An Anthraquinone with Potent Quinone Reductase-Inducing Activity and Other Constituents of the Fruits of *Morinda citrifolia* (Noni)

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Abstract: *Morinda citrifolia*, commonly known as noni, has a long history of utilization throughout much of tropical Polynesia and is considered to be the second most important medicinal plant in the Hawaiian Islands. Recently, the use of noni as a dietary supplement in the United States has greatly increased. Bioassay-guided fractionation of a dichloromethane-soluble partition of a MeOH extract of noni fruits has led to the isolation of an extremely potent quinone reductase inducer, 2-methoxy-1,3,6-trihydroxyanthraquinone (**1**). This new anthraquinone (**1**) was nearly 40 times more potent than a positive control, L-sulforaphane. Furthermore, compound **1** demonstrated no discernible cytotoxicity at the highest dose tested. In addition to compound **1**, 11 known compounds were also isolated and identified in the present investigation. This is the first report of the isolation of anthraquinones from noni fruits.

Currently, the use of *Morinda citrifolia* L. (Rubiaceae), commonly called noni, as a botanical dietary supplement is growing in the United States and elsewhere. The purported uses include for arthritis, cardiovascular disease, cancer, and as a tonic for promoting overall health. Noni is a tree or large bush distributed in tropical areas from India to Hawaii, where it is widely considered a valuable medicinal plant. Although all parts of *M. citrifolia* are used ethnobotanically for various medical ailments, the ripe fruits are currently the predominant part used in the United States.[1]

To date, the majority of studies done on noni have demonstrated a number of potential biological activities, yet the phytochemical constituents responsible for the ascribed activities are largely unknown.[2] In our continuing investigation of botanical dietary supplements for cancer chemopreventive activities,[3] we tested a noni fruit extract for activity in a quinone reductase (QR) induction bioassay.[4,5] QR is a phase II metabolizing enzyme and is induced in conjunction with other protective phase II enzymes by a chemically diverse array of compounds. The induction of phase II enzymes is considered cancer chemopreventive in that potential oxidative and electrophilic molecules can be more readily metabolized and excreted before they can interact with cellular macromolecules such as DNA. QR is also responsible for maintaining the reduced states of antioxidants such as α-tocopherol and coenzyme Q_{10}.[6] Hence, QR inducers are sometimes referred to as "indirect antioxidants", and this activity is considered protective at the initiation stage of carcinogenesis.[7] Herein we describe the isolation, identification, and biological activity of 12 compounds from *M. citrifolia* fruits, including the structure elucidation of one new anthraquinone (**1**) with potent QR induction activity.

The CH_2Cl_2-soluble partition of the MeOH extract of noni fruits exhibited a potent concentration to double QR (CD) activity value of <2.5 $\mu g/mL$. Bioassay-guided fractionation of this extract led to the isolation of one new anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone (**1**), as well as 11 known compounds, 1,8-dihydroxy-2-hydroxymethyl-5-methoxyanthraquinone(**2**),[8] 1,3-dihydroxy-2-methoxyanthraquinone (**3**),[9] 1,6-dihydroxy-5-methoxy-2-methylanthraquinone (**4**),[10] 2-hydroxy-1-methoxyanthraquinone (**5**),[11,12] balanophonin,[13] 4-hydroxy-3-methoxycinnamaldehyde,[14] β-hydroxypropiovanillone,[15] 1-monopalmitin,[16] scopoletin, β-sitosterol, and vanillin. The structures of these known compounds were identified by comparing their physical and spectroscopic data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) with those of published values or by comparing with an authentic sample (β-sitosterol, scopoletin, and vanillin) directly.
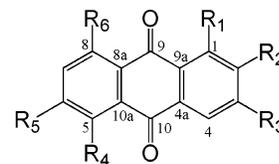
	R_1	R_2	R_3	R_4	R_5	R_6
1	OH	OCH_3	OH	H	OH	H
2	OH	CH_2OH	H	OCH_3	H	OH
3	OH	OCH_3	OH	H	H	H
4	OH	CH_3	H	OCH_3	OH	H
5	OCH_3	OH	H	H	H	H

