Anthraquinone derivatives from the roots of *Morinda elliptica*

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**ABSTRACT**

This work was aimed to investigate the chemical constituents from the methanol extract of *Morinda elliptica* roots. Three anthraquinones (1-3) were isolated from this extract by preparative HPLC using gradient solvent manner of H₂O and MeOH. The structures of all isolated compounds were elucidated by the analyses of 1D and 2D NMR spectroscopic data.

![Chemical structures](attachment:image.png)

**Keywords:** Rubiaceae, *Morinda elliptica*, anthraquinones, spectroscopic data, HPLC
1. INTRODUCTION

The genus *Morinda* belongs to the family Rubiaceae. It consists of about thirteen species in Thailand [1]. Phytochemical investigation of this genus has been reported to obtain several compounds. The structures were identified as anthraquinones [1-5], iridoids [1, 6-11], flavonoids [8-10, 12], coumarins [12] and benzophenones [5].

*Morinda elliptica* Ridl. is a shrub or small tree up to 25 meters in high. The young is greenish and smooth bark while shallow fissure in older. Leaves are elliptic shape with 4-6.5 x 10-19.5 cm. It is widely distributed throughout South-east Asia [6]. This plant have been used as a traditional medicine for loss of appetite, headaches, diarrhea, fever, hemorrhoids, antipyretic and anti-inflammatory agents [2, 6]. In this work we have reported on the isolation and structure determination of the anthraquinone derivatives obtained from the methanol extract of the roots of *M. elliptica*. The preparative HPLC separation resulted in the isolation of three anthraquinones. The spectroscopic data of these compounds associated with comparison to the previous reports were elucidated as damnacanthol (1), rubadin-1-methyl ether (2) and damnacanal (3).

![Chemical structures of compounds](image)

2. MATERIALS AND METHODS

General procedures

Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. UV spectra were recorded by a UV-1700 PharmaSpec spectrophotometer (SHIMADZU). The IR spectra were measured with a Cary 630 ATR FT-IR spectrophotometer (Agilent Technologies). The 1H and 13C NMR spectra were recorded by a FT-NMR Bruker Avance 300 MHz spectrometer using TMS as the internal standard. The Agilent 1100 HPLC system was used for separation purposes with Lichrospher®100 RP-18 endcapped column (Ø 25 x 250 mm, particle size 5 µm, Merck). Column chromatography (CC) was performed on silica gel 100 (70–230 Mesh ASTM, Merck). For thin-layer chromatography (TLC), aluminium sheets of silica gel 60 F 254 (20 × 20 cm, layer thickness 0.2 mm, Merck) was used for analytical purposes. The compounds were visualized under ultraviolet light. All solvents for extraction and chromatography were distilled at their boiling ranges prior to use except for HPLC separation (MeOH) was analytical grade (Merck).

Plant material

The roots of *M. elliptica* were obtained from Pa Phayom District, Phatthalung Province in southern part of Thailand during October 2013. The identification was made by Associate Prof. Dr. Kitichate Sridith and the voucher specimen has been deposited at the Prince of Songkla University Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

Extraction and isolation

The air-dried roots of *M. elliptica* (336.8 g) were chopped and successively macerated with MeOH at room temperature for twice (each for 4 days) to give a dark brown gum (4.2 g). The extract (2.0 g) was subjected to HPLC separation using gradient solvent of MeOH-H2O. The elution was conducted at a flow rate of 3.0 mL/min under linear gradient conditions of 20% MeOH up to 100% MeOH in 180 min, then 100% MeOH for a further 60 min to afford 80 fractions (3 min each). Fractions 49 (17.6 mg), 58 (19.9 mg) and 62 (13.6 mg) were washed with CH2Cl2 to yield brownish powder of compound 1 (9.5 mg, 0.0059% dry wt), yellowish powder of compound 2 (8.3 mg, 0.0052% dry wt) and compound 3 as yellow powder (4.3 mg, 0.0027% dry wt).

