Anthraquinones with Quinone Reductase-Inducing Activity and Benzophenones from *Morinda citrifolia* (Noni) Roots

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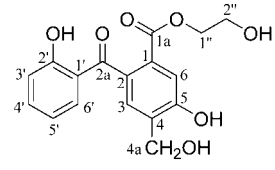
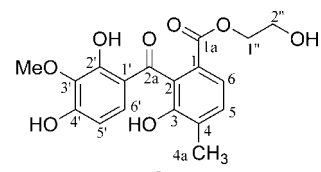
Two new benzophenones, morintrifolins A (**1**) and B (**2**), together with 14 known anthraquinones and four other known compounds, were isolated from a chloroform-soluble extract of *Morinda citrifolia* roots. Of the isolated compounds, four known anthraquinones, namely, 1,2-dihydroxyanthraquinone (**3**), 1,3-dihydroxy-2-methylanthraquinone (**4**), 2-hydroxy-3-(hydroxymethyl)anthraquinone (**5**), and 1,3,6-trihydroxy-2-methylanthraquinone (**6**), exhibited quinone reductase (QR)- inducing activity in Hepa lclc7 cells, with concentrations required to double QR activity of 12.0, 8.1, 0.94, and 0.56 μM, respectively.

Morinda citrifolia L. (Rubiaceae), commonly known as "noni", is a shrub or small tree widely distributed in southern Asia and the Pacific islands and has been subjected to recent review.[1–4] There is a long history of the use of *M. citrifolia* as an important medicinal plant, for the treatment of asthma, bone fractures, cancer, cholecystitis, dysentery, lumbago, menstrual cramps, urinary difficulties, and many other ailments.[1–4] According to statistical data published by the *Nutrition Business Journal* in 2006, in recent years noni juice has become increasingly a best-selling herbal and botanical dietary supplement on the U.S. market.[5] Phytochemical studies have indicated that various plant parts of noni contain anthraquinones and anthraquinone glycosides, fatty acid glycosides, iridoids and iridoid glycosides, lignans, and triterpenoids.[1–4] Recent studies have indicated the activities of constituents of noni in a wide range of biological assays, such as the inhibition of angiogenesis,[6] cyclooxygenase-1,[7] 5- and 15-lipoxygenase,[8] phorbol ester-induced inflammation,[9] and tyrosine kinase,[10] as well as cytotoxicity against Jurkat cells when combined with a tumor necrosis factor-related apoptosis-inducing ligand.[11]

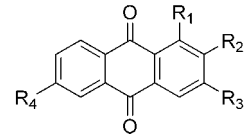
Previous studies by our group have led to the isolation and identification of antioxidant lignans[12] and a potent quinone reductase-inducing anthraquinone from the powdered fruits of *M. citrifolia*.[13] Since *M. citrifolia* roots are known to contain abundant anthraquinones and anthraquinone glycosides,[4] the roots of the plant were selected for further investigation for potential QR-inducing components,[13] with the intent of finding the potent inducer of this enzyme, 2-methoxy-1,3,6-trihydroxyanthraquinone, in larger quantities than in the fruits. This substance was found to be present only as a trace constituent in *M. citrifolia* fruits (0.000018% w/w).[13] As a result of the present study, two new benzophenone derivatives, named morintrifolins A (**1**) and B (**2**), together with 18 known compounds, have been isolated from the chloroform-soluble partition of a methanol extract of *M. citrifolia* roots. The known compounds identified included 14 anthraquinones, namely, 1,2-dihydroxyanthraquinone (alizarin) (**3**),[14] 1,8-dihydroxy-2-(hydroxymethyl)-5-methoxyanthraquinone (hydyotanthraquinone),[15] 1,3-dihydroxy-2-methoxyanthraquinone,[16] 1,3-dihydroxy-2-methylanthraquinone (rubiadin) (**4**),[17] 1,6-dihydroxy-2-methylanthraquinone (soranjidiol),[18] 1-hydroxyanthraquinone,[19] 2-hydroxy-3-(hydroxymethyl)anthraquinone (**5**),[20] 1-hydroxy-2-(hydroxymethyl)-3-methoxyanthraquinone,[21] 3-hydroxy-2-(hydroxymethyl)-1-methoxyanthraquinone (damnacanthol),[21] 1-hydroxy-2-methoxyanthraquinone,[22] 2-hydroxy-3-methoxyanthraquinone, 6-hydroxy-1-methoxy-2-methyanthraquinone,[23] 1,2,5-trihydroxy-3-methylanthraquinone,[24] and 1,3,6-trihydroxy-2-methylanthraquinone (**6**).[25] Also obtained were four other types of compounds, comprising ciwujiatone (a lignan),[26] 4-hydroxy-3-methoxycinnamaldehyde, 6-methoxy-7-hydroxycoumarin (isoscopoletin), and pomolic acid (a triterpenoid).[27]



