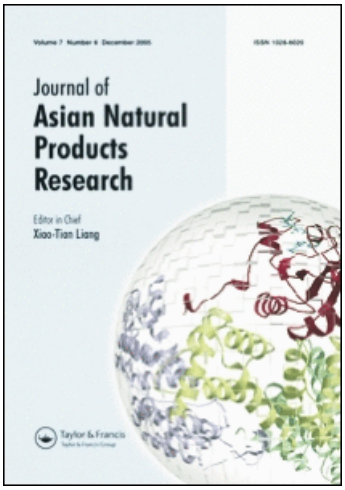

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Four anthraquinones from *Hedyotis diffusa*

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A new anthraquinone has been isolated from the 95% EtOH extract of *Hedyotis diffusa* and characterized as 2-hydroxy-3-methoxy-6-methyl-9,10-anthraquinone (**1**) by extensive spectral analysis. The known compounds isolated for the first time from this plant have been identified as 2-hydroxy-3-methoxy-7-methyl-9,10-anthraquinone (**2**), 2-hydroxy-6-methylanthraquinone (**3**), and 1,3-dimethoxy-2-hydroxy-9,10-anthraquinone (**4**).

Keywords: rubiaceae; *Hedyotis diffusa*; anthraquinones; 9,10-anthraquinone

1. Introduction

Hedyotis diffusa Willd. (Rubiaceae), an annual herb distributed growing throughout India and China, is known in oriental folk medicine to have anticancer, antimicrobial, and anti-inflammatory activities and is used to treat pneumonia in children, appendicitis, pelvitis, and some tumors [1]. Previous phytochemical studies revealed the presence of acylated iridoid glycosides [2,3] and anthraquinones [4,5]. During the course of our search for bioactive ingredients from the traditional medicinal plant, a new anthraquinone (**1**) was obtained together with three known compounds and their structures were elucidated on the basis of IR, ^{1}H and ^{13}C NMR, HMQC, HMBC, ROESY and mass spectroscopic methods. This note describes the isolation and characterization of the new compound 2-hydroxy-3-methoxy-6-methyl-9,10-anthraquinone (**1**) from the whole plants of this species. Three known compounds have been identified as 2-hydroxy-3-methoxy-7-methyl-9,10-anthraquinone (**2**) [6], 2-hydroxy-6- methylanthraquinone (**3**) [7], and 1,3-dimethoxy-2-hydroxy-9,10-anthraquinone (**4**) (Figure 1) [8] by comparing their spectral data with those reported in the literature.

2. Results and discussion

By successive column chromatography (CC) on silica gel and Sephadex LH-20, a 95% ethanolic extract of air-dried *H. diffusa* (Wild) afforded a new anthraquinone (**1**). The identification of **1** was made by spectroscopic data.

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula, $C_{16}H_{12}O_4$, was determined from the $[M - H]^-$ ion peak at *m/z* 267.0657 in the HRESIMS. The IR spectrum indicated that **1** possessed hydroxyl (3330 cm^{-1}) and two conjugated carbonyls (1668 and 1675 cm^{-1}). In its ^{1}H NMR spectrum the aromatic protons resonated at δ_H 8.00 (d, 1H, H-8, $J = 8.0$ Hz), 7.80 (d, 1H, H-5, $J = 1.3$ Hz), 7.64 (dd, 1H, H-7, $J = 8.0, 1.3$ Hz), 7.56 (s, 1H, H-4), and 7.49 (s, 1H, H-1). The methoxyl at C-3 and the methyl at C-6 resonated at δ_H 3.97 and 2.48,

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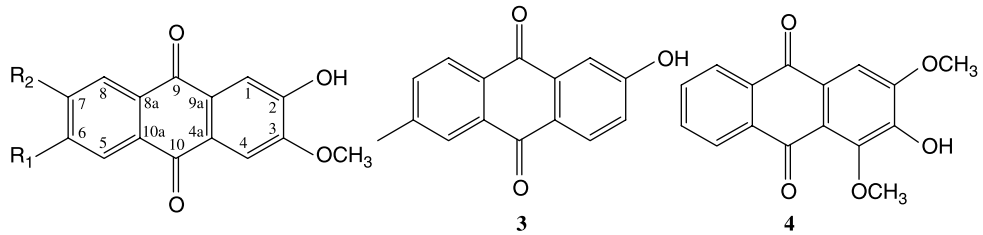


1 R_1=CH_3 R_2=H
2 R_1=H R_2=CH_3

Figure 1. Structures of compounds **1**–**4**.

respectively, as singlets. The phenolic proton appeared as a singlet at δ_H 10.70. The ^{13}C NMR and HMQC spectra gave the signals showing that **1** has one methyl, one methoxyl, fourteen sp^2 quaternary carbons (Table 1) [6]. Detailed analysis of the HMQC and HMBC spectra indicated that **1** possessed the framework as 9,10-anthraquinone. The structure elucidation was assisted by analyses of the HMBC experiments (Figure 2). The HMBC correlations between H-1 (δ_H 7.49) and C-3 (δ_C 152.2), C-9 (δ_C 181.7), C-4a (δ_C 126.5), and H-4 (δ_H 7.56) with C-2 (δ_C 152.6), C-9a (δ_C 127.9), and C-10 (δ_C 181.1) confirmed that the hydroxyl and the methoxyl were connected to C-2 and C-3, respectively. The HMBC correlations between H-5 (δ_H 7.80) and C-7 (δ_C 134.5), C-10 (δ_C 181.1), C-8a (δ_C 132.9), H-8 (δ_H 8.00) and C-6 (δ_C 144.2), C-9 (δ_C 181.7), C-10a (δ_C 130.8), and CH_3-6 (δ_H 2.48) and C-7 (δ_C 134.5), C-5 (δ_C 126.4) secured that the methyl was fixed to C-6. Additionally, the ROESY correlations (Figure 2) between OH-2 (δ_H 10.70) and H-1 (δ_H 7.49), between OCH_3-3 (δ_H 3.97) and H-4 (δ_H 7.56) and between CH_3-6 (δ_H 2.48) with H-5 (δ_H 7.80), H-7 (δ_H 7.64) confirmed the above results. Accordingly, the planar structure of **1** was established as 2-hydroxy-3-methoxy-6-methyl-9,10-anthraquinone.