Structure identification

**Compound 1**: brownish powder, mp. 290-291°C; UV (MeOH) λmax nm (log ε) 230 (4.45), 250 (4.10), 273 (4.50), 368 (3.52); FT-IR (ATR) νmax (cm⁻¹) 3282 (OH stretching), 1672 (C=O stretching); 1H NMR (300 MHz, DMSO-d6) δ 8.18 (dd, J = 8.0 and 1.2 Hz, H-8), 8.14 (dd, J = 8.0 and 1.2 Hz, H-5), 7.88 and 7.83 (m, H-6 and H-7), 7.52 (s, H-4), 4.60 (s, H-11), 3.90 (s, 1-OCH3); 13C NMR (75 MHz, DMSO-d6) δ 185.4 (C-10), 182.9 (C-9), 165.2
and 165.1 (C-1 and C-3), 140.0 (C-4a), 137.8 (C-7), 136.7 (C-6), 136.5 (C-8a), 135.4 (C-10a), 131.8 (C-2), 130.0 (C-8), 129.7 (C-5), 120.8 (C-9a), 113.0 (C-4), 65.2 (1-OCH₃), 56.0 (C-11)

**Compound 2**: yellowish powder, mp. 289-290°C; UV (MeOH) λ_{max} nm (log ε) 234 (4.40), 248 (4.25), 279 (4.62), 370 (3.70); FT-IR (ATR) ν_{max} (cm⁻¹) 3296 (OH stretching), 1671 (C=O stretching); ¹H NMR (300 MHz, DMSO-d₆) δ 8.16 (dd, J = 7.9 and 1.0 Hz, H-8), 8.11 (dd, J = 7.9 and 1.0 Hz, H-5), 7.90 and 7.84 (m, H-6 and H-7), 7.51 (s, H-4), 3.60 (s, 1-OCH₃), 2.20 (s, H-11); ¹³C NMR (75 MHz, DMSO-d₆) δ 183.0 (C-10), 180.9 (C-9), 162.0 (C-3), 161.1 (C-1), 135.1 (C-7), 134.0 (C-4a), 139.8 (C-10a), 133.6 (C-6), 132.5 (C-8a), 127.0 (C-8), 126.8 (C-2), 126.5 (C-5), 118.8 (C-9a), 110.0 (C-4), 61.1 (1-OCH₃), 9.6 (C-11)

**Compound 3**: yellow powder, mp. 210-211°C; UV (MeOH) λ_{max} nm (log ε) 248 (4.20), 279 (4.32), 312 (4.00), 373 (3.50); FT-IR (ATR) ν_{max} (cm⁻¹) 3300 (OH stretching), 1670 (C=O stretching); ¹H NMR (300 MHz, CDCl₃) δ 12.28 (s, 3-OH), 10.48 (s, H-11), 8.30 (dd, J = 7.8 and 1.4 Hz, H-8), 8.25 (dd, J = 7.8 and 1.4 Hz, H-5), 7.84 and 7.78 (m, H-6 and H-7), 7.69 (s, H-4), 4.13 (s, 1-OCH₃); ¹³C NMR (75 MHz, CDCl₃) δ 195.6 (C-11), 182.0 (C-10), 180.1 (C-9), 168.6 and 166.7 (C-1 and C-3), 141.7 (C-4a), 134.8 (C-7), 133.7 (C-6), 133.6 (C-8a), 132.4 (C-10a), 127.4 (C-8), 127.0 (C-5), 118.0 (C-2), 117.8 (C-9a), 113.0 (C-4), 64.8 (1-OCH₃)

### 3. RESULTS

Chemical studies of the constituents from the MeOH extract of *M. elliptica* roots had led to the isolation of three anthraquinones, damnacanthol (1), rubiadin-1-methyl ether (2) and damnacanal (3). The structures of all isolated compounds were elucidated using the analyses of spectroscopic data especially 1D and 2D NMR techniques.