Compound **1** was obtained as a red amorphous powder, and the solubility of this isolate was very limited in common organic solvents such as acetone, $CHCl_3$, and MeOH. A molecular formula of $C_{15}H_{10}O_6$ was determined for compound **1** on the basis of the observed sodiated molecular ion peak at *m/z* 309.0370 (calcd for $C_{15}H_{10}O_6$-Na, 309.0375) in its HRESIMS. The NMR spectra (both 1D and 2D) of **1** were acquired using a mixture of $CDCl_3$ and CD_3OD (ca. 5:1) as the solvents. The ^{1}H NMR spectrum of compound **1** displayed signals for a 1,2,4-trisubstituted aromatic ring at δ_H 8.15 (1H, d, *J* = 8.4 Hz, H-8), 7.55 (1H, br s, H-5), and 7.17 (1H, br d, H-7), an aromatic singlet at δ_H 7.32 (1H, s, H-4), and a three-proton singlet at δ_H 4.02 (3H, s, OMe-2) typical for an aromatic methoxy group. In addition to a characteristic methoxy group signal at δ_C 60.9, the ^{13}C NMR spectrum of **1** displayed 14 carbon signals. The chemical shifts of these 14 resonance signals suggested the presence of two aromatic rings and two doubly conjugated carbonyl carbons (δ_C 187.1, 183.2) in the molecule of **1**. These NMR data suggested that compound **1** is an

Table 1. Biological Activity of Compounds from *Morinda citrifolia* Fruits in the Quinone Reductase (QR) Induction Assay

compound	CD, μM (μg/mL)	IC$_{50}$, μM (μg/mL)	CI
1	0.009 (0.0027)	>69.9 (>20)	>7770
2	1.67 (0.52)	>66.6 (>20)	>39.9
L-sulforaphane[b]	0.34 (0.061)	9.77 (1.73)	28.7

[a] CD, concentration required to double QR activity; IC$_{50}$, concentration inhibiting cell growth by 50%; CI, chemoprevention index (=IC$_{50}$/CD). Compounds with CD values of <5 μg/mL are considered significantly active. *[b]* Positive control used.

anthraquinone with one monosubstituted and one trisubstituted benzene ring.[17] The four substituents of **1** are inclusive of three hydroxyl groups and one methoxy group, which could be deduced on the basis of the determined molecular formula. The locations of these functionalities were assigned by the observed HMBC correlations from H-4 to C-2, C-3, C-9a, and C-10, from H-5 to C-7, C-8a, and C-10, and from H-8 to C-6, C-9, and C-10a. Accordingly, the structure of this new isolate (**1**) was determined as 2-methoxy-1,3,6-trihydroxyanthraquinone.

The roots of *M. citrifolia* are well known to contain anthraquinones, and the traditional use of the roots and bark stems for dyeing is attributed to these compounds;[18] however, this is the first report of anthraquinones (**1**−**5**) from noni fruits. All compounds obtained in the present investigation were evaluated for their cancer chemopreventive activity in terms of their ability to induce quinone reductase (QR) activity with cultured murine hepatoma cells.[4,5] The present investigation demonstrated that the new anthraquinone (**1**) is an extremely potent QR inducer (Table 1). The concentration required to double QR activity of compound **1** is almost 40 times less than that of the positive control L-sulforaphane (Table 1), a well-known cancer chemopreventive agent initially isolated from broccoli.[19] Furthermore, this biologically potent lead compound (**1**) demonstrated no discernible cytotoxicity at the highest dose tested and hence is an outstanding lead for further investigation as a potential cancer chemopreventive agent.[20] The potent QR-inducing activity of compound **1** was highly sensitive to structural modifications since the other four anthraquinones isolated and biologically evaluated were only moderately active (**2**) or inactive (**3**−**5**). This may help explain the limited previous interest in anthraquinones as a structural chemotype of phase II enzyme inducers despite the potent activity of compound **1**. In addition, there have been two recent reports linking the ingestion of noni juice with possible hepatotoxicity in three individuals and the authors surmised that it may be due to the presence of anthraquinones.[21,22] This possibility of hepatoxicity warrants further investigation, particularly in light of the recent increase in noni usage.