1



2



3 R_1, R_2 = OH, R_3, R_4 = H
4 R_1, R_3 = OH, R_2 = Me, R_4 = H
5 R_1, R_4 = H, R_2 = CH_2OH, R_3 =OH
6 R_1, R_3, R_4 = OH, R_2 = Me

The isolation of **5**, **6**, 1-hydroxyanthraquinone, 1-hydroxy-2-(hydroxymethyl)-3-methoxyanthraquinone, 2-hydroxy-3-methoxyanthraquinone, 6-hydroxy-1-methoxy-2-methyanthraquinone, 1,2,5-trihydroxy-3-methylanthraquinone, and ciwujiatone from *M. citrifolia* has not been reported in previous studies. It is pertinent to note that 1-hydroxyanthraquinone has been found to be mutagenic[28] and to produce colon adenomas, and adenocarcinomas as well as liver neoplasms in rats when fed in the diet containing 1% of this compound.[29] However, 1-hydroxyanthraquinone was found in the present study only at a low yield (0.0002% w/w), and it has been suggested that its mutagenic activity in bacteria may be due to contamination by one or more byproducts when this anthraquinone is produced by synthesis.[30]

Compound **1** was obtained as a colorless gum, and its molecular formula was determined as $C_{17}H_{16}O_7$, on the basis of the sodiated molecular ion peak at *m/z* 355.0793 (calcd for $C_{17}H_{16}O_7Na$, 355.0794) in the HRESIMS. The ^{1}H NMR spectrum of **1** showed resonance signals at δ_H 7.48 (td, J = 7.8, 1.6 Hz, H-4′), 7.20 (dd, J = 7.8, 1.6 Hz, H-6′), 6.99 (dd, J = 7.8, 0.5 Hz, H-3′), and 6.80 (td, J = 7.8, 0.5 Hz, H-5′), assignable to an aromatic ring with two *ortho*-substituted groups. Two singlet peaks at δ_H 7.44 (H-6) and 7.43 (H-3) suggested the presence of another 1,2,4,5-tetrasubstituted aromatic ring system in the molecule of **1**. The remaining signals in the ^{1}H NMR spectrum of this isolate could be assigned to hydroxymethyl (δ_H 4.72, 2H, s, H-4a) and ethylene glycol (δ_H 4.08

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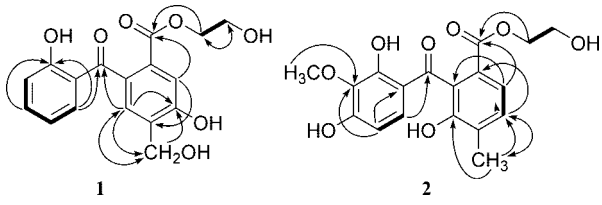

Figure 1. Selected $^1H-^1H$ COSY (—), HMBC (↷), and NOESY (↔) correlations observed for **1** and **2**.

and 3.61) groups. The 17 resonance signals in the ^{13}C NMR spectrum were ascribed to a ketone carbonyl (δ_C 204.6, C-2a), an ester carbonyl (δ_C 167.4, C-1a), two aromatic rings (δ_C 163.5–116.8), an ethylene glycol functionality (δ_C 67.7, C-1″ and 60.6, C-2″), and a hydroxymethyl (δ_C 60.0) group. In the HMBC spectrum (Figure 1), the correlations of δ_H 7.20 (H-6′) with δ_C 204.6 (C-2a) and 163.5 (C-2′) suggested that the first aromatic ring is substituted with a hydroxy group and a ketone group. The correlations of δ_H 7.43 (H-3) with δ_C 204.6 (C-2a), 157.5 (C-5), and 60.6 (C-4a) suggested that the second aromatic ring is also connected to the same ketone group, as well as being substituted with a hydroxy and a hydroxymethyl group. The presence of the ethylene glycol ester group was supported by the correlation between resonances at δ_H 4.08 (H-1″) and δ_C 167.4 (C-1a), and this ester group was located at C-1 by the observation in the HMBC spectrum of a correlation between signals at δ_H 7.44 (H-6) and δ_C 167.4 (C-1a). The correlation of δ_H 7.43 (H-3) with δ_H 4.72 (H-4a) in the NOESY spectrum confirmed that the hydroxymethyl group is positioned at C-4. Other 2D NMR spectroscopic correlations (Figure 1) were supportive of the structure proposed. Thus, the structure of the new benzophenone **1** was established as 5-hydroxy-2-(2-hydroxybenzoyl)-4-(hydroxymethyl)benzoic acid 2-hydroxyethyl ester, which has been assigned the trivial name morintrifolin A.

