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Morindaquinone, a new bianthraquinone from *Morinda coreia* roots

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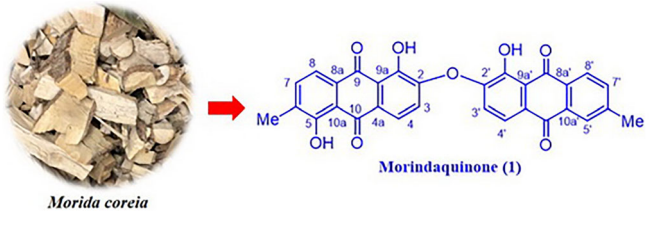
ABSTRACT

Phytochemical investigation of the roots of *Morinda coreia* led to the isolation of one new bianthraquinone, morindaquinone (**1**), together with 12 known compounds, soranjidiol (**2**), rubiadin-1-methyl ether (**3**), 2-methoxy-1,3,6-trihydroxyanthraquinone (**4**), 1-hydroxy-2-methylanthraquinone (**5**), tectoquinone (**6**), nordamnacanthal (**7**), damnacanthal (**8**), 2-formylanthraquinone (**9**), 3-hydroxy-2-hydroxymethylanthraquinone (**10**), lucidin-ω-methyl ether (**11**), scopoletin (**12**) and (+)-mellein (**13**). The structures of these compounds were determined on the basis of extensive spectroscopic analyses, as well as by comparison with literature reports. Compound **1** was the first example of bianthraquinone found in the genus *Morinda*, whereas compound **13** was firstly isolated from this genus. Among them, compounds **2**, **7**, **8** and **10** exhibited moderate to weak cytotoxicity against human cervical (HeLa), human colon (HT 29) and human breast (MCF-7) cell lines, while compounds **6** and **9** – **11** showed weak anti-acetylcholinesterase activity.



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1. Introduction

The genus *Morinda* (Rubiaceae) comprises about 80 species of small ever green trees or shrubs and is found exclusively in tropical climate zones (Phakhodee 2012). Almost all parts of plants of this genus have been used as traditional folk medicine for the treatment of many diseases including diabetes, hypertension and cancer (Xiang et al.

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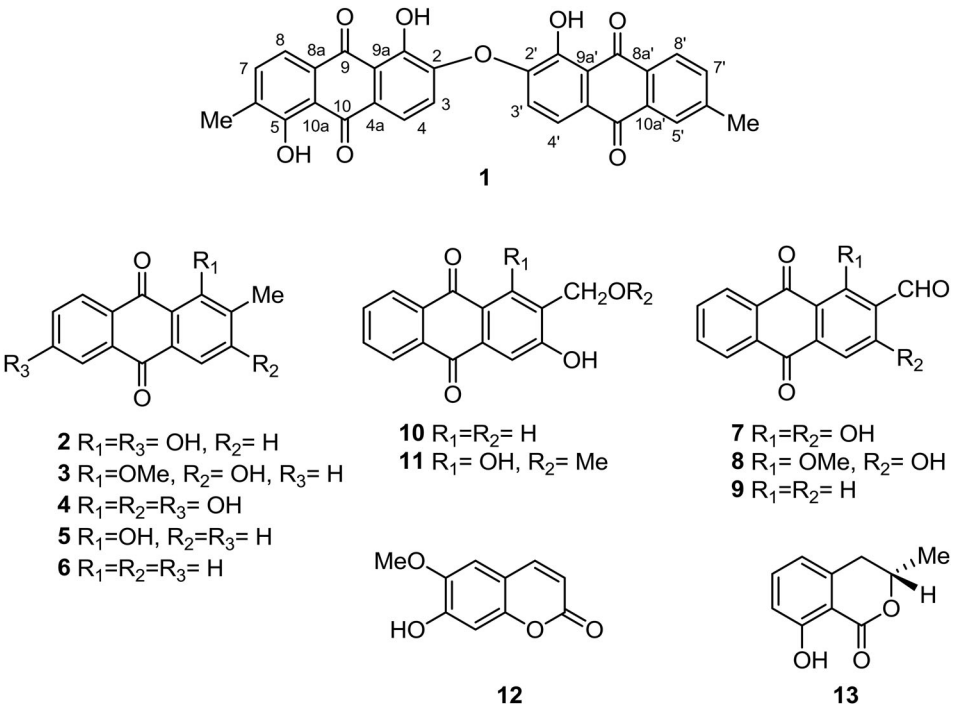

Figure 1. Structures of compounds **1–13** from *M. coreia*.

