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# ANTHRAQUINONES FROM *Morinda officinalis* HOW. (RUBIACEAE) ROOTS, COLLECTED IN QUANG NINH PROVINCE, VIETNAM

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**Abstract**. Investigation on the chemical constituents of methanol extract from roots of *Morinda officinalis* How. (Rubiaceae) has resulted in the isolation of three anthraquinones: anthragallol 2-methyl ether (**1**), physcion (**2**), and emodin (**3**). Their structures were determined by spectroscopic methods. All three compounds were isolated from Vietnamese *Morinda officinalis* for the first time.

***Keywords***: *Morinda officinalis*, anthragallol 2-methyl ether, physcion, emodin.

## 1. Introduction

*Morinda officinalis* How. (Rubiaceae), named as “ba kich tim”, is a small vine, widely grows in tropical and subtropical regions, including Vietnam, Laos, Myanmar, China. According to Vietnam traditional medicine, the roots of this plant have been used to help strengthen the bones, kidney, enhance the immune system function, treat impotence, menstrual disorders [1-3]. A variety of biological activities of *Morinda officinalis* How. roots have been reported, including anti-depressant [4], anti-oxidation [5], anti-osteoporosis [6]. Phytochemical investigations have demonstrated the presence of polysaccharides and oligosaccharides [4, 5], anthraquinones [6], iridoid glucosides [7], triterpenoids [8] in *Morinda officinalis* How. roots. This paper describes the isolation and structural elucidation of three anthraquinones from the roots of *Morinda officinalis* How., collected in Quang Ninh province.

## 2. Content

### 2.1. Materials and methods

***Plant material***

Roots of *Morinda officinalis* How. were collected in Phieng Chieng village, Dong Tam commune, Binh Lieu distr., Quang Ninh province (July 2018) and identified by

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Dr. Do Huu Thu (Institute of Ecology, Natural Resource, and Biology, VAST, Vietnam). Voucher specimens are deposited at the faculty of Chemistry, Hanoi University of Education (MO201807).

***General procedure***

Thin-layer chromatography (TLC) was carried out on precoated Si gel $GF_{254}$ (Merck Co., Germany) and TLC spots were viewed at 254, 302 and 366 nm and visualized by spraying with vanillin-10% $H_2SO_4$ solution. Column chromatography was carried out on silica gel 60 (60-100 μM, Merck), preparative HPLC was performed on a JASCO PU-2087 instrument with a UV-2070 and RI-2031 detectors using a Waters 5 SL-II column (10.0 mm x 250 mm), the flow rate of 1.0 mL/min. NMR ($^1H$, $^{13}C$ NMR, DEPT, HSQC, and HMBC) spectra were recorded on a Bruker Avance 500MHz instrument. The chemical shift ($\delta$) values are given in ppm with TMS as internal standard, coupling constant $J$ - by Hz. Mass spectra, including high-resolution MS were recorded on an HP 5989B mass spectrometer and FT-ICR-MS (Varian 910-MS TQFTMS-7 Tesla).

***Extraction and Isolation***

Dried powder of *M. officinalis* roots (3,000 g) was extracted with methanol. The methanolic extract was concentrated to give a residue (250 g) which was further partitioned into *n*-hexane, EtOAc, BuOH, and water. The ethyl acetate crude extract (13.7 g) was subjected to column chromatography over silica gel and eluted gradient with *n*-hexane – ethyl acetate from 4:1 to 1:1, ethyl acetate - methanol from 10:1 to 0: 10. Eight fractions were successively obtained. Fraction 3 (37 mg) was chromatographed on Sephadex LH-20 column, using $CHCl_3$-MeOH (1-1) as a solvent system, followed by prep. HPLC, using *n*-hexane-EtOAc (4-1) to yield compounds **1** (10 mg), **2** (4 mg) and **3** (4 mg).

*Compound 1:* light yellow powder; ESI-MS: positive *m/z* $[M+H]^+$ calcd. for $C_{15}H_{11}O_5$: 271.0 found 270.8, $[M-H_2O+H]^+$ calcd. for $C_{15}H_9O_4$ 253.0 found 252.8; $^1H$ NMR (500 MHz, $CDCl_3$) and $^{13}C$ NMR (125 MHz, $CDCl_3$): Table 1.

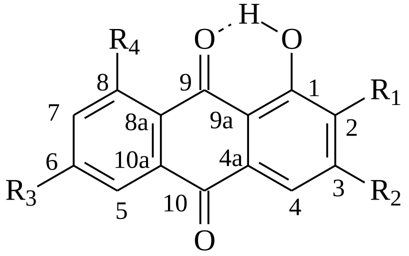
*Compound 2:* yellow powder; negative *m/z* $[M-OH]^-$ calcld. for $C_{16}H_{11}O_4$: 267.0 found: 266.8. $^1H$ NMR (500 MHz, $CDCl_3$) and $^{13}C$ NMR (125 MHz, $CDCl_3$): Table 1.