Compound **2** was obtained as a colorless gum. A molecular formula of $C_{18}H_{18}O_8$ was suggested by a sodiated molecular ion peak at *m/z* 385.0913 (calcd for $C_{18}H_{18}O_8Na$, 385.0899) in the HRESIMS. Both the ^1H and ^{13}C NMR spectra of compound **2** were similar to those of **1**, indicating that they have the same skeleton but with different substituents. The ^1H NMR spectrum of **2** exhibited signals for four *ortho*-coupled aromatic protons present in two different rings at δ_H 7.59 (1H, d, $J = 7.9$ Hz, H-6) and 7.32 (1H, d, $J = 7.9$, H-5), as well as at δ_H 6.75 (1H, d, $J = 8.9$ Hz, H-5′) and 6.25 (1H, d, $J = 8.9$ Hz, H-6′), respectively. The ^1H NMR spectrum also indicated the presence of an ethylene glycol ester (δ_H 4.12, 2H, t, $J = 5.1$ Hz, H-1″ and 3.64, 2H, t, $J = 5.1$ Hz, H-2″), a methoxy substituent (δ_H 3.88 3H, s), and a methyl group (δ_H 2.31, 3H, s, H-4a). The ^{13}C NMR spectrum showed a signal for a ketone group at δ_C 202.5 and 12 resonance signals attributable to two aromatic rings in the range δ_C 158.7 to 109.2, an ethylene glycol ester group (δ_C 167.1, C-1a; 67.5, C-1″ and 60.8, C-2″), a methoxy group (δ_C 60.7), and a methyl group (δ_C 16.9). The correlations of δ_H 6.75 (H-5′) with δ_C 136.0 (C-3′), 116.6 (C-1′), and 129.9 (C-6′), as well as δ_H 6.25 (H-6′) with δ_C 158.7 (C-2′ or 4′) and 158.3 (C-2′ or 4′), in the HMBC spectrum indicated that these carbons are included in the same ring, with ^{13}C NMR chemical shifts for a modified pyrogallol-type functionality. Correlation of the resonance at δ_H 3.88 with the signal at δ_C 136.0 (C-3′) in the HMBC spectrum suggested that the methoxy group in **2** is located at the C-3′ position. The resonance signal at δ_C 153.5 belonging to another aromatic ring indicated the presence of a hydroxy group, which was inferred from deducing all the other functionalities from the molecular weight. The positions of the substituents were assigned by careful inspection of 2D-NMR spectroscopic data from $^1H-^1H$ COSY, HSQC, HMBC, and NOESY experiments, as depicted in Figure 1. Therefore, the structure of **2** (morintrifolin B) was elucidated as 2-(2,4-dihydroxy-3-methoxybenzoyl)-3-hydroxy-4-methylbenzoic acid 2-hydroxyethyl ester.

Table 1. Biological Activity of Compounds from *M. citrifolia* Roots in a Quinone Reductase (QR) Induction Assay

	QR		
compound	CD,[a] μM (μg/mL)	IC_{50},[b] μM (μg/mL)	CI[c]
3	12.0 (2.9)	62.0 (14.9)	5.1
4	8.1 (2.2)	>74 (>20)	>9.0
5	0.94 (0.24)	>78 (>20)	>83
6	0.56 (0.15)	47.0 (12.8)	85
isoliquiritigenin[d]	4.3 (1.1)	39.0 (10.1)	9.2

[a] CD, concentration required to double QR activity. [b] IC_{50}, concentration inhibiting cell growth by 50%. [c] CI, chemoprevention index (=IC_{50}/CD). [d] Positive control.