Table 1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** in DMSO-d_6.[a,b]

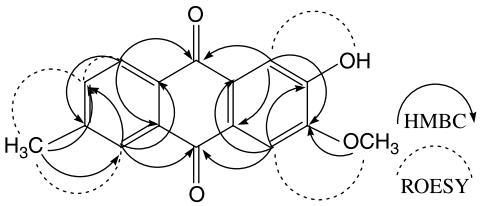
	1	
Position	^1H	^{13}C
1	7.49 (s)	112.3
2		152.6
3		152.2
4	7.56 (s)	108.7
4a		126.5
5	7.80 (d, *J* 1.3 Hz)	126.4
6		144.2
7	7.64 (dd, *J* 8.0, 1.3 Hz)	134.5
8	8.00 (d, *J* 8.0 Hz)	126.5
8a		132.9
9		181.7
9a		127.9
10		181.1
10a		130.8
2-OH	10.70 (brs)	
3-OCH_3	3.97 (s)	55.9
6-CH_3	2.48 (s)	21.2

[a]TMS was used as an internal standard in spectral experiments.
[b]Assignments based on HMQC and HMBC experiments.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a Beckman DU 640 spectrophotometer. IR spectra were measured on a SHIMADZU FT/IR 8900 spectrophotometer. NMR spectra were generated on a Bruker ACF-500 spectrometer at 300 K with TMS as internal standard. The ^1H chemical shift in DMSO-d_6 was referenced to the residual at 3.28 ppm and the ^{13}C chemical shift in DMSO-d_6 was referenced to the solvent resonance at 39.7 ppm. HRESIMS spectra were recorded by using a Wiff Agilent TOF mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, China). Sephadex LH-20



1

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

(Amersham Biosciences, Sweden) and silica gel (100–200 and 200–300 mesh) (Qingdao Haiyang Chemical Co. Ltd, Qingdao, China) were used for CC. Precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) were used for TLC.

3.2 Plant material

The dried herbs were collected from Suichuan county of Jiangxi Province, China, in September 2006, and identified by Dr Li You-bin (Jiangsu Provincial Institute of Traditional Chinese Medicine, China). A voucher specimen has been deposited in the Laboratory of Phytochemistry, Jiangsu Provincial Institute of Traditional Chinese Medicine (accession number: 2006-09-09).

3.3 Extraction and isolation

The dried herbs of *H. diffusa* (20 kg) were extracted by 95%EtOH at 80°C, and the crude extract (1200 g) was suspended in water and then extracted successively with petroleum ether (70–95°C) (5 l × 6), EtOAc (5 l × 5), and *n*-BuOH (5 l × 7) to give corresponding fractions P (370 g), C (296 g), and E (328 g).The EtOAc soluble fraction (296 g) was separated by silica gel (100–200 mesh) CC, eluted with a gradient of $CHCl_3$/MeOH (50:1 to 0:1) to give six fractions (A–F). Fraction C (75 g) was then subjected to CC of silica gel (200–300 mesh), eluting with a gradient of $CHCl_3$/MeOH (10:1, 8:1, and 5:1), to give seven parts (C1–C7). Subfraction C5 (8 g) was chromatographed on a Sephadex LH-20 column ($CHCl_3$/MeOH, 50:50) to give four parts (C5a–C5d). Fraction C5c (1.0 g) was purified over Sephadex LH-20 column ($CHCl_3$/MeOH, 50:50) to afford **1** (36 mg). Subfraction C6 (12 g) was chromatographed on a Sephadex LH-20 column ($CHCl_3$/MeOH, 50:50) to give five parts (C6a–C6e). Fraction C6b (1.3 g) was purified over Sephadex LH-20 column ($CHCl_3$/-MeOH, 50:50) to afford **2** (61 mg). Fraction C6d (1.6 g) was further separated over Sephadex LH-20 column ($CHCl_3$/MeOH, 50:50) to yield **3** (28 mg) and **4** (17 mg).

*3.3.1 2-Hydroxy-3-methoxy-6-methyl-9,10-anthraquinone (**1**)*

Yellow amorphous powder; UV (MeOH) λ_{max} (log ε) 216 (3.80), 273 (3.86), 294 (sh) (3.79), 376 (4.16), 388 (3.45) nm; IR (KBr) υ_{max} 3330, 1675, 1668, 1650, 1597, 1465, 1338, 1288, 1047, 804, 748 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1; negative mode ESIMS *m/z* 267 $[M - H]^-$ (100), 252 $[M\text{-}CH_3]^-$ (84), HRESIMS *m/z* 267.0657 $[M - H]^-$ (calcd for $C_{16}H_{11}O_4$, 267.0662).

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