*Compound 3:* yellow powder; ESI-MS: positive *m/z* $[M+H]^+$ calcd. for $C_{15}H_{11}O_5$: 271.0, found 271.0, negative $[M-H]^-$ calcd. for $C_{15}H_9O_5$: 269.0, found 268.8. $^1H$ NMR (500 MHz, DMSO-$d_6$) and $^{13}C$ NMR (125 MHz, DMSO-$d_6$): Table 1.

## 2.2. Results and discussion

Compound **1** was obtained from ethyl acetate extract of *M.officinalis* roots. Its molecular formula was identified as $C_{15}H_{10}O_5$ based on its *pseudo* molecular ion peak from ESI-MS, $^1H$- and $^{13}C$ NMR data. Its $^1H$ NMR spectrum (Table 1) has signals of five aromatic protons [including one singlet proton at 7.46 (1H, s), two pair of *nearly equivalent* protons at 7.78 m and 8.27 m]; one acidic proton at 13.13 (1H, s) from HO group, that participates in forming hydrogen bond with neighboring >C=O group; and three singlet protons of methoxy group at 4.15 (3H, s), suggesting that **1** should be an anthraquinone with three substituents in ring C. $^{13}C$ NMR (Table 1) and HSQC spectra show the presence of 15 carbons, including two carbonyl carbons at 181.7 and 187.7;

twelve aromatic carbons, from 108.0 to 155.8 [three of them are bonded with oxygens at 138.7, 155.0, 155.8]; one methoxy carbon at 61.1 ppm. In the HMBC spectrum there are crosslink peaks between acidic HO and >C=O group, between methoxy protons and C-2. Thus, compound **1** has very similar spectral data with those of anthragallol 2-methyl ether [9,10]. Therefore, compound **1** was determined as anthragallol 2-methyl ether and was isolated for the first time from Vietnamese *Morinda officinalis* How.



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **1** | $OCH_3$ | OH | H | H |
| **2** | H | $OCH_3$ | $CH_3$ | OH |
| **3** | H | OH | $CH_3$ | OH |

***Figure 1. Structures of compounds 1, 2 and 3***

The molecular formula of compound **2** was found to be $C_{16}H_{12}O_5$ by ESI-MS, $^1$H NMR and $^{13}$C NMR data. Analysis of its $^1$H NMR spectra (Table 1) revealed that it has four aromatic protons [two pair of *ortho*-protons, at 6.68 (d, *J*=2.5 Hz)/7.36 (d, *J*=2.5 Hz) and 7.07 (d, *J*=1.0 Hz)/7.62 (d, *J*=1.0 Hz)]; three methoxy protons at 3.93 (3H, s); three methyl protons at 2.44 (3H, s); two acidic protons from two HO groups, those form hydrogen bonds with neighboring >C=O group, at 12.08 and 12.29 based on its $^{13}$C NMR spectrum. It means that compound **2** should be an anthraquinone with two HO groups, neighboring to a carbonyl group. The $^{13}$C NMR spectrum of **2** (Table 1) has resonances of 16 carbons, those are similar to compound **1**, except for one more methyl carbon at 22.15. Compound **2** has very similar spectral data with those of physcion [11]. Therefore, compound **2** was determined as physcion and in the first time isolated from Vietnamese *Morinda officinalis* How.

***Table 1. $^1$H NMR and $^{13}$C NMR data of compounds 1, 2, and 3***

| **No.** | **$^1$H NMR** (500 MHz, $\sigma$ ppm, *J* Hz) | | | **$^{13}$C NMR** (125 MHz, $\sigma$ ppm) | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **1** | **2** | **3** |
| 1 | - | - | - | 155.8 | 165.2 | 165.4 |
| 2 | - | 6.67 d 2.5 | 6.59 d 2.5 | 138.7 | 106.8 | 107.9 |
| 3 | - | - | - | 155.0 | 165.6 | 165.5 |
| 4 | 7.46 s | 7.34 d 2.5 | 7.12 d 2.5 | 108.0 | 108.2 | 108.8 |
| 4a | - | - | - | 129.7 | 135.3 | 135.1 |
| 5 | 8.27 m | 7.60 d 1.0 | 7.49 d 1.5 | 127.7 | 121.3 | 120.4 |
| 6 | 7.78 m | - | - | 134.3 | 148.4 | 148.2 |
| 7 | 7.78 m | 7.06 d 1.0 | 7.16 d 0.5 | 134.0 | 124.5 | 124.1 |
| 8 | 8.27 m | - | - | 126.7 | 162.5 | 161.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8a | - | - | - | 133.6 | 113.7 | 113.4 |
| 9 | - | - | - | 187.7 | 190.8 | 189.7 |
| 9a | - | - | - | 111.8 | 110.3 | 109.0 |
| 10 | - | - | - | 181.7 | 182.0 | 181.4 |
| 10a | - | - | - | 134.1 | 133.3 | 132.8 |
| 2-$OCH_3$ | 4.15 s | - | - | 61.1 | - | - |
| 3-$OCH_3$ | - | 3.93 s | - | - | 56.1 | - |
| 6-$CH_3$ | - | 2.44 s | 2.41 s | - | 22.2 | 21.5 |
| 1-OH | 13.13 s | 12.29 s | 12.08 s | - | - | - |
| 3-OH | 6.52 s | - | 11.40 brd s | - | - | - |
| 8-OH | - | 12.08 s | 12.01 s | - | - | - |