Experimental Section

Plant Material. The freeze-dried powder of *M. citrifolia* fruits (lot number 0110122) used in this study was obtained from Nature's Sunshine Products, Inc. A representative sample (#N0002) was deposited in the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University. The *n*-BuOH extract of this sample gave a HPLC trace comparable to that reported previously.[3]

Extraction and Isolation. The freeze-dried fruit powder (9 kg) was extracted by maceration with MeOH three times (3 × 20 L) at room temperature, for 2 days each. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract was suspended in H$_2$O (1500 mL), then partitioned in turn with hexane (4 × 2000

mL), CH$_2$Cl$_2$ (3 × 2000 mL), and *n*-BuOH (3 × 2000 mL), to afford 32.7 g of a quinone reductase (QR)-inducing (CD value < 2.5 μg/mL) CH$_2$Cl$_2$ partition.

Therefore, the QR-active CH$_2$Cl$_2$-soluble extract was subjected to vacuum chromatography over a silica gel column (9 × 25 cm), eluted with CHCl$_3$−MeOH (1:0 to 1:3, then pure MeOH), to give 10 fractions (F01−F10). Fraction F04 (CD value < 2.5 μg/mL) was further fractionated using a silica gel vacuum column (9 × 25 cm), eluted with hexane−EtOAc−MeOH (1:0:0 to 1:1:0 to 1:1:0.1 to 1:1:2, then pure MeOH). Further purification of the most active subfractions (F04-04 and -05) using MeOH in a Sephadex LH-20 column yielded compounds **1** (1.6 mg) and **2** (1.8 mg). Balanophonin, β-hydroxypropiovanillone, vanillin, 4-hydroxy-3-methoxycinnamaldehyde, and 1-monopalmitin were also isolated from fraction F04. Compounds **3**−**5** were isolated from F02 (CD < 2.5 μg/mL) after repeated chromatography and were finally purified by reversed-phase HPLC. Scopoletin and β-sitosterol crystallized from F03 and F04, respectively.

2-Methoxy-1,3,6-trihydroxyanthraquinone (1): red amorphous powder; UV (MeOH) λ_{max} (log ϵ) 212 (4.22), 280 (4.37), 301 (3.97, sh), 414 (3.57, br) nm; IR (dried film) ν_{max} 3391, 2926, 2853, 1731, 1595, 1455, 1393, 1365, 1290, 1130, 1092 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 8.15 (1H, d, J = 8.4 Hz, H-8), 7.55 (1H, br s, H-5), 7.32 (1H, s, H-4), 7.17 (1H, br d, H-7), 4.02 (3H, s, OMe-2); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 187.1 (C, C-9), 183.2 (C, C-10), 163.6 (C, C-6), 157.1 (C, C-3), 156.9 (C, C-1), 140.3 (C, C-2), 136.0 (C, C-10a), 130.0 (C, C-4a), 129.8 (CH, C-8), 126.0 (C, C-8a), 121.5 (CH, C-7), 113.5 (CH, C-5), 111.1 (C, C-9a), 109.5 (CH, C-4), 60.9 (CH$_3$, OMe-2); LREIMS (70 eV) *m/z* 286 [M]$^+$ (15), 201 (37), 103 (100); HREIMS *m/z* 309.0370 [M + Na]$^+$ (calcd for C$_{15}$H$_{10}$O$_6$Na, 309.0375).

Quinone Reductase Assay. Using Hepa 1c1c7 murine hepatoma cells, this bioassay was performed as previously described for all extracts, fractions, and pure compounds.[4,5] Briefly, the cells were seeded onto 96-well plates at a density of 1.5 × 10^4 cells/well in 190 μL of media/well and incubated for 24 h. The cells were then dosed with test samples including L-sulforaphane as the positive control and DMSO as the negative control and incubated for 48 h. Two plates are used for each test sample in order to determine both quinone reductase induction activity, by measuring NADPH-dependent menadiol-mediated reduction of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and cytotoxicity, using crystal violet staining. Both the cytotoxicity (IC$_{50}$) and quinone reductase activity (concentration to double enzyme-inducing activity, CD) were measured at 595 nm with an ELISA plate reader.

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