Benzophenone derivatives (**1** and **2**) have been isolated for the first time from noni (*M. citrifolia*), and these two new compounds can be considered as seco-anthraquinones from a biogenetic point of view.[31] A number of other natural products have been reported bearing an ethylene glycol functionality.[32–34]

One of the strategies of cancer chemoprevention for protecting cells from carcinogenesis is to induce phase II enzymes that deactivate harmful radicals and electrophiles. Since quinone reductase (QR) is a phase II metabolizing enzyme that reversibly catalyzes the oxidation of NADH or NADPH and consequently decreases the amount of harmful radicals and electrophiles, the induced QR may exert cancer chemopreventive properties.[35,36] A QR induction assay using Hepa lclc7 murine hepatoma cells was employed to evaluate the activities of all compounds isolated from *M. citrifolia* roots, except for 1-hydroxy-2-(hydroxymethyl)-3-methoxyanthraquinone, 4-hydroxy-3-methoxycinnamaldehyde, hydyotanthraquinone, isoscopoletin, and pomolic acid. Of the tested compounds, **3**–**6** exhibited QR induction activity with CD values of 12, 8.1, 0.94, and 0.56 μM, respectively, as summarized in Table 1. The structure of the most potent compound (**6**, 1,3,6-trihydroxy-2-methylanthraquinone) obtained in the present study is similar to the previously reported 2-methoxy-1,3,6-trihydroxyanthraquinone,[13] but the latter compound exhibited more potent QR induction activity with a CD value of 0.009 μM and no evidence of cytotoxicity at up to 20 μg/mL (69.9 μM). The only difference between these two molecules is in the substitution at the C-2 position, which leads to both differential potency and cytotoxicity in the QR induction assay using Hepa lclc7 cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Perkin-Elmer Lambda 10 UV/vis spectrometer. IR spectra were run on a Nicolet Protégé 460 FT-IR spectrometer. NMR spectroscopic data were recorded at room temperature on a Bruker Avance DPX-300 or DRX-400 spectrometer. Column chromatography was performed with 65–250 or 230–400 mesh silica gel (Sorbent Technologies, Atlanta, GA). Analytical thin-layer chromatography was conducted on 250 μm thickness Partisil silica gel 60 F_{254} glass plates (Whatman, Clifton, NJ). Analytical and semipreparative HPLC were carried out on a Waters system composed of a 600 controller, a 717 Plus autosampler, and a 2487 dual wavelength absorbance detector, with Waters Sunfire C_{18} columns (4.6 × 150 or 19 × 150 mm, respectively).

Plant Material. The freeze-dried root powder of *Morinda citrifolia* (lot number 0125412) was obtained from Nature's Sunshine Products, Inc. (Spanish Fork, Utah) in February 2006. A representative sample (OSUADK-CCP0005) has been deposited at the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University.

Extraction and Isolation. The freeze-dried root powder of *M. citrifolia* (5.0 kg) was extracted by percolation with MeOH (80 L) at room temperature for 7 days. The solvent was removed under a vacuum below 45 °C to give 400 g of a dark brown syrup. Next, the MeOH extract was suspended in 3 L of a 10% methanol–water solution and then partitioned in turn with hexanes (3 × 2000 mL) and chloroform (3 × 2000 mL), to afford a defatted chloroform-soluble extract (11 g).

This chloroform-soluble extract was chromatographed over a silica gel gravity column (65–250 mesh, 9 × 45 cm), eluted with a solvent gradient of increasing polarity (CH_2Cl_2−MeOH, 20:1 to 1:1, then pure MeOH) to give 14 fractions (F1−F14).

