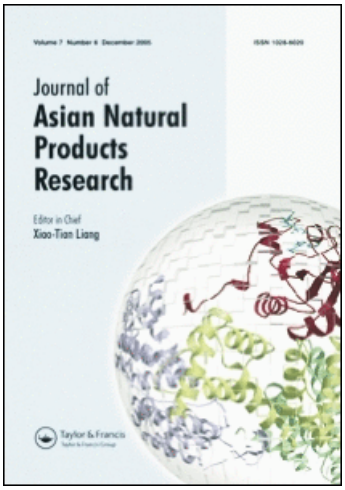

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Xing-Dong Kang[a]; Xian Li[a]; Chun-Chao Zhao[a]; Yu Mao[a]
[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China

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Two new anthraquinones from *Hedyotis diffusa* W.

XING-DONG KANG, XIAN LI*, CHUN-CHAO ZHAO and YU MAO

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China

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Two new anthraquinones, 2,6-dihydroxy-3-methyl-4-methoxyanthraquinone (**1**) and 2-hydroxy-7-hydroxymethyl-3-methoxyanthraquinone (**2**), were isolated from *Hedyotis diffusa* W. Their structures were elucidated by means of spectroscopic evidence.

Keywords: *Hedyotis diffusa* W; Rubiaceae; Anthraquinones; Structure elucidation

1. Introduction

Hedyotis diffusa W. (Rubiaceae), widely distributed in China, is used as a folk medicine for the treatment of gastroenteritis and appendicitis [1]. Phytochemical investigations on *H. diffusa* have led to the isolation of several classes of compounds such as anthraquinones, iridoids [2] and flavonoids [3]. In our extended research, two new anthraquinones, compounds **1** and **2**, were obtained. In this paper, we report the isolation and structural elucidation of the two new anthraquinones.

2. Results and discussion

The $CHCl_3$ parts of the 70% EtOH extract of *H. diffusa* W. were subjected to silica gel column chromatography and RP-HPLC (ODS) to afford two new compounds **1** and **2**. On the basis of spectral data and by comparison with those reported in the literature, the structures of these anthraquinones were established.

Compound **1** was obtained as orange-coloured needles. The molecular formula of $C_{16}H_{12}O_5$ was determined by EI-MS at *m/z* 284.0 $[M]^+$ and HRESI-MS at *m/z* 307.0584 $[M + Na]^+$, which was compatible with NMR analysis. It showed a positive Borntrager's reaction and $FeCl_3$ reaction, suggesting the presence of an anthraquinone skeleton. The ^1H NMR spectrum of **1** (table 1, figure 1) showed the presence of two β-hydroxyl groups at about δ 10.90 and

*Corresponding author. Email: proflixian@163.com

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Table 1. NMR (DMSO, 600 MHz) spectral data of **1** and **2**.

	1					***2***			
NO	δ_C	δ_H	*HMBC*	*NOESY*	*NO*	δ_C	δ_H	*HMBC*	*NOESY*
1	125.5	7.79 (1H, s)	1a, 2, 9-C		1	112.5	7.54 (1H, s)	1a, 2, 9-C	
1a	124.6				1a	126.7			
2	154.9				2	152.9			
3	131.8				3	152.7			
4	146.9				4	108.7	7.60 (1H, s)	3, 4a, 10-C	C_3 —OCH_3
4a	124.2				4a	128.2			
5	112.1	7.42 (1H, d, 2.4 Hz)	5a,10-C		5	126.5	8.12 (1H, d, 8.6 Hz)	5a, 6,10-C	C_6-H
5a	125.3				5a	131.6			
6	162.8				6	133.1	7.78 (1H, br d, 8.6 Hz)	5, 8-C	C_5-H, C_7-CH_2OH
7	120.9	7.16 (1H, dd, 2.4, 8.5 Hz)	5-C		7	149.4			
8	129.2	7.98 (1H, d, 8.5 Hz)	6, 8a, 9-C		8	123.9	8.10 (1H, br s)	6, 7, 8a, 9-C	C_7—CH_2OH
8a	136.6				8a	131.9			
9	180.6				9	182.1			
10	181.9				10	181.4			
C_2—OH		10.90*			C_2—OH		5.57		
C_6—OH		10.10*			C_3—OCH_3	56.1	3.98	2-C	C_4-H
C_3—CH_3	16.6	2.28	2, 3-C	C_4—OCH_3	C_7—CH_2OH	62.3	4.68	6, 7, 8-C	C_6,C_8-H
C_4—OCH_3	61.2	3.78	4-C	C_3—CH_3					