2008). *Morinda* species are well known for the chemical diversities of anthraquinones, iridoids, saccharide fatty acid esters, and lignans. However, chemical composition differs largely depending on the part of the plants (Phakhodee 2012).

Morinda coreia, known in Thai as Yo-Paa, is a tree distributed in the Southeast Asia region. Its bark and wood are used to treat fever and as an antimalarial agent in northeastern Thai (Isarn) traditional medicine. Previous studies on the chemical components of *M. coreia* reported eight iridiod glucosides, one phenolic glycoside, one secoiridoid glucoside and one anthraquinone glycoside from leaves and branches (Kanchanapoom et al. 2002), as well as seven anthraquinones and one anthraquinone glycoside from roots (Ruksilp et al. 2013).

Our on-going investigation of *M. coreia* roots has resulted in the isolation of one new bianthraquinone, morindaquinone (**1**), together with 12 known compounds, soranjidiol (**2**) (Nunez Montoya et al. 2006), rubiadin-1-methyl ether (**3**) (Rath et al. 1995), 2-methoxy-1,3,6-trihydroxyanthraquinone (**4**) (Itokawa et al. 1983), 1-hydroxy-2-methylanthraquinone (**5**) (Rath et al. 1995), tectoquinone (**6**) (Rath et al. 1995), nordamnacanthal (**7**) (Rath et al. 1995), damnacanthal (**8**) (Rath et al. 1995), 2-formylanthraquinone (**9**) (Hou and Wan 2008), 3-hydroxy-2-hydroxymethylanthraquinone (**10**) (Rath et al. 1995), lucidin-ω-methyl ether (**11**) (Chang and Lee 1984), scopoletin (**12**) (Mofiz Uddin Khan and Sugar Hossain 2015) and (+)-mellein (**13**) (Chacon-Morales et al. 2013) (Figure 1). Compound **13** was identified for the first time from the genus *Morinda*. In addition, compounds **3–6** and **9–13** were firstly isolated from this plant. We report herein the structure of the new bianthraquinone and the evaluation of anti-acetylcholinesterase and cytotoxic activities of some isolated compounds.

