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9,10-Dihydrophenanthrene derivatives and one 1,4-anthraquinone firstly isolated from *Dioscorea zingiberensis* C. H. Wright and their biological activities

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ABSTRACT

Two new phenanthrene derivatives, 2,5,7-trimethoxy-9,10-dihydrophenanthrene-1,4-dione (1) and 2,5,6-trihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (3), one new anthracenedione, 2,5,7-trimethoxyanthracene-1,4-dione (2), together with two known 9,10-dihydrophenanthrenes (4–5) were isolated from the rhizomes of *Dioscorea zingiberensis* C. H. Wright. The structures of these new compounds were established based on extensive NMR spectroscopy. Several isolated compounds were evaluated for the inhibition against nitric oxide (NO) production in the lipopolysaccharide (LPS)-activated RAW 264.7 macrophage cell line, DPPH radical scavenging, and inhibitory activity on Free Fatty Acids (FFAs) induced triglyceride accumulation in HepG2 cells. Compound 2 exhibited moderate anti-inflammatory activity, compound 3 possessed comparable DPPH radical scavenging activity as Vitamin C, compounds 2 and 4 showed potent inhibitory activities on triglyceride accumulation.

1. Introduction

Dioscorea, a genus in Dioscoreaceae family, is comprised of about 600 species. Most of the *Dioscorea* plants are known for their steroid saponin rich content [1]. Only a total of 31 phenanthrenes have been isolated to date, mainly from *Dioscorea opposita*, *Dioscorea nipponica*, and *Dioscorea bulbifera* [2–4]. The rhizomes of *Dioscorea zingiberensis* C. H. Wright have been traditionally used in China as herbal medicine for a long period. It is used for the treatment of cough with lung heat, pyretic stranguria, anthracia, coronary heart disease, swelling, ulcer, and sprain [5]. Previous chemical and pharmacological investigation of the title plant established that *D. zingiberensis* possesses many spirostane and furostane glycosides with anti-tumor activities [6–10]. However, there has been no trial conducted to find certain biological phenol ingredients from the low-polarity fraction.

As a part of our ongoing project to search for new biological constituents from the dichloromethane-soluble fraction of *D. zingiberensis*, we systematically examined the non-steroid compounds and isolated five phenols, including three new ones, 2,5,7-trimethoxy-9,10-dihydro phenanthrene-1,4-dione (1), 2,5,7-trimethoxyanthracene-1,4-dione (2), and 2,5,6-trihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (3), as well as two known compounds 5,6-dihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene (4) and 2,5-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene (5). This paper deals with the isolation, structural elucidation, inhibitory effect on Nitric Oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, DPPH radical scavenging, and inhibitory activity on Free Fatty Acids (FFAs) induced triglyceride accumulation on HepG2 cells of the selected isolates.

2. Experimental procedure

2.1. General experimental procedures

The UV spectra were recorded using a Beckman Coulter DU-800 spectrometer (Beckman, US). IR spectra were recorded on an AVATER-360 spectrometer (Nicolet, USA). NMR spectra were run on an AV II spectrometer (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR) (Bruker, Germany). HR-ESIMS were recorded on a Q-TOF-premier mass analyzer (Waters company, US). ODS RP (50 μm, Fuji Silysia Chemical Company, Japan), and Sephadex LH-20 (GE healthcare biosciences AB, Sweden) were used for column chromatography. TLC was carried out on plates

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(0.25 mm thickness), and spots were visualized by heating after spraying with anisaldehyde–H_2SO_4. Analysis HPLC was performed using a LC-20A instrument (Shimadzu, Japan) with an Inertsil® ODS-SP column (150 mm × 4.6 mm, 5 μm; Shimadzu, Japan). Prepared HPLC was performed with a LC-6AD pump, using a SPD-20A detector, and a ODS-A column (250 mm × 20 mm, 5 μm; YMC, Japan).

2.2. Plant material

The fresh rhizomes of *D. zingiberensis* were purchased from Tianhe Pharmaceutical Company in Yunxi city of Hubei province of China in April 2012, and were identified by Prof. Guangzhi Wang of Chengdu university of Traditional Chinese Medicine. A voucher specimen (NO.201206HJ) has been deposited in the Herbarium of Laboratory of Ethnopharmacology of West China Hospital/West China Medical School of Sichuan University.

2.3. Extraction and isolation

Dry rhizomes (10 kg) of *D. zingiberensis* were extracted three times with EtOH at room temperature to obtain a crude extract (650 g). The crude extract was suspended in distilled water and partitioned with CH_2Cl_2. The dichloromethane soluble fraction (10.3 g) was subjected to Sephadex LH-20 (MeOH–CH_2Cl_2 = 1:1) to yield 4 fractions. Fraction 3 (5.2 g) was subjected to MPLC (C_{18} column, 1 × 50 cm; detection, UV at 210 nm; 10 mL/min) with a gradient elution of MeOH–H_2O (5:95 → 95:5) to yield 14 fractions (A–N). Fraction I (0.52 g) was subjected to HPLC (YMC-pack ODS-A C_{18}, 250 × 10 mm; eluent, MeCN–H_2O (60:40)); detection, UV at 210 nm; flow rate, 6 mL/min) to yield Compounds 1 (3 mg, t_R = 16.7 min), 2 (2 mg, t_R = 17.8 min), and 4 (20 mg, t_R = 27.4 min). Compound 3 (42 mg, t_R = 26.4 min) from fraction F was isolated by HPLC (YMC-pack ODS-A C_{18}, 250 × 10 mm; eluent, MeCN–H2O (62:38); detection, UV at 210 nm; flow rate, 6 mL/min). Compound 5 (4 mg, t_R = 22.6 min) from fraction G was isolated by prepared HPLC (YMC-pack ODS-A C_{18}, 250 × 10 mm; eluent, MeCN–H_2O (52:48); detection, UV at 210 nm; flow rate, 5 mL/min).