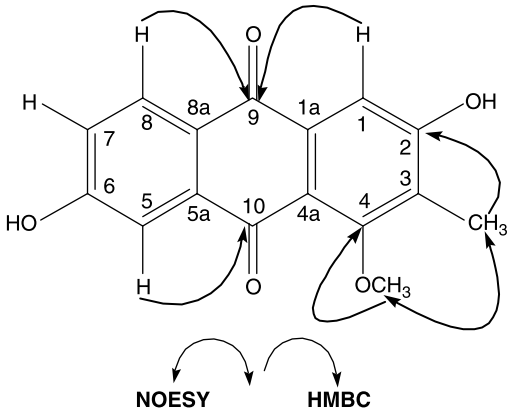

Figure 1. Key HMBC and NOESY correlations of compound **1**.

10.10, a methoxyl group at δ 3.78, a methyl group at δ 2.28, a proton singlet at δ 7.79 and three coupled proton signals at δ 7.98 (1H, d, $J = 8.5$ Hz, 8-H), 7.16 (1H, dd, $J = 8.5$, 2.4 Hz, 7-H), 7.42 (1H, d, $J = 2.4$ Hz, 5-H). The ^{13}C NMR spectrum (figure 1) revealed 16 carbon signals for two quinone carbonyls, 12 aromatic carbons, a methyl carbon and a methoxyl carbon. A comparison of the ^{13}C NMR spectral data of **1** with the compound 1,3,6-trihydroxy-2-methylanthraquinone [4] indicated that both compounds have similar structures and the difference was the presence of a methoxyl group in **1** (δ 3.78, 3H) instead of the hydroxyl group. The HMBC correlations between H-8 (δ 7.98) and C-9 (δ 180.6), H-1 (δ 7.79) and C-9 (δ 180.6) justified the location of one β-hydroxyl at C-6. The HMBC correlations between H-1 (δ 7.79) and C-3 (δ 131.8), -OCH_3 (δ 3.78) and C-4 (δ 146.9), as well as —CH_3 (δ 2.28) and C-2 (δ 154.9), C-4 (δ 146.9) indicated that the methyl group was located at C-3 and the methoxyl group at C-4. In addition, the NOESY spectrum showed a correlation between —OCH_3 (δ 3.78) and —CH_3 (δ 2.28) also supported that the methyl group was located at C-3 and the methoxyl group at C-4. And, another β-hydroxyl was located at C-2. Therefore, compound **1** was finally elucidated as 2, 6-dihydroxy-3-methyl-4-methoxyanthra-quinone.

Compound **2** was obtained as orange-coloured needles. Its molecular formula was determined as $C_{16}H_{12}O_5$ by EI-MS at *m/z* 284.0 $[M]^+$ and HRESI-MS at *m/z* 307.0579 $[M + Na]^+$. It showed a positive Borntrager's reaction and $FeCl_3$ reaction, suggesting the presence of an anthraquinone skeleton. The ^{1}H NMR spectrum of **2** (table 1, figure 2) showed the presence of a β-hydroxyl at δ 5.57, a methoxyl group at δ 3.98, a hydroxymethyl group at δ 4.68, two proton singlets at δ 7.60 and 7.54, three coupled proton signals at δ 8.12 (1H, d, $J = 8.6$ Hz, 5-H), 7.78 (1H, br d, $J = 8.6$ Hz, 6-H), 8.10 (1H, br s, 8-H). The ^{13}C NMR spectrum revealed 16 carbon signals for two quinone carbonyls, twelve aromatic carbons, a hydroxymethyl carbon and a methoxyl carbon. Comparison of **2** with a known compound 2-hydroxy-7-methyl-3-methoxy-anthraquinone [5] showed that both compounds have the same substituent pattern, and the difference was the presence of a hydroxymethyl group in compound **2**, instead of a methyl group. Furthermore, compound **2** is ascertained as 2-hydroxy-7-hydroxymethyl-3-methoxyanthraquinone or 2-hydroxy-6-hydroxylmethyl-3-methoxy-anthraquinone. The proposed structure of **2** and the assignment of NMR spectral data were corroborated by the HMBC and NOESY spectra. The HMBC correlations between H-8 (δ 8.10), H-1 (δ 7.54) with C-9 (δ 181.4), and —CH_2OH (δ 4.68) with C-6 (δ 133.1), C-7 (δ 149.4), C-8 (δ 123.9) justified the location of the hydroxymethyl group at C-7, and the

