Anti-inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of Morinda citrifolia (Noni)

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A new anthraquinone, 1,5,15-tri-O-methylmorindol (1), and two new saccharide fatty acid esters, 2-O-(β-D-glucopyranosyl)-1-O-hexanoyl-β-D-glucopyranose (4) and 2-O-(β-D-glucopyranosyl)-1-O-octanoyl-β-D-glucopyranose (5), have been isolated from a methanol extract of the fruits of Morinda citrifolia (noni) along with 10 known compounds, namely, two anthraquinones (2, 3), six saccharide fatty acid esters (6–11), an iridoid glycoside (12), and a flavanol glycoside (13). Upon evaluation of six compounds (5–7, 9, 10, and 13) for inhibitory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice, four saccharide fatty acid esters, 5–7 and 9, exhibited potent anti-inflammatory activity, with ID50 values of 0.46–0.79 mg per ear. In addition, when compounds 1–13 were evaluated against the Epstein–Barr virus early antigen (EBV-EA) activation induced by TPA, all of the compounds exhibited moderate inhibitory effects (IC50 values of 386–578 mol ratio/32 pmol TPA).

Morinda citrifolia L. (Rubiaceae), known as “noni”, is a small tree that grows widely across Polynesia. The roots, barks, stems, leaves, and fruits have been used traditionally as a folk medicine for the treatment of many diseases including diabetes, high blood pressure, and cancer. Furthermore, “noni juice”, which is made from the fruits of this plant, is widely consumed today for the purported prevention of lifestyle-related diseases such as diabetes, high blood pressure, cardiopathy, and cerebral apoplexy caused by arteriosclerosis. In this paper, we report the isolation and characterization of three new compounds, 1,5,15-tri-O-methylmorindol (1), 2-O-(β-D-glucopyranosyl)-1-O-hexanoyl-β-D-glucopyranose (4), and 2-O-(β-D-glucopyranosyl)-1-O-octanoyl-β-D-glucopyranose (5), and 10 known compounds, 2, 3, and 6–13, from a methanol (MeOH) extract of the fruits of M. citrifolia L., as well as their inhibitory effects on TPA-induced inflammation in mice and on the EBV-EA activation induced by TPA. This is only the third report of anthraquinones in the fruits of M. citrifolia.

Results and Discussion

Three anthraquinones, 1–3, eight saccharide fatty acid esters, 4–11, an iridoid glycoside, 12, and a flavonol glycoside, 13, were isolated from the MeOH extract of M. citrifolia fruits as described in the Experimental Section.

The molecular formula of 1 was determined to be C18H32O12 on the basis of the [M – H]+ ion observed at m/z 327.0861 in the negative HRESIMS. The 13C NMR spectrum indicated 18 carbon signals, including three methoxy carbons (δC 58.9, 62.2, 62.3), one methylene carbon (δC 69.0), and two carbonyl carbons (δC 181.5, 182.5). In the 1H NMR spectrum, two pairs of ortho-coupled signals [one at δH 7.35 and 8.08 (each 1H, d, J = 8.6 Hz) and the other at δH 7.85 and 8.10 (each 1H, d, J = 8.6 Hz)] were observed. In addition, the presence of three methoxyl groups and one methylene group was suggested from the 1H NMR resonances of δH 3.94, and 4.02 (each 3H, s) and 4.64 (2H, s), respectively. The

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The remaining 13 C NMR signals for the two glucose moieties were glycosylation shifts of the C-2 signal, on comparison with the signal presented in Figure 1. The observation of the ability to induce quinone reductase (QR) activity for an another anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone, from noni fruits has been suggested recently from the analysis of the 1 H and 13 C NMR signals for 3,5,7,3'-di-(O-beta-D-glucopyranosyl)-1-O-beta-D-glucopyranose, which exhibited potent inhibitory activity in the mouse ear edema assay, in addition to an anthraquinone, 1, which showed potent inhibitory effect against EBV-EA activation induced by TPA, may be potential inhibitors of tumor promotion (potential cancer chemopreventive agent). Potent cancer chemopreventive activity for another anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone, from noni fruits has been suggested recently from the observation of the ability to induce quinone reductase (QR) activity with cultured murine hepatoma cells. In view of the widespread use of noni fruits as a botanical dietary supplement and the few reports that have described the chemical constituents of the fruits, it might be worthwhile to undertake further investigation of the bioactive constituents of noni fruits including those with potential anti-inflammatory and cancer chemopreventive activities.