2. Results and discussion

Compound **1** was obtained as an orange amorphous powder. The IR spectrum indicated the presence of hydroxyl groups (3448 cm^{-1}), unchelated and chelated carbonyl groups (1626 and 1599 cm^{-1}) and aromatic ring (1570 and 1503 cm^{-1}). The UV spectrum showed absorption maxima at λ 298, 259 and 230 nm. The HR-ESI-MS showed $[M+H]^+$ at *m/z* 507.2725, corresponding to the molecular formula of $C_{30}H_{18}O_8$ (22 degrees of unsaturation). The ^{1}H NMR spectrum of **1** revealed the presence of three sets of AB type aromatic protons at $\delta_{\Η}$ 7.11 (d, $J = 8.4$ Hz, H-3), 7.73 (d, $J = 8.4$ Hz, H-4), 7.43 (d, $J = 7.8$ Hz, H-7), 7.66 (d, $J = 7.8$ Hz, H-8), 7.12 (d, $J = 8.4$ Hz, H-3′) and 7.72 (d, $J = 8.4$ Hz, H-4′), one set of ABX type aromatic protons at $\delta_{\Η}$ 8.00 (br s, H-5′), 7.50 (dd, $J = 7.8$ and 0.8 Hz, H-7′) and 8.10 (d, $J = 7.8$ Hz, H-8′), three chelated hydroxyl groups at $\delta_{\Η}$ 12.93 (1-OH), 13.19 (5-OH), and 12.87 (1′-OH), and two aromatic methyl groups at $\delta_{\Η}$ 2.30 (6-Me) and 2.46 (6′-Me). The ^{13}C NMR spectrum of **1** exhibited 29 signals (with two overlapping peaks) of two aromatic methyl groups at δ_C 16.1 (6-Me) and 21.8 (6′-Me), 9 methines, 19 quaternary carbons including three chelated carbonyl groups at δ_C 188.5 (C-9), 187.2 (C-10) and 189.0 (C-9′) and one unchelated carbonyl group at δ_C 181.8 (C-10′), as well as five oxygenated aromatic carbons at δ_C 150.1 (C-1), 151.8 (C-2), 160.8 (C-5), 149.8 (C-1′) and 152.3 (C-2′). Moreover, the presence of four carbonyl groups and three chelated hydroxyl groups for five oxygenated aromatic carbons strongly suggested that **1** was a dimer of anthraquinone linked by an oxygenated bridge. This bridge was located at C-2/C-2′ from the interpretation of the HMBC spectrum which showed correlations from H-4 ($\delta_{\Η}$ 7.73) to C-2 (δ_C 151.8) and C-10 (δ_C 187.2) and from H-4′ ($\delta_{\Η}$ 7.72) to C-2′ (δ_C 152.3) and C-10′ (δ_C 181.8). In this spectrum, the correlations from OH proton at $\delta_{\Η}$ 12.93 to C-1 (δ_C 150.1), from OH proton at $\delta_{\Η}$ 13.19 to C-5 (δ_C 160.8), C-6 (δ_C 135.7) and C-10a (δ_C 115.2) as well as from OH proton at $\delta_{\Η}$ 12.87 to C-1′ (δ_C 149.8) and C-9a′ (δ_C 116.2) gave evidence of the attachment of these hydroxyl groups at C-1, C-5 and C-1′, respectively. The long-range correlations from H-8 ($\delta_{\Η}$ 7.66) to C-6 (δ_C 135.7), C-9 (δ_C 188.5) and C-10a (δ_C 115.2) as well as from H-8′ ($\delta_{\Η}$ 8.10) to C-6′ (δ_C 146.1), C-9′ (δ_C 189.0) and C-10a′ (δ_C 133.9) allowed assignments of these aromatic protons to C-8 and C-8′, respectively. The position of the two aromatic methyl groups at C-6 and C-6′ was established from the long-range correlations between the methyl protons at $\delta_{\Η}$ 2.30 and the carbons C-5 (δ_C 160.8), C-6 (δ_C 135.7) and C-7 (δ_C 136.4) as well as between the methyl protons at $\delta_{\Η}$ 2.46 and the carbons C-5′ (δ_C 127.6), C-6′ (δ_C 146.1) and C-7′ (δ_C 134.4). Further HMBC correlations were established and the important HMBC and COSY correlations were shown in Figure S12. Therefore, compound **1** was characterized as 1,5,1′-trihydroxy-6,6′-dimethyl-2,2′-*O*-bianthraquinone, trivially named morindaquinone. This compound is being reported for the first time and it is the first example of a bianthraquinone isolated from genus *Morinda*.

Some isolated compounds were evaluated for their anti-acetylcholinesterase activity and cytotoxicity against three human cancer cell lines, HeLa (human cervical cancer cells), HT29 (human colon cancer cells) and MFC-7 (human breast cancer cells) (Table S1). Compounds **6** and **9**–**11** showed weak anti-acetylcholinesterase activity with IC_{50} values of 195.11–304.90 μM while the other compounds were inactive. For cytotoxic activity, compound **10** exhibited moderate activity against HeLa and

MCF-7 cell lines with IC_{50} values of 7.89 and 10.88 μM, respectively, whereas compounds **2**, **7** and **8** showed weak activity against these cell lines with IC_{50} values of 25.78–40.63 μM. Compounds **2**, **7**, **8** and **10** displayed weak cytotoxicity against HT 29 cell lines with IC_{50} values of 21.17–56.74 μM. In addition, compounds **1**, **4**, **11** and **12** were inactive against all tested cell lines.

3. Experimental

3.1. General experimental procedures

UV spectra were measured on an UV-1800 Shimadsu UV spectrophotometer. ATR-FTIR spectra were obtained using a Perkin Elmer FT-IR spectrum 400 spectrometer. 1 D and 2 D NMR spectra were recorded on a Bruker ASCEND 400 NMR spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ES-MS and HR-ESI-MS were recorded on a Finnigan LC-Q mass spectrometer and a micrOTOP-II mass spectrometer, respectively. Column chromatography (CC) was carried out using SaliCycle SiliaFlash silica gel 60 (<60–200 μm) and Amersham Biosciences Sephadex LH-20. For TLC, Merck precoated silica gel 60 F254 plates were used. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

3.2. Plant material

The roots of *M. coreia* were collected from Sanom district, Surin province, in January 2017. A voucher specimen (Thitima Rukachaisirikul, No. 014) was deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

