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ANTHRAQUINONES FROM *HEDYOTIS PINIFOLIA*

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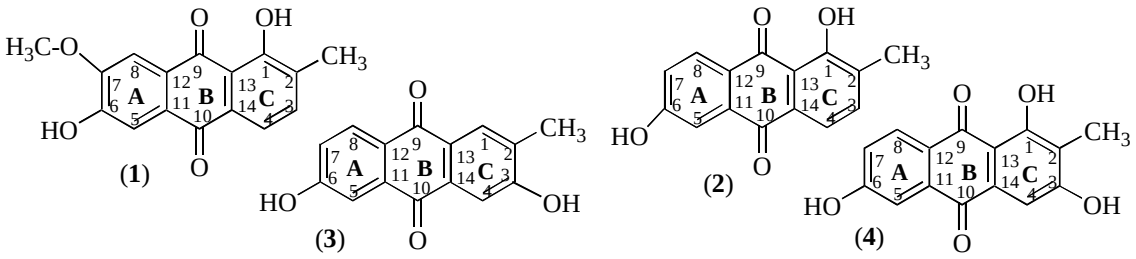
ABSTRACT

*Hedyotis pinifolia Wall ex G.Don (Vietnamsese name An điền lá thông), family of Rubiaceae, has not yet been chemically studied. From the aerial parts of H. pinifolia, four anthraquinones had been isolated: 1,6-dihydroxy-7-methoxy-2-methylanthraquinone **(1)**, 1,6-dihydroxy-2-methylanthraquinone **(2)**, 3,6-dihydroxy-2-methylanthraquinon **(3)** and 1,3,6-trihydroxy-2-methylanthraquinone **(4)**. Their chemical structures were established by spectroscopic analysis.*



Keywords: Rubiaceae, *Hedyotis pinifolia*, anthraquinone.

I - INTRODUCTION

Several species of *Hedyotis* genus (Rubiaceae) are used in traditional medicine in a number of Asian countries including Vietnam [1, 2]. There are approximately 180 species recorded of which 56 were identified in Vietnam [2, 3]. *Hedyotis pinifolia* Wall ex G. Don is a small herb (up to 25 cm tall) commonly found in sandy areas from Hue to the South of Vietnam[2]. Previous studies showed that the genus *Hedyotis* contained anthraquinones[1-6], but there have been no anthraquinone compounds isolated from *Hedyotis pinifolia*. From the aerial parts of *Hedyotis pinifolia*, we isolated four anthraquinones **(1-4)**. The structural elucidation of these compounds was reported.

II - EXPERIMENTAL

1. General procedures

Melting points were determined on a Maquene hot stage apparatus and were uncorrected. MS, ^{1}H-, ^{13}C-, DEPT- and 2D-NMR, H-H COSY were recorded in The Institute of Chemistry, Vietnamese Academy of Science and Technology, Cau Giay Dist., Hanoi. For analytical and preparative TLC, Merck TLC aluminium sheets silica gel 60 GF_{254} were utilized. For column chromatography (CC), silica gel Merck 60 GF_{254} and silica gel Merck 60 (0.040 - 0.063 mm) were used.

2. Plant material

The aerial parts of *H. pinifolia* were collected at the seaside of Long Hai commune,

Ba Ria — Vung Tau province, Vietnam in December 2005. A voucher specimen was prepared and deposited by Msc. Vo Thi Phi Giao, Faculty of Biology, University of Natural Sciences, Vietnam National University of Ho Chi Minh City.

3. Extraction and isolation

2.6 kg of ground, air-dried aerial parts of *H. pinifolia* were macerated in ethanol for 24 h and the extraction was repeated many times. After removal of the solvent under reduced pressure, 80.0 g of crude ethanol extract was obtained. This extract was then subjected to fast dry column chromatography to obtain different fractions: petroleum ether extracts A (6.97 g) and B (1.53 g), chloroform extract (18.09 g), ethyl acetate extracts A (5.73 g) and B (12.68 g) and methanol extracts A (29.46 g) and B (1.39 g), respectively. The chloroform extract was rechromatographed on CC, purified by TLC and recrystallized in acetone to yield compounds **1** and **2**. The same work for ethyl acetate extract A and a mixture of **3** and **4** with the ratio of (1:1) was isolated.