Experimental Section

General Experimental Procedures. Crystallizations were performed in EtOAc–MeOH, and melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 polarimeter in MeOH at 25 °C. IR spectra were recorded in KBr disks. NMR spectra were recorded on a JEOL ECA-600 (1 H, 600 MHz; 13 C, 150 MHz) or with a JEOL LA-500 (1 H, 500 MHz; 13 C, 125 MHz) spectrometer in CD3OD or in CDCl3 with tetramethylsilane as an internal standard. ESIMS and HRESIMS were recorded on an Agilent 1100 LC/MSD TOF (time-of-flight) system [ionization mode: positive; nebulizing gas (N2) at m/z 491.2110, suggesting the molecular formula C20 H36 O12. Compound was determined as 2-O-(beta-D-glucopyranosyl)-1-O-beta-D-glucopyranopyranose. The positive HRESIMS of compound 5 exhibited a sodiated molecular ion [M + Na]+ at m/z 491.2110, suggesting the molecular formula C20 H36 O12. Compound 5 exhibited two anomic signals at ðH 4.56 and 5.60 in the 1 H NMR spectrum, and this spectrum was almost identical with that of 4. Only slight differences were observed in the high-field region, where, instead of the signals for a hexanoyl moiety, signals for an octanoyl moiety were observed. This observation was supported by the 13 C NMR spectrum, which showed signals at ðC 14.4 (q), 23.6 (t), 25.5 (t), 30.1 (t, 2 × C), 32.8 (t), 34.9 (t), and 174.0 (s), assignable to an octanoyl moiety. The remaining 13 C NMR signals for the two glucose moieties were almost identical with those of 4. Analysis of the 1 H–1 H COSY, NOESY, HMQC, and HMBC spectra led to the assignment of all of the 1 H and 13 C NMR signals for 5. Thus, compound 5 was determined as 2-O-(beta-D-glucopyranosyl)-1-O-octanoyl-beta-D-glucopyranopyranose.

Ten other compounds isolated from the MeOH extract of M. citrifolia fruits were identified as the known compounds, 5,15-di-0-methylmorindol (2), anthragallol 2-methyl ether (3), 6-O-(beta-D-glucopyranosyl)-1-O-hexanoyl-beta-D-glucopyranose (6), 6-O-(beta-D-glucopyranosyl)-1-O-octanoyl-beta-D-glucopyranose (7), 2,6-Di-O-(beta-D-glucopyranosyl)-1-O-hexanoyl-beta-D-glucopyranose (8), 2,6-Di-O-(beta-D-glucopyranosyl)-1-O-octanoyl-beta-D-glucopyranose (9), 3-methylbut-3-enyl-beta-D-glucopyranopyranose (10), 3-methylbut-3-enyl-6-O-beta-D-glucopyranosyl-beta-D-glucopyranose (11), 3,4'-pentahydroxyflavone), a known inhibitor of TPA-induced inflammation in mice, and indomethacin, a commercially available anti-inflammatory drug, as shown in Table 1. Four saccharide fatty acid esters, 5–7 and 9, exhibited potent inhibitory activity, with ID50 (50% inhibitory dose) values of 0.46–0.79 mg/ear, which were more highly inhibitory than quercetin (ID50 1.6 mg/ear) while less inhibitory than indomethacin (ID50 0.30 mg/ear).

The inhibitory effect on EBV-EA activation induced by TPA was further examined as a preliminary evaluation of the potential anti-tumor-promoting effects of the 13 compounds, 1–13. The results are shown in Table 1, together with comparable data for quercetin as well as beta-carotene, a vitamin A precursor that has been intensively studied in cancer chemoprevention by using in vitro, in vivo, and epidemiological test systems. All of the compounds tested showed inhibitory effects, with IC50 values of 386–512 mol ratio/32 pmol TPA, which were almost comparable with or more inhibitory than quercetin (IC50 560 mol ratio/32 pmol TPA) while, except for 1 (386 mol ratio/32 pmol TPA), less inhibitory than beta-carotene (397 mol ratio/32 pmol TPA).