***Note.*** *The multiplicity "m" in $^1$H NMR spectrum of H-5 to H-8 from compound* ***1*** *instead of doublet or doublet of doublet should be explained by overlapping signals of nearly equivalent two pairs H-5/H-8, H6/H7.*

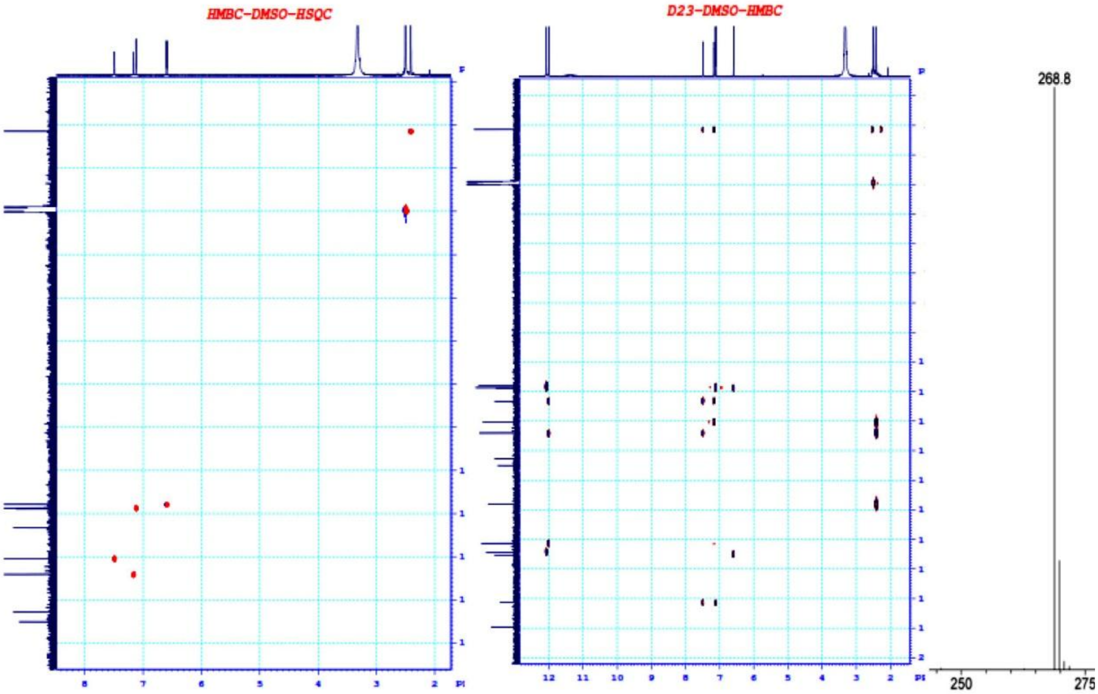


*Figure 2. HSQC, HMBC and MS spectra of compound 3*

From the ESI-MS negative data of **3** was afforded *m/z* 268.8 $[M-H]^-$, founded molecular mass is 270.0, corresponding to a molecular formula of $C_{15}H_{10}O_5$. The $^1H$ NMR spectrum of **3** looks like of **2**, with four aromatic protons [two pair of *ortho*-protons, at 6.59 (d, *J*=2.5 Hz)/7.12 (d, *J*=2.5 Hz) and 7.16 (d, *J*=1.5 Hz)/7.12 (d, *J*=1.2 Hz)]; three methyl protons at 2.41 (3H, s); two acidic protons from two HO groups, those form hydrogen bonds with neighboring >C=O group, at 12.01 and 12.08 based on its $^{13}C$ NMR spectrum. The $^{13}C$ NMR spectrum revealed that compound **3** has 15 carbons, like compound **2** (Table 1) except one less methoxy group at C-3. Compound **3** has very similar spectral data with those of emodin [11]. Therefore, compound **3** was determined as emodin and for the first time isolated from Vietnamese *Morinda officinalis* How.

## 3. Conclusions

Chemical composition of *methanol* extract of the roots of *Morinda officinalis* collected in Quang Ninh province has been investigated. Three anthraquinones, anthragallol 2-methyl ether (**1**), physcion (**2**), and emodin (**3**) were isolated for the first time from Vietnamese *Morinda officinalis* and structurally elucidated by MS, 1D, and 2D NMR spectroscopies.

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