4. 1,6-Dihydroxy-7-methoxy-2-methylanthrquinone (1)

2 mg, orange needles (recrystallized in acetone). Melting point: 189-190^{0}C. ESI-MS *m/z*: 284.9 $[M+H]^+$ (calc. for $C_{16}H_{12}O_5$). ^{1}H-NMR (500 MHz, DMSO), δppm: 12.92 (1H, *s*, 1-OH), 7.65 (1H, *d*, *J*=7.5 Hz, H-3), 7.59 (1H, *d*, *J*=7.5 Hz, H-4), 7.58 (1H, *s*, H-5), 7.55 (1H, *s*, H-8), 3.98 (3H, *s*, 7-OCH$_3$), 2.29 (3H, *s*, 2-CH$_3$). ^{13}C-NMR (125 MHz, DMSO) see table 1. HMBC see figure 1.

5. 1,6-Dihydroxy-2-methylanthraquinone (2)

2.5 mg, orange needles (recrystallized in acetone). Melting point: 182-184^{0}C. ESI-MS *m/z*: 255.0 $[M+H]^+$ (calc. for $C_{15}H_{10}O_4$). ^{1}H-NMR (500 MHz, acetone-d_6), δppm: 13.19 (1H, *s*, 1-OH), 7.64 (1H, *d*, *J* = 7.5 Hz, H-3), 7.68 (1H, *d*, *J* = 7.5 Hz, H-4), 7.63 (1H, *d*, *J* = 2.5 Hz, H-5), 7.33 (1H, *dd*, *J* = 8.5, 2.5 Hz, H-7), 8.22 (1H, *d*, *J* = 8.5 Hz, H-8), 2.06 (3H, *s*, 2-CH$_3$). ^{13}C-NMR (125 MHz, acetone-d_6) see table 1. HMBC see figure 1.

6. Anthraquinones 3 and 4

12 mg, orange needles (recrystallized in acetone); ESI-MS *m/z* 254.9 $[M+H]^+$, 253.0 $[M-H]^-$ (calc. for $C_{15}H_{10}O_4$ for **3**); *m/z* 270.9 $[M+H]^+$, 269.0 $[M-H]^-$ (calc. for $C_{15}H_{10}O_5$ for **4**). ^{1}H-NMR (500 MHz, acetone-d_6) and ^{13}C-NMR (125 MHz, acetone-d_6) see Table 1. HMBC see figure 2. H-H COSY see figure 3.