3.3. Extraction and isolation

The air-dried, powdered roots of *M. corriea* (0.7 kg) were extracted successively with n-hexane, EtOAc and MeOH at room temperature. The hexane, EtOAc and MeOH extracts were filtered and concentrated to dryness under reduced pressure. The hexane extract (14.9 g) was subjected to CC using gradient solvent system of hexane, hexane-EtOAc and EtOAc to give 6 fractions (H1-H6). Fr. H2 (0.26 g) was purified by CC (20% EtOAc in hexane) to give 5 subfractions (H2.1-H2.5). Subfr. H2.2 furnished **8** (3.5 mg). Subfr. H2.3 (31.1 mg) was further purified by CC (5% EtOAc in hexane) to yield **7** (1.7 mg). Fr. H3 (1.40 g) was rechromatographed by CC (5% EtOAc in hexane) to obtain 6 subfractions (H3.1-H3.6). Subfr. H3.2 (0.25 g) was purified by CC (CH_2Cl_2) to afford **1** (101.5 mg). Fr. H3.4 (0.40 g) was separated by CC (40% CH_2Cl_2 in hexane) to give **2** (80.7 mg) and **9** (2.3 mg). The EtOAc extract (14.2 g) was subjected to CC using gradient solvent system of hexane, hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH to give 6 fractions (E1-E6). Fr. E2 (0.37 g) was separated by CC twice in succession (20% CH_2Cl_2 in hexane) to afford **13** (1.0 mg). Fr. E4 (4.26 g) was fractionated by CC (1% MeOH in CH_2Cl_2) to afford 5 subfractions (E4.1-E4.5). Subfr. E4.1 yielded **3** (153.0 mg). Subfr. E4.2 (2.68 g) was rechromatographed by CC (1% MeOH in CH_2Cl_2) to afford 7 subfractions (E4.2.1-E4.2.7). Subfr. E4.2.3 (0.25 g) was purified by CC (40% EtOAc

in hexane) to give **6** (1.2 mg), whereas subfr. E4.2.5 was further purified by CC (20% EtOAc in hexane) to furnish **10** (1.9 mg). Subfr. E4.3 (92.0 mg) was purified by CC (20% hexane in CH_2Cl_2) to give 4 subfractions (E4.3.1-E4.3.4). Subfr. E4.3.1 (50.2 mg) was rechromatographed by CC (CH_2Cl_2) to afford **12** (10.6 mg), whereas subfr. E4.3.2 yielded **4** (5.3 mg). The MeOH extract (70.47 g) was subjected to CC using gradient solvent system of hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH to give 7 fractions (M1-M7). Fr. M4 (0.39 g) was rechromatographed by CC twice in succession (20% hexane in CH_2Cl_2 and 40% CH_2Cl_2 in hexane) to afford **5** (13.4 mg). Fr. M5 (0.47 g) was purified on Sephadex LH-20 (MeOH) to give **11** (89.5 mg).

Morindaquinone (**1**): UV (MeOH) λ_{max} (log ε): 230 (4.79), 259 (4.85), 298 (4.46) nm; IR (ATR) ν_{max} 3448, 3096, 2967, 2923, 2845, 1626, 1599, 1570, 1503, 1446, 1356, 1276, 1258, 1218, 1190, 1172, 1154, 1078, 1065, 1017, 969, 933, 792, 767, 749, 732 cm^{-1}, ^1H NMR ($CDCl_3$ + 1 drop of CD_3OD, 400 MHz): δ 13.19 (s, 5-OH), 12.93 (s, 1-OH), 12.87 (s, 1′-OH), 8.10 (d, $J = 7.8$ Hz, H-8′), 8.00 (s, H-5′), 7.73[a] (d, $J = 8.4$ Hz, H-4), 7.72[a] (d, $J = 8.4$ Hz, H-4′), 7.66 (d, $J = 7.8$ Hz, H-8), 7.50 (dd, $J = 7.8$, 0.8 Hz, H-7′), 7.43 (d, $J = 7.8$ Hz, H-7), 7.12[b] (d, $J = 8.4$ Hz, H-3′), 7.11[b] (d, $J = 8.4$ Hz, H-3), 2.46 (s, 6′-Me), 2.30 (s, 6-Me); ^{13}C NMR ($CDCl_3$ + 1 drop of CD_3OD, 125 MHz): 189.0 (C-9′), 188.5 (C-9),187.2 (C-10), 181.8 (C-10′), 160.8 (C-5), 152.3 (C-2′), 151.8 (C-2), 150.1 (C-1), 149.8 (C-1′), 146.1 (C-6′), 136.4 (C-7), 135.7 (C-6), 134.4 (C-7′), 133.9 (C-10a′), 130.9[c] (C-8a), 130.8[c] (C-8a′), 127.6 (C-5′), 126.9 (C-8′), 124.8[d] (C-4a′), 124.3[d] (C-4a), 121.5[e] (C-4′), 121.4[e] (C-4), 120.3 (C-3, C-3′), 118.8 (C-8), 116.2 (C-9a, C-9a′), 115.2 (C-10a), 21.8 (6′-Me), 16.1 (6-Me); [a–e]Assignment with the same superscript may be interchanged.; HR-ESI-MS *m/z* 507.2725 $[M + H]^+$ (calcd. for $C_{30}H_{18}O_8$, 507.2727).

