,3′	99.6	6.97 (1H, s)	C-2,2′,4,4′	C-1,1′,4a,4a′
4,4′	157.9			
4a,4a′	114.2			
4b,4b′	124.2			
5,5′	109.2	9.08 (1H, s)	C-4b,4b′,6,6′	C-4a,4a′,7,7′,8a,8a′
6,6′	147.9			
7,7′	145.1			
8,8′	111.8	7.08 (1H, s)	C-7,7′,8a,8a′	C-4b,4b′,6,6′,9,9′
8a,8a′	126.4			
9,9′	126.4	7.26 (1H, d, J = 9.0 Hz)	C-8a,8a′	C-4b,4b′,8,8′,10a,10a′
10,10′	122.7	6.79 (1H, d, J = 9.0 Hz)	C-10a,10a′	C-1,1′,4a,4a′,8a,8a′
10a,10a′	133.9			
2,2′-OH		9.01 (1H, s)	C-2,2′	C-1,1′,3,3′
4,4′-OCH_3	55.9	4.14 (3H, s)		C-4,4′
6,6′-OCH_3	55.5	3.98 (3H, s)		C-6,6′
7,7′-OH		9.29 (1H, s)	C-7,7′	C-6,6′,8,8′

IR data were taken on an AVATAR 330FT-IR (Nicolet, Madison, WI, USA), and UV data on a UV-1700 (Shimadzu, Kyoto, Japan). HR-MS spectra were recorded using Ionspec 7.0T FT-ICR-MS (IonSpec Corporation, Lake Forest, CA, USA). NMR spectra were measured at 500 MHz for ^{1}H NMR, 125.8 MHz for ^{13}C NMR, and 500 MHz for HMBC on a Bruker Avance-500 spectrometer (Karlsruhe, Germany). NMR spectra were measured in CD_3OD (for **1**, **3**, and **4**), DMSO-d_6 (for **2**), and $CDCl_3$ (for **5**), using TMS as the internal standard (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). Chemical shifts (δ) are expressed in ppm. Silica gel H (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China) was used in column chromatography. Also, silica gel G plates (Qingdao Marine Chemical, Inc.) were used in thin layer chromatography.

3.2 Plant material

The stems and leaves of *D. nipponica* Makino were collected in the Jingyu County of Jilin Province (China) in October 2005, and identified by Researcher Jin-Min Zhang. A voucher specimen is deposited at the Institute of Frontier Medical Science (No. 2005005), Jilin University, China.

3.3 Extraction and isolation

The air-dried stems and leaves of *D. nipponica* (5 kg) were pulverized and then extracted with 70% ethyl alcohol. The extract was concentrated under reduced pressure to 300 ml, diluted with water (1000 ml), and extracted with petroleum ether, and then with chloroform. The chloroform fraction was concentrated under reduced pressure to afford a residue (110 g). A portion (80 g) of the residue was subjected to repeated silica gel column chromatography with $CHCl_3$–CH_3OH (from 1:20 to 1:5), yielding **1** (26 mg), **2** (43 mg), **3** (115 mg), **4** (22 mg), and **5** (35 mg).

3.3.1 7-Hydroxy-2,3,5-trimethoxy-9,10-dihydrophenanthrene (**1**)

Brown amorphous powder (acetone); mp 179–181°C; UV λ_{max}^{EtOH} (nm): 266, 300, 313, 337, 353, 372; IR ν_{max}^{KBr} (cm^{-1}): 3419 (OH), 2945, 2931 (CH_2), 1613, 1579, 1510, 1488, 1461 (benzene ring). For ^{1}H and ^{13}C NMR spectroscopic data, see Table 1. HR-MS *m/z*: 285.1146 $[M-H]^-$ (calcd for $C_{17}H_{17}O_4$, 286.1205).