Anthraquinones appear to be rare in the fruits of M. citrifolia, whereas the roots of noni are well known to contain these compounds. Since the inhibitory effect against TPA-induced inflammation has been demonstrated to closely parallel that of the inhibition of tumor promotion in two-stage carcinogenesis initiated by 7,12-dimethylbenz[a]anthracene (DMBA) and promoted by TPA in a mouse skin model, four saccharide fatty acid esters, 5–7 and 9, which exhibited potent inhibitory activity in the mouse ear edema assay, in addition to an anthraquinone, 1, which showed potent inhibitory effect against EBV-EA activation induced by TPA, may be potential inhibitors of tumor promotion (potential cancer chemopreventive agent). Potent cancer chemopreventive activity for another anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone, from noni fruits has been suggested recently from the observation of the ability to induce quinone reductase (QR) activity with cultured murine hepatoma cells. In view of the widespread use of noni fruits as a botanical dietary supplement and the few reports that have described the chemical constituents of the fruits, it might be worthwhile to undertake further investigation of the bioactive constituents of noni fruits including those with potential anti-inflammatory and cancer chemopreventive activities.

Plant Material. Morinda citrifolia L. (Rubiaceae) was cultivated on a farm at Nakajo (Okinawa prefecture, Japan), and the fruit was harvested from a 2-year-old tree in April 2004. The plant was...
authenticated by one (H.K.) of the authors, and a voucher specimen (No. 024040) has been deposited in the Research Laboratory, Nakazeki Co. Ltd.

Chemicals and Reagents. Chemicals were purchased as follows: TPA from ChemSyn Laboratories (Lenexa, KS), quercetin, indomethacin, hydrocortisone, and β-carotene from Sigma Chemical Co. (St. Louis, MO), and the EBV cell culture reagents and n-butanonic acid from Nacalai Tesque, Inc. (Kyoto, Japan).

Extraction and Isolation. Air-dried and powdered fruits (1.31 kg) from the fresh fruits (24.6 kg) of *M. citrifolia* were extracted three times with MeOH (reflux, 3 h) to yield a MeOH extract (228 g). This extract was suspended in water and partitioned successively with CHCl₃, hydrocortisone, and TPA from ChemSyn Laboratories (Lenexa, KS), quercetin, indomethacin, hydrocortisone, and β-carotene from Sigma Chemical Co. (St. Louis, MO), and the EBV cell culture reagents and n-butanonic acid from Nacalai Tesque, Inc. (Kyoto, Japan).

<table>
<thead>
<tr>
<th>compound</th>
<th>inhibition of inflammation</th>
<th>percentage of EBV-EA induction*</th>
<th>IC₅₀&lt;sup&gt;†&lt;/sup&gt; (mol ratio/32 pmol TPA)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ID₅₀&lt;sup&gt;‡&lt;/sup&gt; (mg/ear)</td>
<td>concentration (mol ratio/32 pmol TPA)</td>
<td></td>
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<tr>
<td>1</td>
<td>1,5,15-tri-O-methylmorindol</td>
<td>10.1 (60)</td>
<td>7.56</td>
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<tr>
<td>2</td>
<td>5,15-di-O-methyl morindol</td>
<td>13.7 (60)</td>
<td>4.51</td>
</tr>
<tr>
<td>3</td>
<td>anthragallol 2-methyl ether</td>
<td>14.3 (60)</td>
<td>46.7</td>
</tr>
<tr>
<td>4</td>
<td>2-O-[β-D-glucopyranosyl]-1-O-hexanyl-β-D-glucopyranose</td>
<td>16.0 (70)</td>
<td>61.5</td>
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<td>5</td>
<td>2-O-[β-D-glucopyranosyl]-1-O-octanoyl-β-D-glucopyranose</td>
<td>15.3 (60)</td>
<td>56.4</td>
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<td>6</td>
<td>6-O-[β-D-glucopyranosyl]-1-O-hexanoyl-β-D-glucopyranose</td>
<td>15.6 (60)</td>
<td>59.2</td>
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<td>6-O-[β-D-glucopyranosyl]-1-O-octanoyl-β-D-glucopyranose</td>
<td>15.3 (60)</td>
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<td>8</td>
<td>2,6-di-O-[β-D-glucopyranosyl]-1-O-hexanoyl-β-D-glucopyranose</td>
<td>14.1 (60)</td>
<td>58.6</td>
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<tr>
<td>9</td>
<td>2,6-di-O-[β-D-glucopyranosyl]-1-O-octanoyl-β-D-glucopyranose</td>
<td>16.1 (70)</td>
<td>61.9</td>
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<td>10</td>
<td>3-methylbut-3-enyl-β-D-glucopyranose</td>
<td>&gt;1.0</td>
<td>10.5 (60)</td>
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<td>11</td>
<td>12-methylbut-3-enyl-6-O-[β-D-glucopyranosyl]-β-D-glucopyranose</td>
<td>14.2 (60)</td>
<td>58.3</td>
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<tr>
<td>12</td>
<td>asperulosidic acid</td>
<td>13.5 (60)</td>
<td>47.2</td>
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<tr>
<td>13</td>
<td>rutin</td>
<td>&gt;1.0</td>
<td>16.2 (70)</td>
</tr>
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</table>