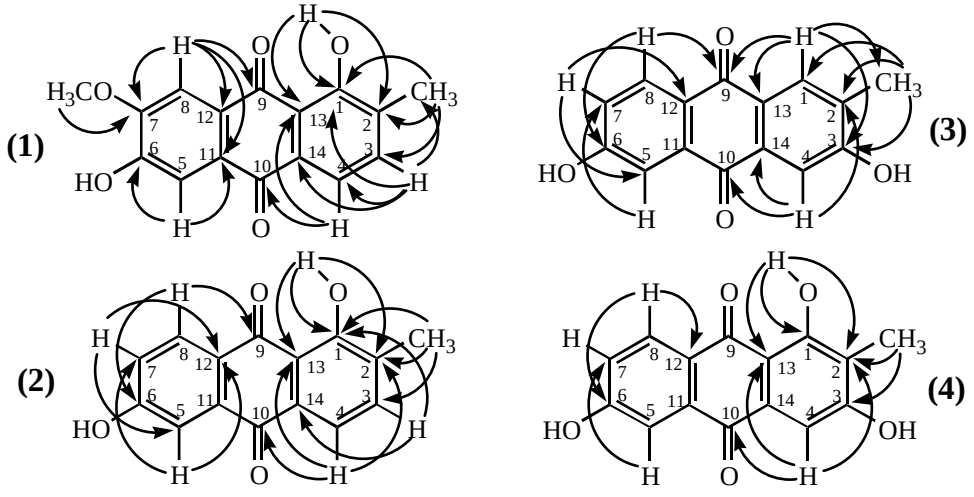


Figure 1: HMBC correlations of compounds **1 - 4**

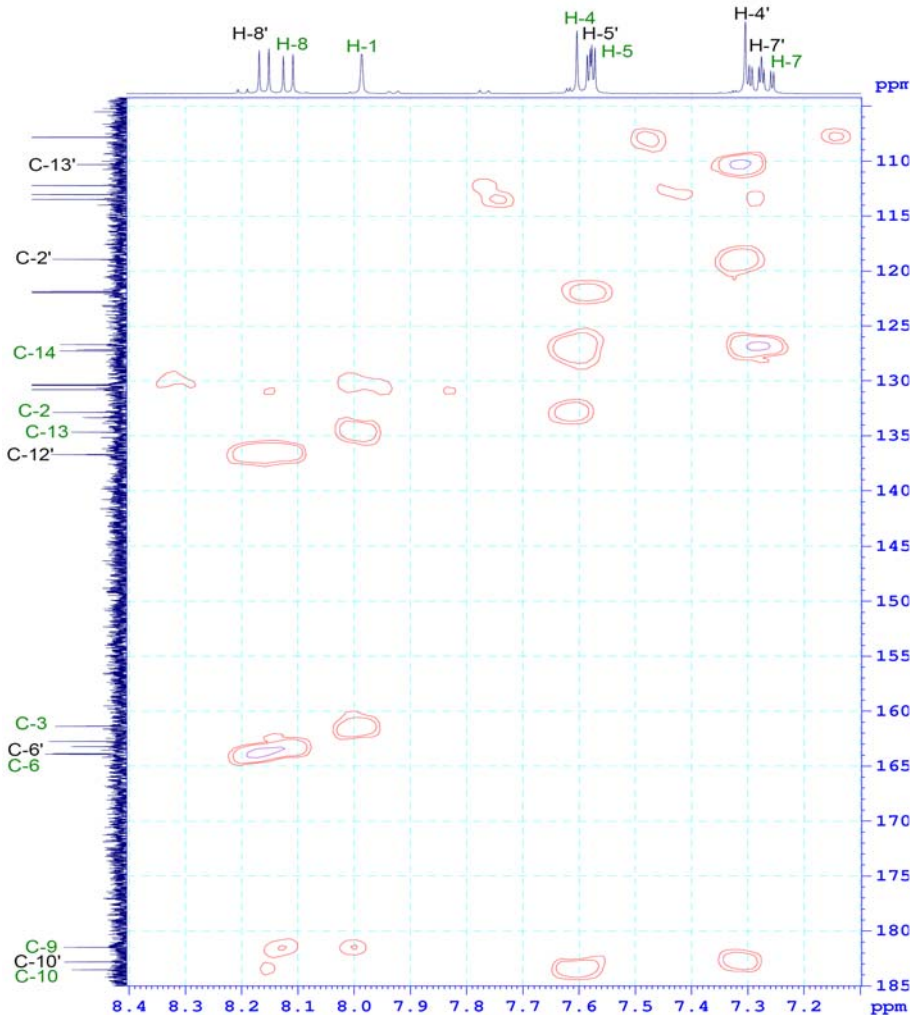
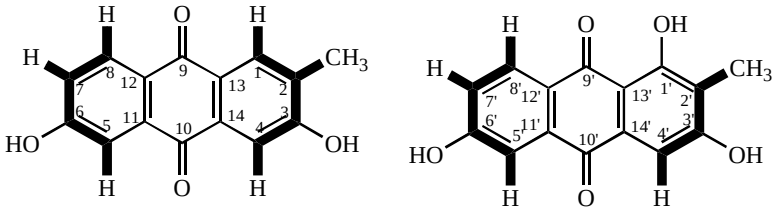