3.3.2 2,2′,7,7′-Tetrahydroxy-4,4′,6,6′-tetramethoxy-1,1′-biphenanthrenes (**2**)

Light brown amorphous powder (acetone); UV λ_{max}^{EtOH} (nm): 218, 278, 302; IR ν_{max}^{KBr} (cm^{-1}): 3394 (OH), 1625, 1590, 1505, 1480, 1450 (benzene ring). For ^{1}H and ^{13}C NMR spectroscopic data, see Table 2. HR-MS *m/z*: 537.1535 $[M-H]^-$ (calcd for $C_{32}H_{26}O_8$, 538.1628).

3.3.3 2,7-Dihydroxy-3,5-dimethoxy-9,10-dihydrophenanthrene (6-methoxycoelonin, **3**)

Brown needles (MeOH); mp 118–120°C. A fresh green spot was visualized on a silica gel plate after spraying with 10% H_2SO_4 (ethanol solution), and then heating at 105°C for 2 min. ^{1}H NMR (500 MHz, CD_3OD): δ 6.53 (1H, s, H-1), 7.74 (1H, s, H-4), 6.30 (1H, d, $J = 2.0$ Hz, H-6), 6.20 (1H, d, $J = 2.0$ Hz, H-8), 2.51 (2H, m, H-9), 2.48 (2H, m, H-10), 3.74 (6H, s, 3-OCH_3, 5-OCH_3); ^{13}C NMR (125.8 MHz, CD_3OD): δ 115.3 (C-1), 145.3 (C-2), 146.7 (C-3), 113.6 (C-4), 126.2 (C-4a), 116.9 (C-4b), 159.0 (C-5), 99.4 (C-6), 157.7 (C-7), 108.6 (C-8), 142.0 (C-8a), 32.1 (C-9), 30.2 (C-10), 132.3 (C-10a), 56.7 (3-OCH_3), 56.1 (5-OCH_3).

3.3.4 4,7-Dihydroxy-2,3,6-trimethoxyphenanthrene (**4**)

Brown amorphous powder ($CHCl_3$); ^{1}H NMR (500 MHz, CD_3 OD): δ 6.95 (1H, s,

H-1), 8.91 (1H, s, H-5), 7.06 (1H, s, H-8), 7.31 (1H, d, $J = 9.0$ Hz, H-9), 7.26 (1H, d, $J = 9.0$ Hz, H-10), 3.89 (3H, s, 2-OCH_3), 3.91 (3H, s, 3-OCH_3), 3.94 (3H, s, 6-OCH_3); ^{13}C NMR (125.8 MHz, CD_3OD): δ 110.0 (C-1), 152.6 (C-2), 143.0 (C-3), 150.3 (C-4), 119.2 (C-4a), 125.2 (C-4b), 108.6 (C-5), 149.3 (C-6), 146.6 (C-7), 113.0 (C-8), 128.9 (C-8a), 126.9 (C-9), 125.5 (C-10), 131.2 (C-10a), 60.7 (2-OCH_3), 61.5 (3-OCH_3), 56.3 (6-OCH_3).

*3.3.5 3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene (**5**)*

Brown amorphous powder ($CHCl_3$); ^{1}H NMR (500 MHz, CD_3Cl): δ 6.96 (1H, s, H-1), 8.90 (1H, s, H-5), 7.23 (1H, s, H-8), 7.39 (2H, s, H-9, 10), 3.93 (3H, s, 2-OCH_3), 3.88 (3H, s, 4-OCH_3), 3.97 (3H, s, 6-OCH_3); ^{13}C NMR (125.8 MHz, CD_3Cl): δ 104.6 (C-1), 146.5 (C-2), 138.7 (C-3), 143.5 (C-4), 118.5 (C-4a), 123.4 (C-4b), 106.7 (C-5), 146.5 (C-6), 144.6 (C-7), 111.0 (C-8), 127.8 (C-8a), 124.9 (C-9), 124.7 (C-10), 126.1 (C-10a), 59.9 (2-OCH_3), 56.0 (4-OCH_3), 55.8 (6-OCH_3).

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