Fraction F1 (180 mg) was subjected to silica gel column chromatography (230–400 mesh, 1.8 × 30 cm), eluted with hexanes−acetone (10:1), to give four subfractions (F1F1−F1F4). Subfraction F1F1 (15 mg) was further purified on a silica gel column (230–400 mesh, 1 × 15 cm), using chloroform−acetone (20:1) for elution, to give 1-hydroxyanthraquinone[19] (10 mg). Fraction F2 (850 mg) was subjected to a silica gel column (65–250 mesh, 2 × 35 cm) using chloroform−acetone (15:1) as eluting solvent to give five subfractions (F2F1−F2F5). Subfraction F2F3 (310 mg) was purified over a Sephadex LH-20 column (1 × 50 cm) with methanol to afford 1,2-dihydroxyanthraquinone[14] (**3**, 5 mg) and rubiadin[17] (**4**, 2 mg). Fraction F6 (90 mg) was subjected to passage over a silica gel column (230–400 mesh, 1 × 30 cm) eluted with hexane−acetone (10:1) to give two subfractions (F6F1 and F6F2), with subfraction F6F2 (40 mg) further purified over a Sephadex LH-20 column (1 × 50 cm), using pure MeOH as solvent, to furnish soranjidiol[18] (23 mg) and 6-hydroxy-1-methoxy-2-methylanthraquinone[23] (15 mg). Fractions F8 and F9 were combined (1.1 g), and the pooled fraction was subjected to passage over a Sephadex LH-20 column (4.5 × 50 cm) eluted with pure MeOH to give four fractions (F8F1−F8F4). Subfraction F8F2 (150 mg) was further separated using a silica gel column (230–400 mesh, 1.8 × 45 cm) to furnish **6**[25] (20 mg) and pomolic acid[27] (4 mg). Subfraction F8F3 (80 mg) was chromatographed over a silica gel column (230–400 mesh, 1 × 50 cm), using $CHCl_3$−MeOH (30:1) as solvent, to yield 1-hydroxy-2-(hydroxymethyl)-3-methoxyanthraquinone (6 mg). Subfraction F8F4 (210 mg) was isolated on a silica gel column (230–400 mesh, 1.8 × 40 cm), eluted with $CHCl_3$−MeOH (30:1), to give **5**[20] (8 mg) and isoscopoletin (2 mg). Fraction F10 (500 mg) was further purified with a Sephadex LH-20 column (4 × 50 cm) eluted with pure MeOH to give five subfractions (F10F1–F10F5). Subfraction F10F2 (70 mg) was partially purified on a silica gel column (230–400 mesh, 1 × 30 cm) using chloroform−MeOH (50:1) as eluting solvent, then finally purified by semipreparative HPLC using MeOH−H_2O−MeCN (15:70:15, 6 mL/min) as solvent, to give ciwujiatone[26] (t_R 35.5 min, 8 mg). Another peak with a retention time of 43.8 min was collected and further purified by semipreparative HPLC using H_2O−MeCN (75:25, 8 mL/min) as solvent to give the new compound **2** (t_R 21.5 min, 3 mg). Subfraction F10F3 (300 mg) was subjected to passage over a RP-18 open column (2 × 40 cm) eluted with gradient H_2O−MeOH mixtures (60:40, 50:50, and 40:60, 400 mL each) to afford three subfractions (F10F3F1−F10F3F3). The second subfraction, F10F3F2 (100 mg), was separated using a silica gel column (230–400 mesh, 1 × 20 cm) to give damnacanthol[21] (1.5 mg) and 1-hydroxy-2-methoxyanthraquinone[22] (8 mg), and subfraction F10F4 (45 mg) was separated in a similar column, using $CHCl_3$−MeOH (30:1) as eluting solvent, to give 4-hydroxy-3-methoxycinnamaldehyde (2 mg).

Fractions F11, F12, and F13 were combined, and the new bulked fraction F11′ (1.2 g) was chromatographed over a Sephadex LH-20 column (4 × 50 cm) column using pure MeOH as eluting solvent to give eight subfractions (F11′F1−F11′F8). Subfraction F11′F4 (50 mg) was separated by semipreparative HPLC, using MeCN−H_2O (30:70, 8 mL/min) as solvent system, to afford the new compound **1** (t_R 35.5 min, 5 mg). F11′F5 (70 mg) was passaged over a silica gel column (230–400 mesh, 1 × 40 cm), eluted with $CHCl_3$−MeOH (20:1), to give hydyotanthraquinone[15] (4 mg), and F11′F6 (220 mg) was chromatographed over a silica gel column (230–400 mesh, 1.8 × 45 cm) using $CHCl_3$−MeOH (20:1) to give six subfractions (F11F6F1−F11F6F6). Then, subfraction F11′F6F2 (10 mg) was purified on a RP-18 column (1 × 20 cm), with elution by MeOH−H_2O (60:40), to give 1,3-dihydroxy-2-methoxyanthraquinone[16] (2 mg). The constituents of subfraction F11′F7 (12 mg) were isolated by semipreparative HPLC, using MeOH−H_2O (80:20, 8 mL/min) as eluting solvent, running initially for 12 min, then changing to pure MeOH at the same flow rate, to give 1,2,5-trihydroxy-3-methylanthraquinone[24] (t_R 8.7 min, 4 mg) and 2-hydroxy-3-methoxyanthraquinone (t_R 18.6 min, 2 mg).