3.4. Anti-acetylcholinesterase assay

Anticholinesterase activity testing. Acetylcholinesterase (AChE) inhibitions were determined spectrophotometrically using acetylthiocholine as substrate, by modifying the method of Ellman (Ellman et al. 1961; Bolognesi et al. 2007). Briefly, in the 96-well plates, 140 μL of 10 mM sodium phosphate buffer (pH 8.0), 20 μL of a solution of AChE (0.2 units/mL in 10 mM sodium phosphate buffer, pH 8.0) and 20 μL of test compound solution dissolved in 80% methanol (a final concentration of 0.1 mg/mL) were mixed and incubated at room temperature for 15 min. The reaction was started by adding 20 μL of mixture solution of 5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in 10 mM sodium phosphate buffer (pH 8.0), containing 0.1% bovine serum albumin (BSA) and 5 mM acetylcholine iodine (ATCI) in 10 mM sodium phosphate buffer, pH 8.0 (5:1). The hydrolysis of acetylthiocholine was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion as result of reaction with DTNB and thiocholines, catalyzed by enzymes at a wavelength of 405 nm and the absorbance was measured after 2 minutes of incubation at room temperature. Percentage of inhibition was calculated by comparing the rate of enzymatic hydrolysis of ATCI for the sample to that of the blank (80% methanol in buffer). Galanthamine was used as a reference standard. Every experiment was done in triplicate.

3.5. Cytotoxicity assay

Human colon adenocarcinoma (HT29) and cervical epithelial adenocarcinoma (HeLa) cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 mg/mL) and l-glutamine (4 mM) at 37 °C, 5% CO_2. Human breast adenocarcinoma (MCF-7) was also grown as mentioned above except that the medium contained 1% of insulin. For the cytotoxicity assay, the cells were seeded up to 1×104 cells/well in a 96-well plate, to give 50–70% confluence to be used on the next day. Monolayers of cancer cells were treated with various concentrations of pure compound in DMSO for 48 h. Cell viability was determined by MTT assay. Cells were incubated with 0.5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution and incubated at 37 °C under a humidified 5% CO_2 incubator for 4 h. Then, the culture medium was removed and 100 μL of DMSO was added before the measurement of absorbance at 550 nm by Tecan U.S., Durham, NC, USA. Half maximal inhibitory concentration (IC_{50}) values were determined by regression analysis (Radchatawedchakoon et al. 2015).

4. Conclusion

A new bianthraquinone, morindaquinone (**1**), together with 12 known compounds, soranjidiol (**2**), rubiadin-1-methyl ether (**3**), 2-methoxy-1,3,6-trihydroxyanthraquinone (**4**), 1-hydroxy-2-methylanthraquinone (**5**), tectoquinone (**6**), nordamnacanthal (**7**), damnacanthal (**8**), 2-formylanthraquinone (**9**), 3-hydroxy-2-hydroxymethyl-anthraquinone (**10**), lucidin-ω-methyl ether (**11**), scopoletin (**12**) and (+)-mellein (**13**) were isolated from the roots of *Morinda coreia*. To our knowledge, compound **1** was the first example of bianthraquinone found in the genus *Morinda*, whereas compound **13** was isolated from the genus *Morinda* for the first time. Moreover, eight compounds were firstly obtained from this plant. Among them, compounds **2**, **7**, **8** and **10** exhibited moderate to weak cytotoxicity against HeLa, HT 29 and MCF-7 cell lines, while compounds **6** and **9**– **11** showed weak anti-acetylcholinesterase activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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