* Values represent percentages relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells. †IC₅₀ represents the molar ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA.

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Table 1. Inhibitory Effects of Compounds from *Morinda citrifolia* Fruits and Reference Compounds on TPA-Induced Inflammation in Mice and on the Induction of Epstein–Barr Virus Early Antigen

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1,5,15-Tri-O-(1-hydroxy-1,5-dimethoxy-2-methoxy-methylamthraquinone) (1); yellow-brown, fine needles; mp 186–190 °C; UV (MeOH) λ<sub>max</sub> (log ε) 218 (4.18), 248 (4.15), 270 (4.12), 355 (3.57) nm; IR (KBr) ν<sub>max</sub> 3388 (OH), 2927, 1666 (C=O), 1576 cm<sup>−1</sup>; 1<sup>H</sup> NMR (500 MHz, CDCl₃) δ 8.10 (1H, d, J = 8.6 Hz, H-4), 8.08 (1H, d, J = 8.6 Hz, H-8), 7.85 (1H, d, J = 8.6 Hz, H-3), 7.35 (1H, d, J = 8.6 Hz, H-7), 4.64 (2H, s, H-15), 4.02 (3H, s, OMe-5), 3.94 (3H, s, OMe-1), 3.50 (3H, s, OMe-15); 1<sup>3</sup>C NMR (125 MHz, CDCl₃) δ 182.5 (s, C-10), 181.5 (s, C-19), 150.0 (s, C-14), 135.9 (s, C-6), 141.6 (s, C-5), 135.0 (s, C-2), 138.5 (s, C-13), 133.6 (d, C-3), 128.8 (s, C-12), 125.6 (d, C-8), 125.0 (s, C-13), 124.9 (s, C-11), 123.4 (d, C-4), 120.4 (d, C-7), 69.0 (t, C-15), 58.2 (q, OMe-5), 58.9 (q, OMe-15); HMBC data, see Table S1; negative HRESIMS m/z 327.0861 [M – H]<sup>+</sup> (calcld for C₁₈H₁₅O₆, 327.0868).

