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 known as 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.  

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. In our continuing investigation of botanical dietary supplements for cancer chemopreventive activities, we tested a noni fruit extract for activity in a quinone reductase (QR) induction bioassay. 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 Q10. Hence, QR inducers are sometimes referred to as "indirect antioxidants", and this activity is considered protective at the initiation stage of carcinogenesis. 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 CH2Cl2-soluble partition of the MeOH extract of noni fruits exhibited a potent concentration to double QR (CD) activity value of <2.5 μg/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), 1,3-dihydroxy-2-methoxyanthraquinone (3), 1,6-dihydroxy-5-methoxy-2-methylanthraquinone (4), 2-hydroxy-1-methoxyanthraquinone (5), balanophonin, 4-hydroxy-3-methoxyinnamaldehyde, β-hydroxypropiovanillone, 1-monopalmatin, scopoletin, β-sitosterol, and vanillin. The structures of these known compounds were identified by comparing their physical and spectroscopic data (1H NMR, 13C NMR, DEPT, 2D NMR, and MS) with those of published values or by comparing with an authentic sample (β-sitosterol, scopoletin, and vanillin) directly.

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, CHCl3, and MeOH. A molecular formula of C15H10O6 was determined for compound 1 on the basis of the observed sodiated molecular ion peak at m/z 309.0370 (calcd for C15H10O6 Na, 309.0375) in its HRESIMS. The NMR spectra (both 1D and 2D) of compound 1 were acquired using a mixture of CDC13 and CD3OD (ca. 5:1) as the solvents. The 1H NMR spectrum of compound 1 displayed signals for a 1,2,4-trisubstituted aromatic ring at δH 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 δH 7.32 (1H, s, H-4), and a three-proton singlet at δH 4.02 (3H, s, OMe-2) typical for an aromatic methoxy group. In addition to a characteristic methoxy group signal at δH 60.9, the 13C 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 (δC 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>CD, μM (μg/mL)</th>
<th>IC50, μM (μg/mL)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.009 (0.0027)</td>
<td>&gt;69.9 (&gt;20)</td>
<td>&gt;7770</td>
</tr>
<tr>
<td>2</td>
<td>1.67 (0.52)</td>
<td>&gt;66.6 (&gt;20)</td>
<td>&gt;39.9</td>
</tr>
<tr>
<td>L-sulfonaphane</td>
<td>0.34 (0.061)</td>
<td>9.77 (1.73)</td>
<td>28.7</td>
</tr>
</tbody>
</table>

* a CD, concentration required to double QR activity; IC50, concentration inhibiting cell growth by 50%; CI, chemoprevention index (1/IC50/CD). Compounds with CD values of >5 μg/mL are considered significantly active. Positive control used.

2-Methoxy-1,3,6-trihydroxyanthraquinone (1): red amorphous powder; UV (MeOH) λmax (log ε) 212 (4.22), 280 (4.37), 301 (3.97, sh), 414 (3.57, br) nm; IR (dried film) 3389, 2926, 1731, 1595, 1455, 1393, 1365, 1290, 1130, 1092 cm−1; 1H NMR (CDCl3, 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); 13C NMR (CDCl3, 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), 126.0 (CH), 125.2 (CH), 121.5 (CH, C-7), 111.1 (C, C-9a), 109.5 (CH, C-4a), 60.9 (CH, OMe-2); LREIMS (70 eV) m/z 286 [M]+ (15), 201 (37), 103 (100); HREIMS m/z 309.0370 [M + Na]+ (calcd for C15H10O6Na, 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. Briefly, the cells were seeded onto 96-well plates at a density of 1.5 × 104 cells/well in 190 μL of media/well and incubated for 24 h. The cells were then dosed with test samples including L-sulfonaphane 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-dimethoxy-2-yl-2,5-diphenyltetrazolium bromide (MTT), and cytotoxicity, using crystal violet staining. Both the cytotoxicity (IC50) 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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References and Notes


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