Morintrifolin A (1): colorless gum; UV (MeOH) λ_{max} (log ϵ) 214 (3.98), 252 (3.61), 313 (3.27, br) nm; IR (film) ν_{max} 3253, 1718, 1626, 1603, 1330, 1257, 1057, 897 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.48 (1H, td, J = 7.8, 1.6 Hz, H-4′), 7.44 (1H, s, H-6), 7.43 (1H, s, H-3), 7.20 (1H, dd, J = 7.8, 1.6 Hz, H-6′), 6.99 (1H, dd, J = 7.8, 0.5 Hz, H-3′), 6.80 (1H, td, J = 7.8, 0.5 Hz, H-5′), 4.72 (2H, s, H-4a), 4.08 (2H, t, J = 5.1 Hz, H-1″), 3.61 (2H, t, J = 5.1 Hz, H-2″); ^{13}C NMR (100 MHz, CD_3OD) δ 204.6 (C, C-2a), 167.4 (C, C-1a), 163.5 (C, C-2′), 157.5 (C, C-5), 137.3 (CH, C-4′), 134.2 (C, C-4), 133.9 (CH, C-6′), 132.4 (C, C-1), 130.7 (C, C-2), 128.3 (CH, C-3), 122.1 (C, C-1′), 120.0 (CH, C-5′), 118.8 (CH, C-3′), 116.8 (CH, C-6), 67.7 (CH_2, C-1″), 60.6 (CH_2, C-2″), 60.0 (CH_2, C-4a); HRESIMS m/z 355.0793 [M + Na]$^+$ (calcd for $C_{17}H_{16}O_7Na$, 355.0794).

Morintrifolin B (2): colorless gum; UV (MeOH) λ_{max} (log ϵ) 211 (3.96), 235 (3.58), 295 (3.56, br) nm; IR (film) ν_{max} 3253, 1718, 1626, 1603, 1330, 1057, 897 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.59 (1H, d, J = 7.9 Hz, H-6), 7.32 (1H, d, J = 7.9, H-5), 6.75 (1H, d, J = 8.9 Hz, H-5′), 6.25 (1H, d, J = 8.9 Hz, H-6′), 4.12 (2H, t, J = 5.1 Hz, H-1″), 3.88 (3H, s, −OCH_3), 3.64 (2H, t, J = 5.1 Hz, H-2″), 2.31 (3H, s, H-4a); ^{13}C NMR (100 MHz, CD_3OD) δ 202.5 (C, C-2a), 167.1 (C, C-1a), 158.7* (C, C-4′), 158.3* (C, C-2′), 153.5 (C, C-3), 136.0 (C, C-3′), 133.2 (C, C-4), 132.5 (CH, C-5), 129.9 (CH, C-6′), 129.5 (C, C-2), 128.3 (C, C-1), 123.1 (CH, C-6), 116.6 (C, C-1′), 109.2 (CH, C-5′), 67.5 (CH_2, C-1″), 60.8 (CH_2, C-2″), 60.7 (CH_3, −OCH_3), 16.9 (CH_3, C-4a) (*assignments are interchangeable); HRESIMS m/z 385.0913 [M + Na]$^+$ (calcd for $C_{18}H_{18}O_8Na$, 385.0899).

Quinone Reductase Induction Assay. A quinone reductase assay using Hepa lclc7 murine hepatoma cells[13] was employed to evaluate the activities of the compounds isolated from *M. citrifolia* roots, using isoliquiritigenin as a positive control.[36,37] However, for the present work, Hepa lclc7 cells (CRL-2026) were purchased from American Type Culture Collection (Manassas, VA). Compounds with CD (concentration required to double QR activity) values of <5 μg/mL are considered active.[13,38]

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Supporting Information Available: ^1H, ^{13}C, $^1H-^1H$ COSY, HSQC, HMBC, and NOESY NMR spectra of compounds **1** and **2**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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