Compound 1 was isolated as brownish powder. The UV spectrum showed absorption bands maxima at 230, 250, 273 and 368 nm which expected for an anthraquinone skeleton [2]. The FT-IR spectrum exhibited the absorption bands at 3282 and 1672 cm⁻¹ assignable for hydroxyl and conjugated carbonyl groups stretching, respectively. The ¹H NMR spectrum revealed the resonances of four adjacent aromatic protons at 8.18 (dd, J = 8.0 and 1.2 Hz), 8.14 (dd, J = 8.0 and 1.2 Hz), 7.88 (m) and 7.83 (m) indicated that one of the aromatic rings was an unsubstituted anthraquinone [3]. These coupled protons were clearly confirmed by the ¹H-¹H COSY experiment. The spectrum showed further a singlet aromatic proton at δ 7.52, an oxygenated methylene group at δ 4.60 (s) and a methoxyl group at 3.90 (s). Data suggested the other anthraquinone ring was a trisubstituted moiety. The presence of the oxygenated methylene and methoxyl groups corresponded to the signals at δ 5.60 and 65.2 in the ¹³C NMR spectrum, respectively. Among these two carbons, the spectrum exhibited additional signals due to the resonances of two carbonyl carbons at δ 185.4 and 182.9, seven quaternary carbons at δ 140.0, 136.5, 135.4, 131.8, 120.8 including the oxygenated carbons at δ 165.2 and 165.1 and five aromatic methine carbons at δ 137.8, 136.7, 130.0, 129.7 and 113.0. In the HMBC experiment, the signals resonated for the oxygenated methylene protons (δ 4.60) showed the correlation peaks with the two oxygenated quaternary carbons at δ 165.2 and 165.1 indicating that the hydroxymethyl group was located at the C-2 position (δ 131.8). The aromatic proton H-4 (δ 7.52) displayed the ¹J HMBC cross peaks to the carbon signals at δ 185.4 (C-10), 131.8 (C-2) and 120.8 (C-9a) and the ¹J correlations with the signals at δ 165.2 (C-3) and 140.0 (C-4a) confirmed the location of this proton at the C-4 position and suggestively oriented the same side with the carbonyl carbons C-10. In addition, the ¹H NMR spectrum showed the methoxyl group at δ 3.90 and disappeared a chelated hydroxyl signal at the down field region indicated that the C-1 position was replaced by the methoxyl group. Consequently the free hydroxyl group was positioned at the carbon C-3. These concluded the structure of compound 1 as 3-hydroxy-2-hydroxymethyl-1-methoxyanthraquinone or known as damnacanal [3, 13]. The HMBC correlations were fully supported this assigned anthraquinone structure.

Compound 2 was obtained as yellowish powder. The UV spectrum displayed absorption bands maxima at 234, 248, 279 and 370 nm indicating the same chromophore as for compound 1. The FT-IR spectrum showed the absorption bands for hydroxyl group at 3296 cm⁻¹ and for conjugated carbonyl group at 1671 cm⁻¹. The ¹H NMR spectrum of anthraquinone 2 exhibited similar signals to those of compound 1 consisting of the unsubstituted aromatic ring [δ 8.16 (dd, J = 7.9 and 1.0 Hz, H-8), 8.11 (dd, J = 7.9 and 1.0 Hz, H-5), 7.90 and 7.84 (m, H-6 and H-7)] and the trisubstituted aromatic ring [δ 7.51 (s, H-4), 3.60 (s, 1-OCH₃), 2.20 (s, H-11)]. The only difference was the presence of a singlet methyl group at δ 2.20 in compound 2 in stead of the hydroxymethyl group in compound 1. The C-2 of compound 2 was then placed by the methyl group. The ¹³C NMR and HMBC spectra were obviously confirmed this conclusion. Therefor compound 2 was identified to be 3-hydroxy-1-methoxy-2-methylanthraquinone or rubiadin-1-methyl ether [13, 14].

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Compound 3 was isolated as yellow powder. The UV and FT-IR spectra showed the similar absorption bands to those of compound 1 indicating the same anthraquinone structure. The \( ^1 \)H NMR spectrum also demonstrated the resonance signals closely to compound 1 except for the signals of the chelated hydroxyl and aldehyde protons at \( \delta 12.28 \) (s) and 10.48 (s), respectively. The \( ^{13} \)C NMR signal resonated at \( \delta 195.6 \) supported the existence of the formyl group. The \( ^3 \)J HMBC correlations of the formyl proton (\( \delta 10.48 \)) with the oxygenated quaternary carbons at \( \delta 166.8 \) and 166.7 (C-1 and C-3) suggested the placement of this formyl group at the C-2 position. The HMBC correlations of the chelated hydroxyl group (\( \delta 12.28 \)) to the carbons resonated at \( \delta 118.0 \) (C-2) and 113.0 (C-4) indicated the location of this hydroxyl group at the C-3 position. Consequently the methoxy group (\( \delta 4.13 \)) was clearly substituted at the C-1. Comparison the spectroscopic data with the previous works concluded that compound 3 was closely corresponded to 2-formyl-3-hydroxy-1-methoxyanthraquinone or widely known as damnacanthal [13,14].

4. CONCLUSIONS

Investigation of chemical constituents from the methanol extract of \( M. \) elliptica roots performed by reversed phase preparative HPLC separation using MeOH-H2O gradient solvents resulted in the isolation of three known anthraquinones, damnacanthol (1), rubiadin-1-methyl ether (2) and damnacanthal (3).

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REFERENCES