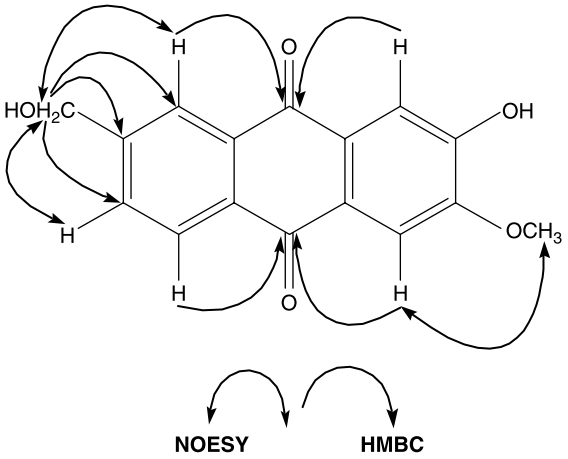

Figure 2. Key HMBC and NOESY correlations of compound **2**.

correlation between H-4 (δ 7.60) and C-10 (δ 181.4) indicated that the location of the methoxyl was at C-3. In addition, the cross-peak correlation between —OCH_3 (δ 3.98) and H-4 (δ 7.60) in the NOESY experiment was observed. These results confirmed the structure of compound **2** as 2-hydroxy-7-hydroxymethyl -3-methoxyanthraquinone.

3. Experimental

3.1 General experimental procedures

Melting points were determined on Yanaco MP-S3 melting point apparatus and are uncorrected. The UV spectrum was recorded on a Shimadzu UV-260 UV-Vis instrument. The IR spectrum was recorded on a Bruker IFS-55 instrument. NMR spectra were reported with a Bruker ARX-300 or Bruker ARX-600. EI-MS was performed on VG-5050E mass spectrometer. HRESI-MS was performed on QSTAR LCQ mass spectrometer. RP-HPLC was Shimadzu CTO-6A model with ultraviolet detector.

3.2 Plant material

The plant material of *Hedyotis diffusa* W. was collected in Nanchang city, Jiangxi Province, China, in March 2005, and identified by Professor Qishi Sun (Shenyang Pharmaceutical University). A voucher specimen (No. 20050920) is deposited in Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The air-dried herbs (5 kg) of *Hedyotis diffusa* W. were extracted with 70% ethanol (100 L $\times$ 3) for 2 h. Extracts were concentrated *in vacuo* to give a residue (0.8 kg), which was partitioned with petroleum ether, $CHCl_3$ and n-BuOH successively. The $CHCl_3$ extract (40 g) was subjected to column chromatographic separation (400 g), gradiently eluted with

petroleum ether/acetone (each 500 ml) to obtain fraction 14 (100:16). Fraction 14 (225 mg) was submitted to preparative thin layer chromatography using petroleum ether/EtOAc/acetone (4:1:1) to afford compound **1** (8 mg) and a mixture containing compound **2** (60 mg). The mixture was separated on RP-HPLC with an ODS column (150 × 4 mm, flow rate 1.0 ml/min) with MeCN/H_2O (90:10) to yield **2** (10 mg) (t_R = 8.5 min).

3.3.1 Compound 1. Orange-coloured needles (MeOH), mp 226–228°C; UV (CH_3OH) λ_{max} (log ε) 245 (0.13), 279 (0.50) nm; IR (KBr) ν_{max} 3395 (OH), 2925, 2853, 1667 (C═O), 1583, 1474, 1381 cm^{-1}. NMR spectral data: see table 1; HRESI-MS: *m/z* 307.0584 (calcd for $C_{16}H_{12}O_5Na$, 307.0582); EI-MS *m/z* 284 $[M]^+$(48), 266 $[M-H_2O]^+$(47), 238 $[M-H_2O\text{-}CO]^+$(100), 213 $[M-CH_3\text{-}2CO]^+$(24), 197 $[M-OCH_3\text{-}2CO]^+$(23).

3.3.2 Compound 2. Orange-coloured needles (MeOH), mp 223–225°C; UV (CH_3OH) λ_{max} (log ε) 246 (0.30), 284 (0.86) nm; IR (KBr) ν_{max} 3391 (OH), 2920, 2850, 1668 (C═O), 1590, 1517, 1451 cm^{-1}. NMR spectral data: see table 1; HRESI-MS: *m/z* 307.0579 (calcd for $C_{16}H_{12}O_5Na$, 307.0582); EI-MS *m/z* 284 $[M]^+$(100), 267 $[M-OH]^+$(3), 255 $[M-COH]^+$(43), 241 $[M-COCH_3]^+$(11), 225 $[M-CO\text{-}OCH_3]^+$(9), 213 $[M-CO\text{-}COCH_3]^+$(9).

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