2.4. Spectroscopic data

2,5,7-Trimethoxy-9,10-dihydrophenanthrene-1,4-dione (1). Purple amorphous powder; UV (MeOH) λ_{max} nm (log ε) 210 (4.03), 235 (3.70), 266 (3.65); IR (neat) ν_{max} 3410, 2930, 2848, 1658, 1603, 1460, 1429, 1348, 1318, 1285, 1233, 1157, 1091, 841 cm^{-1}; ^1H NMR (CD_3Cl, 600 MHz) and ^{13}C NMR (CD_3Cl, 150 MHz) see Tables 1 and 2; HR-ESIMS *m/z* 323.0894 (calcd for $C_{17}H_{16}O_5Na$, 323.0895).

2,5,7-Trimethoxyanthracene-1,4-dione (2). Reddish amorphous powder; UV (MeOH) λ_{max} nm (log ε) 218 (4.20), 238 (4.12), 263 (3.77), 302 (4.03), 399 (3.64); IR (neat) ν_{max} 3416, 2919, 2849, 1654, 1614, 1601, 1499, 1464, 1437, 1371, 1349, 1266,1238, 1201, 1164, 1088, 841 cm^{-1}; ^1H NMR (CD_3Cl, 600 MHz) and ^{13}C NMR (CD_3Cl, 150 MHz) see Tables 1 and 2; HR-ESIMS *m/z* 321.0738 (calcd for $C_{17}H_{14}O_5Na$, 321.0739).

3,4-Dimethoxy-9,10-dihydrophenanthrene-2,5,6-triol (3). Brownish amorphous powder; UV (MeOH) λ_{max} nm (log ε) 216 (4.37), 246 (3.51), 275 (4.01), 296 (3.77); IR (neat) ν_{max} 3419, 2940, 2836, 1595, 1580, 1485, 1449, 1424, 1347, 1242, 1347, 1242, 1205, 1162, 1144, 1077, 997, 812, 795 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) see Tables 1 and 2; HR-ESIMS *m/z* 311.0894 (calcd for $C_{16}H_{16}O_5Na$, 311.0895).

Table 1
^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for compounds 1 and 3.

Position	1[a]		3[b]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	180.9		113.8	6.69 (1H, s)
2	169.7		151.1	
3	100.3	5.7 (1H, s)	140.7	
4	182.1		150.2	
4a	137.7		119.2	
4b	112.9		121.7	
5	158.4		140.7	
6	97.7	6.39 (1H, d, *J* = 2.4 Hz)	142.0	
7	161.9		114.2	6.71 (1H, d, *J* = 7.8 Hz)
8	105.2	6.38 (1H, d, *J* = 2.4 Hz)	120.4	6.68 (1H, d, *J* = 7.8 Hz)
8a	141.1		131.9	
9	28.8	2.68 (2H, m)	31.4	2.16 (2H, m)
10	21.5	2.59 (2H, m)	32.1	2.15 (2H, m)
10a	141.8		138.3	
2-OCH_3	56.9	3.88 (3H, s)		
3-OCH_3			61.7	3.9 (3H, s)
4-OCH_3			62.7	3.71 (3H, s)
5-OCH_3	55.9	3.76 (3H, s)		
7-OCH_3	55.6	3.82 (3H, s)		

[a] Recorded in $CDCl_3$.
[b] Recorded in CD_3OD.

Table 2
^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for compound 2 in $CDCl_3$.

Position	2	
	δ_C	δ_H (*J* in Hz)
1	185.4	
2	169.7	
3	100.9	5.82 (1H, s)
4	186.7	
4a	130.5	
5	157.9	
6	102.1	6.67 (1H, d, *J* = 2.4 Hz)
7	160.6	
8	98.9	6.73 (1H, d, *J* = 2.4 Hz)
8a	137.8	
9	132.6	7.85 (1H, brs)
9a	133.4	
10	121.6	7.85 (1H, brs)
10a	119.3	
2-OCH_3	57.1	3.88 (3H, s)
5-OCH_3	56.0	3.76 (3H, s)
7-OCH_3	55.7	3.82 (3H, s)

2.5. NO production in RAW 264.7 macrophages

Murine monocytic RAW264.7 macrophages were grown in RPMI-1640 medium (Hyclone) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS, Gibco-Invitrogen) under a humidified atmosphere of 5% CO_2 at 37 °C. The RAW264.7 macrophages were

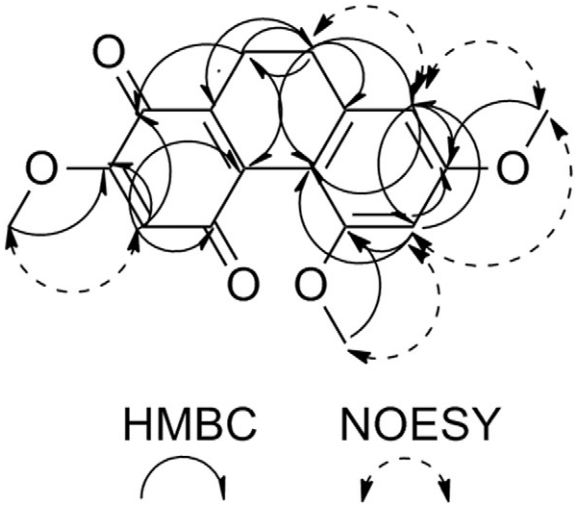

Fig. 1. HMBC and key NOESY correlations of 1.