Figure 2: Part of two-dimensional HMBC spectrum of the mixture **3** and **4**



(3) **(4)**

Figure 3: H-H long-range COSY correlations of **3** and **4**

III - RESULTS AND DISCUSSION

From the chloroform extract, compounds **1** and **2** were isolated. Compound **1** exhibited a melting point of 189 - 190ºC. The MS spectrum showed a pseudomolecular ion peak at *m/z* 284.9 $[M+H]^+$, indicating the molecular formula to be $C_{16}H_{12}O_5$. The ^{1}H-NMR spectrum showed a characteristic downfield signal for the chelated hydroxyl group at δ 12.92 (1H, *s*). The ^{1}H-NMR

spectrum also revealed the presence of four aromatic protons, a methoxyl group at δ 3.98 (3H, *s*) and a methyl group at δ 2.29 (3H, *s*). The ^{13}C-NMR spectrum showed 16 carbon atoms, which includes two carbonyl downfield signals at δ 188.1 and δ 180.7, indicative of the presence of the chelated and nonchelated carbonyl. The exact location of the substituents was established based on HSQC and HMBC. There are four signals in the aromatic region and the pattern suggested an anthraquinone with two disubstituted rings. A set of *ortho* coupled doublets at δ 7.65 (1H, *J* = 7.5 Hz) and 7.59 (1H, *J* = 7.5 Hz) indicated that the two aromatic protons were next to each other. These two aromatic protons combining with one hydroxyl and one methyl group were located on ring C. In ring A, there were two proton singlets at δ 7.58 and 7.55 and also one methoxyl and one hydroxyl group. The data caused us the hesitation in the assignment of the structure of the product: whether it was 1,6-dihydroxy-7-methoxy-2-methylanthraquinone or 1,7-dihydroxy-6-methoxy-2-methylanthraquinone. Later, when we isolated other anthraquinones in this herb and observed that the quinones of this herb always possessed substituents of 2-methyl and 6-hydroxyl, so we proposed **1** was 1,6-dihydroxy-7-methoxy-2-methylanthraquinone.

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) chemical shifts of **1-4**

Position	**1**[a]	**2**[b]	**3**[b]		**4**[b]	
	δ_C		δ_H (*J*, Hz)	δ_C[c]	δ_H (*J*, Hz)	δ_C[c]
1	159.8	161.7	7.99 (*s*)	130.8 (d)	-	163.9 (s)
2	133.6	135.4	-	132.9 (s)	-	118.9 (s)
3	136.9	137.4	-	161.4 (s)	-	163.3 (s)
4	118.5	119.5	7.60 (*s*)	112.2 (d)	7.30 (*s*)	107.9 (d)
5	109.0	113.6	7.57 *d* (2.5)	113.1 (d)	7.58 (*d*, 8.0)	113.5 (d)
6	153.0	164.5	-	163.3 (s)	-	163.9 (s)
7	153.4	122.1	7.27 (*dd*, 8.5 , 2.5)	121.9 (d)	7.29 (*dd*, 8.0 , 2.5)	122.0 (d)
8	112.1	130.7	8.12 (*d*, 8.5)	130.4 (d)	8.16 (*d*, 8.0)	130.4 (d)
9	188.1	189.2	-	181.5 (s)	-	187.3 (s)
10	180.7	182.7	-	183.5 (s)	-	182.8 (s)
11	126.8	127.8	-	127.2 (s)[d]	-	127.2 (s)[d]
12	127.5	126.5	-	126.7 (s)	-	136.7 (s)[d]
13	114.8	115.9	-	134.7 (s)[d]	-	110.3 (s)
14	131.2	132.6	-	127.3 (s)[d]	-	130.4 (s)[d]
1-OH	-	-	-	-	13.37 (*s*)	-
2-CH_3	15.6	16.0	2.36 (*s*)	16.5 (q)	2.16 (*s*)	8.3 (q)
7-OCH_3	56.1	-	-	-	-	-

[a]Spectrum run in DMSO; [b]Spectrum run in acetone-d_6; [c]Multiplicities were determined by DEPT experiment; [d]Determined based on ChemNMR C-13 estimation

Compound **2** exhibited a melting point of 182 - 184ºC. The MS spectrum showed a pseudomolecular ion peak at *m/z* 255.0 $[M+H]^+$, 30 amu less than that of **1**, corresponding to molecular formula of $C_{15}H_{10}O_4$. The ^{1}H-NMR spectra showed signals of one *peri*-hydroxyl at δ 13.19 and one methyl group at δ 2.06. A disubstituted ring C was indicated by the two coupled aromatic protons at δ 7.64 (*d*, *J* = 7.5 Hz, H-3) and δ 7.59 (*d*, *J* = 7.5 Hz, H-4). And a monosubstituted ring A was indicated by the three coupled protons at δ 7.63 (*d*, *J* = 2.5 Hz, H-5), δ 7.33 (*dd*, *J* = 8.5 and 2.5 Hz, H-7) and δ 8.22 (*d*, *J* = 8.5 Hz, H-8). The ^{13}C-NMR

spectrum showed 15 carbon atoms, like **1**, which included two carbonyl groups at δ 189.2 and 182.7, presented of the chelated and nonchelated carbonyl, respectively. The HSQC and HMBC correlation NMR spectra (Figure 1) strongly supported the structure of **2** was 1,6-dihydroxy-2-methylanthraquinone.

The ethyl acetate extract was subjected to a series of chromatographic procedures, leading to the isolation of 12 mg of fine orange needles. Initial inspection of the ^{1}H and ^{13}C-NMR spectra of this product indicated the doubling signals for most protons and carbons. These facts suggested that perhaps the product was a dimeric anthraquinone of more than 29 unique carbon nuclei. LC-MS spectrum of the product revealed two equally intensitive peaks at the time of 11.6 and 12.9 minutes. The mass spectrum positive mode showed that these peaks corresponded to the molecular ion of *m/z* 254.9 $[M+H]^+$ and 270.9 $[M+H]^+$, in agreement with the molecular formula of $C_{15}H_{10}O_4$ and $C_{15}H_{10}O_5$, respectively. This result suggested that the product was a mixture of two structurally related compounds with the ratio of (1:1). Extensive efforts to separate these two compounds by silica gel CC, preparative TLC and to check by C-18 TLC with variety of solvent systems proved unsuccessful. Therefore, the structure elucidation and full ^{1}H and ^{13}C-NMR assignments of compounds **3** and **4** (Table 1) were performed in the inseparative mixture. Compounds **3** and **4** were determined exactly based on the 2D-NMR spectra: HSQC, HMBC and H-H long-range COSY correlations.

Compound **3** had molecular formula of $C_{15}H_{10}O_4$ with the presence of five aromatic protons, one methyl and two hydroxyl groups. In ring C, two proton singlets presented at δ 7.99 (H-1) and 7.60 (H-4). And a monosubstituted ring A was indicated by the three coupled protons at δ 7.57 (*d*, *J* = 2.5 Hz, H-5), 7.27 (*dd*, *J* = 8.5 and 2.5 Hz, H-7) and 8.12 (*d*, *J* = 8.5 Hz, H-8). There were 15 carbon atoms in ^{13}C-NMR spectrum, including two carbonyl groups at δ 183.5 and 181.5, and one methyl group at δ 16.5. The HSQC, HMBC and H-H long-range COSY confirmed the location of methyl group at C-2 and two hydroxyl groups at C-3 and C-6 based on two- and three-bond correlations (Figure 1 and 2). These evident spectra supported the structure of **3** to be 3,6-dihydroxy-2-methylanthraquinone.

Compound **4** had molecular formula of $C_{15}H_{10}O_5$, 16 amu more than the one of **3**, with the presence of four aromatic protons, one methyl group and two hydroxyl groups. In ring C, difference from ring C of **3**, only one proton singlet presented at δ 7.30 (H-4) and one *peri*-hydroxyl group at δ 13.37 (C1-OH). Compound **4** also had a monosubstituted ring A similar to **3,** with three coupled protons at δ 7.58 (*d*, *J* = 2.5 Hz, H-5), 7.29 (*dd*, *J* = 8.0; 2.5 Hz, H-7) and 8.16 (*d*, *J* = 8.0 Hz, H-8). The ^{13}C-NMR spectrum showed 15 carbon atoms which include two downfield carbonyl signals at δ 187.3 and 182.8, indicative of the presence of the chelated and nonchelated carbonyl groups, respectively. The HSQC, HMBC and H-H long-range COSY (Figure 2 and 3) confirmed that the structure of **4** was 1, 3, 6-trihydroxy-2-methylanthraquinone.

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