2-O-[β-D-Glucopyranosyl]-1-O-hexanoyl-β-D-glucopyranose (4); colorless gum; [α]<sub>D</sub><sup>20</sup> +139 (c 1.02, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 204 (2.67) nm; IR (KBr) ν<sub>max</sub> 3378 (OH), 2927, 1754 (C=O), 1641 cm<sup>−1</sup>; 1<sup>H</sup> NMR (600 MHz, CDCl₃) δ 5.61 (1H, d, J = 7.9 Hz, H-1′), 5.56 (1H, d, J = 7.9, 9.3 Hz, H-1′′), 3.83 (2H, d, J = 12.1, 2.1 Hz, Hb-6′′ and Hb-6′), 3.68 (2H, d, J = 4.8, 12.1 Hz, Ha-6′ and Ha-6″), 3.63 (1H, dd, J = 8.2, 9.3 Hz, H-3′), 3.59 (1H, dd, J = 7.9, 9.3 Hz, H-2′), 3.40 (1H, H-5′), 3.38 (1H, dd, J = 7.6, 9.3 Hz, H-4′), 3.36 (1H, dd, J = 8.6, 9.3 Hz, H-3′′), 3.30 (1H, dd, J = 8.2, 9.3 Hz, H-4′′), 3.28 (1H, H-5′′), 3.19 (1H, dd, J = 7.9, 9.3 Hz, H-2″), 3.19 (1H, dd, J = 7.9, 9.3 Hz, H-2″′), 2.47 (1H, d, J = 16.5, 7.6 Hz, H-b2), 2.38 (1H, d, J = 16.5, 7.6 Hz, H-a2), 1.63 (2H, quint., J = 7.3 Hz, H-1″), 1.34 (4H, H-4 and H-5), 0.92 (3H, t, J = 7.0 Hz, H-6); 1<sup>3</sup>C NMR (150 MHz, CDCl₃) δ 174.1 (s, C-1′), 106.4 (d, C-1″′), 94.8 (d, C-1″), 83.3 (d, C-2″), 79.5 (d, C-5″), 79.0 (d, C-5″′), 78.6 (2 × C, d, C-3′ and C-3″′), 76.8 (d, C-2″′), 72.3 (d, C-4″′), 71.6 (d, C-4″), 63.6 (t, C-6″′), 63.1 (d, C-6″), 61.4 (d, C-6″).
35.7 (t, C-2), 33.1 (t, C-4), 26.0 (t, C-3), 24.2 (t, C-5), 15.0 (q, C-6); HMBC data, see Table S2; positive HRESIMS m/z 463.1788 [M + Na]+ (calcd for C₃₆H₅₈O₁₄Na, 463.1791).

2-O-(β-D-Glucopyranosyl)-1-O-octanoyl-β-D-glucopyranose (5): colorless gum; [α]D₂₀ = -2.4 (c 1.95, MeOH); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)) 215 (2.83) nm; IR (KBr) \( \nu_{\text{max}} \) 3402 (OH), 2927, 1745 (C=O), 1641 cm⁻¹; ¹H NMR (600 MHz, CD₃OD) \( \delta \) 6.00 (1H, d, J = 7.5 Hz, H-1′′); 5.56 (1H, d, J = 7.5 Hz, H-1″), 3.83 (2H, dt, J = 2.1, 2.0 Hz, Hb-6″ and Hb-6′); 3.68 (2H, dd, J = 4.5, 12.1 Hz, Ha-6″ and Ha-6′), 3.62 (1H, dd, J = 7.7, 9.2 Hz, H-2′′), 3.59 (1H, dd, J = 7.5, 9.2 Hz, H-3′), 3.38 (1H, dd, J = 7.9, 9.2 Hz, H-3″), 3.27 (1H, H-4″), 3.19 (1H, dd, J = 7.5, 9.2 Hz, H-2″), 2.47 (1H, dt, J = 16.3, 7.4 Hz, Hb-2), 2.38 (1H, dt, J = 16.3, 7.4 Hz, Ha-2), 1.63 (2H, quint., J = 7.2 Hz, H-3), 1.33 (8H, H-4, H-5, H-6, and H-7), 0.90 (t, J = 6.9 Hz, H-8); ¹³C NMR (150 MHz, CD₃OD) \( \delta \) 174.0 (s, C-1), 105.6 (d, C-1″), 94.0 (d, C-1′), 82.5 (d, C-2′′), 78.7 (d, C-5″), 78.2 (d, C-5′′), 77.8 (2 × C, d, C-3′ and C-3″), 76.0 (d, C-2′), 71.4 (d, C-4″), 70.8 (d, C-4′), 62.7 (t, C-6″), 62.2 (d, C-6′), 34.9 (t, C-2), 32.8 (t, C-6), 30.1 (2 × C, t, C-4 and C-5), 25.5 (t, C-3), 23.6 (t, C-7), 14.4 (t, C-8); HMBC data, see Table S2; positive HRESIMS m/z 491.2110 [M + Na]+ (calcd for C₂₀H₃₆O₁₂Na, 491.2104).

Assay of TPA-Induced Inflammation Ear Edema in Mice. For the protocol for this in vivo assay, refer to a previous article.¹⁹

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Supporting Information Available: Tables of HMBC NMR data for compounds 1, 4, and 5. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes