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ANTHRAQUINONES FROM THE ROOTS OF *PAEDERIA SCANDENS*

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ABSTRACT

*Chemical investigation on the ethyl acetate extract of the roots of Vietnamese Paederia scandens resulted in the isolation of one new anthraquinone, 1,3-Dihydroxy-2,4-dimethoxy-9,10-anthraquinone (**1**), together with three known anthraquinones, 2-Hydroxy-1,4-dimethoxy-9,10-anthraquinone (**2**), 1-Methoxy-2-methoxymethyl-3-hydroxy-9,10-anthraquinone (**3**), 1-Hydroxy-2-hydroxymethyl-9,10-anthraquinone (**4**). Their structures were elucidated by the combination of 2D NMR, IR, UV and high resolution EI-MS spectroscopy. These anthraquinones (**1 - 4**) showed strong antimicrobial activity.*

Keywords: Anthraquinone, *Paederia scandens*, Rubiaceae, Antimicrobial.

I - INTRODUCTION

Many species of the Rubiaceae family are known to produce substantial amounts of anthraquinones both free and as glucosides [1]. Anthraquinones were also found from tissue culture of Rubiaceae species. Inoue and co-workers [2] isolated from cell suspension cultures of *Morinda citrifolia* 12 anthraquinones, both aglycones and glucosides. *Paederia scandens* (Lour.) Merrill belonging to Rubiaceae family was reported to elaborate abundant iridoid glucosides, paederoside, paederosidic acid, asperuloside, and scandoside [3]. Some of which showed the inhibitory effect on Epstein-Barr virus activation [4]. So far, no anthraquinone has been isolated from this plant. In the course of our investigation of the biologically active substances from Vietnamese medicinal plants, we studied the chemical constituents of the roots of *P. scandens* and isolated three new dimeric iridoid glucosides and two new iridoid glucosides from its MeOH extract [5]. In continuation, the EtOAc extract of the roots of this plant was investigated and four anthraquinones, which showed strong antimicrobial activity, were isolated and characterized. The outcome of these efforts will be the subject of this paper.

II - EXPERIMENTAL

1. General procedure

NMR spectra were recorded on Varian Unity 600 (600 MHz), using $CDCl_3$ as solvent. Mass spectra including high-resolution mass spectra were recorded on a JOEL JMS AX-500 spectrometer. IR spectra were measured on JASCO FT/IR-5300 spectrophotometer. The UV spectra were obtained on a Shimadzu UV-1650PC in MeOH solution. HPLC was performed on Shimadzu liquid chromatography LC-10AS with RID/6A and SPD-10A detectors using a Waters 5 SL-II column (10x250 mm). TLC was performed on silica gel plates (Kiesegel 60 F254, Merck).

2. Plant materials

Fresh roots of *Paederia scandens* (Lour.) Merrill were collected in Hanoi, Vietnam in July 2000 by DNQ and then identified by Dr. Tran Ngoc Ninh (Institute for Ecology and Natural Resources, Hanoi, Vietnam). The voucher specimen (VN 02001) has been deposited in Faculty of Chemistry, Hanoi University of Education, Hanoi, Vietnam.

3. Extraction and isolation

The dried roots of *P. scandens* (3.2 kg) was extracted with methanol. The MeOH extract was concentrated to give a residue (118.6 g), which was partitioned between EtOAc and water. The EtOAc was evaporated to give (5.97 g), a stick residue, which was further subjected to silica gel column chromatography, using EtOAc/$CHCl_3$ gradient from 10% to 100% EtOAc as a solvent system to give nine fractions. Fraction 3 (1.66 g) was further purified by silica gel column, hexane/EtOAc gradient from 5% to 30% EtOAc afforded eight subfractions. Subfraction 3 (66.9 mg) was separated by Sephadex LH 20 column, $CHCl_3$/MeOH (1:1) following by preparative HPLC, hexane/EtOAc (1:1), flow rate 1 ml/min and preparative TLC, toluene:methanol (10:1) to obtain four anthraquinones **1** (2.3 mg), **2** (4.2 mg), **3** (9.3 mg), and **4** (5.3 mg).

1,3-Dihydroxy-2,4-dimethoxy-9,10-anthraquinone (1). UV (MeOH): λ_{max} (log ε) 415 (3.6), 279 (4.4), 249 (4.3) nm; FT-IR ν_{max}: 3309, 1669, 1627, 1591, 1578, 1453, 1404, 1360, 1272, 1123, 1019, 972 cm^{-1}; EI-MS: 300 (100), 284 (66), 269 (54), 253 (23), 229 (25), 158 (27), 114 (15), 102 (19), 76 (12), 75 (10); HR-EIMS: *m/z* 300.0632 ($C_{16}H_{12}O_6$, requires *m/z* 300.0634). ^1H and ^{13}C NMR (see table 1).

4. Antimicrobial assay

Antimicrobial activity was assayed in analogy to a conventional plate diffusion assay as described in reference [14].

III - RESULTS AND DISCUSSION

The crude methanol extract of *P. scandens* was partitioned between EtOAc and water. The EtOAc layer was concentrated and purified by using a combination of silica gel column chromatography, preparative HPLC, and preparative thin layer chromatography to afford a new anthraquinone (**1**), along with three known compounds 2-hydroxy-1,4-dimethoxy-9,10-anthraquinone (**2**) [6], 1-Methoxy-2-methoxymethyl-3-hydroxy-9,10-anthraquinone (**3**) [7], 1-Hydroxy-2-hydroxymethyl-9,10-anthraquinone (**4**) [8].

Electron impact mass spectrum (EI□MS) of **1** showed the molecular ion peak at *m/z* 300 and the high-resolution EI□MS indicated the molecular formula of $C_{16}H_{12}O_6$, deduced also from ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) analyses. Its UV spectrum of **1** exhibited the absorption maxima at 415, 279 and 249 nm, suggested an anthraquinone basic structure [9], with a single *peri*-hydroxyl group. It was further supported by signal at 13.82 ppm in its ^1H NMR spectrum (table 1) and also IR absorption band at 3309 cm^{-1}. Detail analysis of its ^1H NMR spectrum revealed the presence of four aromatic protons and two methoxyl groups. Two carbonyl groups of an anthraquinone skeleton were also found in its ^{13}C NMR spectrum at 181.3 and 187.8 ppm for the none-chelated and chelated ones, respectively. The HMBC spectrum located two methoxyl groups at C-2 and C-4 by the HMBC correlations between them and C-2 and C-4. In addition, 1-OH coupled to C-1, C-2 in the HMBC spectrum. The spectral data of compound **1** was similar with that of the anthraquinone reported previously [10], only difference from the presence of a hydroxyl group at C-6. From above discussion, **1** was determined to be 1,3-Dihydroxy-2,4-dimethoxy-9,10-anthraquinone as shown in figure 1.

Previous publications showed that anthraquinones exhibited many interesting biological activities, such as inhibition of Epstein-Barr virus [11], antiviral [12] and cytotoxic acitivity [13]. In this paper, antimicrobial activities of **1**-**4** were tested against four bacteria and two fungi, their results are described in table 2. Compound **1** inhibited

strongly *S. enteritidis* and *E. coli* than standards, meanwhile **4** affected not only bacteria but also fungi and especially the strongest one was against *S. aureus*.

This is the first report on the isolation and structural elucidation of antimicrobial anthraquinones from *P. scandens*.

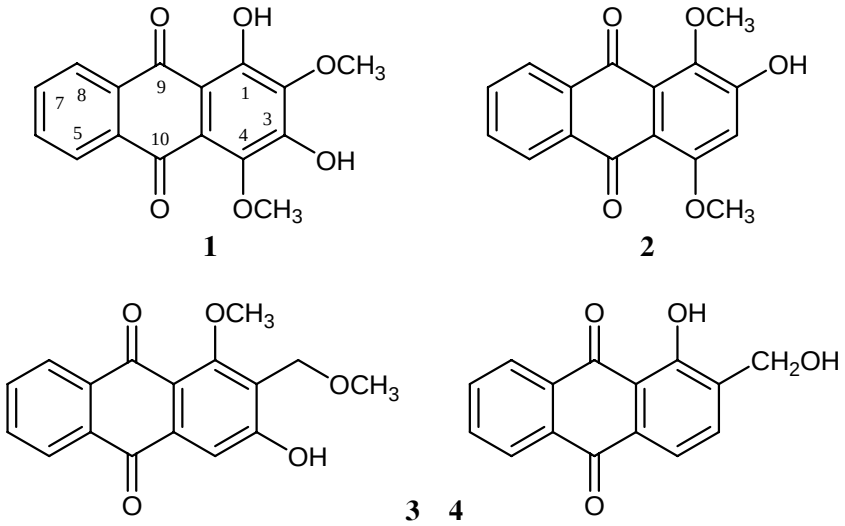

Figure 1: Structures of **1 - 4**

Table 1: ^{1}H and ^{13}C NMR spectra of **1** ($CDCl_3$)

Position	H	C
1		155.1 (s)
2		143.4 (s)
3		150.1 (s)
4		139.7 (s)
5	8.26 m	127.2 (d)
6	7.77 m	134.4 (d)
7	7.77 m	133.6 (d)
8	8.28 m	126.4 (d)
9		187.8 (s)
10		181.3 (s)
1-OH	13.82 (s)	
2-OMe	4.01 (s)	61.2 (q)
4-OMe	4.15 (s)	61.8 (q)

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Table 2: Antimicrobial activity of the compounds **1** - **4** (diameter of the zone of growth inhibition, bactericidal or fungicidal zone in mm, including the diameter of disc, 6 mm)

Microorganism/Sample	**1**	**2**	**3**	**4**	**A1**	**A2**
Escherichia coli	19	16	7	15	16	nt
Klebsiella pneumoniae	-	-	7	15	14	nt
Pseudomonas aeruginosa	12	9	-	16	16	nt
Staphylococcus aureus	10	13	12	19	15	nt
Salmonella enteritidis	19	-	14	18	18	nt
Aspergillus niger	8	14	16	18	nt	18
Candida albicans	11	16	16	16	nt	17

nt: not tested; -: no activity; A1, Gentamicin, standard for bacteria; A2, Nystatin, standard for fungi.

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