Chemical Constituents of the Fruits of *Morinda citrifolia* (Noni) and Their Antioxidant Activity

Bao-Ning Su,[†,‡] Alison D. Pawlus,[†,‡] Hyun-Ah Jung,[§] William J. Keller,[⊥] Jerry L. McLaughlin,[⊥] and A. Douglas Kinghorn*[,†,‡]

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, and Nature's Sunshine Products, Inc., 1655 N. Main Street, Spanish Fork, Utah 84660

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Purification of a *n*-BuOH-soluble partition of the MeOH extract of *Morinda citrifolia* (Noni) fruits led to the isolation of two new iridoid glucosides, 6α-hydroxyadoxoside (**1**) and 6*β*,7*β*-epoxy-8-*epi*-splendoside (**2**), as well as 17 known compounds, americanin A (**3**), narcissoside (**4**), asperuloside, asperulosidic acid, borreriagenin, citrifolinin B epimer a, citrifolinin B epimer b, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, *epi*-dihydrocornin, D-glucose, D-mannitol, methyl α-D-fructofuranoside, methyl *β*-D-fructofuranoside, nicotifloroside, and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside. The structures of the new compounds were determined by spectroscopic data interpretation. Compound **4**, borreriagenin, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, *epi*-dihydrocornin, methyl α-D-fructofuranoside, and methyl *β*-D-fructofuranoside were isolated for the first time from *M. citrifolia*. The antioxidant activity was evaluated for all isolates in terms of both DPPH and $ONOO^-$ bioassays. The neolignan, americanin A (**3**), was found to be a potent antioxidant in these assays.

Morinda citrifolia L. (Rubiaceae), commonly called Noni or Indian mulberry, is a small evergreen tree or shrub of Polynesian origin.[1] *M. citrifolia* bears a lumpy, green to yellowish-white fruit, normally 5 to 10 cm in length, with a surface covered in polygonal-shaped sections.[1,2] *M. citrifolia* has a long history of use as a medicinal plant in parts of Southeast Asia, Polynesia, and Australia and is considered to be the second most important medicinal plant in the Hawaiian Islands.[1,3] The leaves, roots, bark, and fruits have all been used medicinally to treat a wide range of ailments. These include, but are not limited to, diabetes, diarrhea, hypertension, malaria, pain, and topical infections.[1,4] The fruits are also eaten as a food, but primarily only in times of famine.[4]

Today, the use of *M. citrifolia* in the United States is becoming more widespread and Noni products are commercially available in health food stores, chain grocery stores specializing in natural foods, and on the Internet. Both the leaves and the fruits are being sold in tablet, tea, and juice form, although the fruit as a juice is the predominant formulation sold. This growth in popularity in the United States may in part be attributed to claims of Noni being a "cure-all" or aid in relieving symptoms for a host of chronic conditions such as arthritis, cancer, diabetes, and hypertension.[1] A number of in vitro biological activities have been reported, such as angiogenesis inhibition,[5] antioxidant,[6] cyclooxygenases-1 and -2 inhibition,[7,8] and tyrosine kinase inhibition.[9] Most of these studies have involved crude extracts or fractions of *M. citrifolia*, and the compound(s) responsible for the biological activities have not been determined. In addition, an in vivo study using the ethanol-insoluble precipitate of *M. citrifolia* fruits, given intraperitoneally to mice with Lewis lung carcinoma implanted cells, demonstrated a significant life-prolonging effect that was increased when given in conjunction with several chemotherapeutic agents.[10] Currently, a freeze-dried Noni fruit extract is in phase I clinical trials at the Cancer Research Center of Hawaii in cancer patients for which there is no other standard treatment available. The aim of this trial is to determine if Noni extract can be useful to cancer patients for antitumor and/or symptom control.[11]

To date, the major chemical constituents of this plant have been found to be anthraquinones,[12] flavonol glycosides,[13] iridoid glycosides,[13,14] lipid glycosides,[15] and triterpenoids.[14] In the present study, the *n*-BuOH-soluble partition part of the MeOH extract of *M. citrifolia* fruits was found to have moderate antioxidant activity in a free-radical (DPPH) scavenging bioassay. This partition was purified by repeated chromatography, which led to the isolation of two new iridoid glucosides, 6α-hydroxyadoxoside (**1**) and 6*β*,7*β*-epoxy-8-*epi*-splendoside (**2**), and 17 known compounds. All isolates obtained in this study were evaluated for their antioxidant activity, and the neolignan, americanin A (**3**), demonstrated significant antioxidant activity in two antioxidant bioassays.

Compound **1**, $[\alpha]^{23}_D$ −50.7° (*c* 0.28, MeOH), was obtained as a colorless gum by repeated chromatography and finally purified by reversed-phased HPLC. A molecular formula of $C_{17}H_{26}O_{11}$ was established for **1** on the basis of the observed sodiated molecular ion peak at *m/z* 429.1378 $[M + Na]^+$ in its HRESIMS (calcd for $C_{17}H_{26}O_{11}Na$, 429.1373). The IR absorption bands at 3355 and 1692 cm^{-1}, respectively, indicated the presence of hydroxyl groups and a carbonyl group in the molecule. The characteristic ^1H NMR (in CD_3OD) signals at δ_H 7.46 (1H, d, J = 1.2 Hz, H-3), 5.15 (1H, d, J = 6.0 Hz, H-1), and 4.65 (1H, d, J = 7.9 Hz, Glc-1) and ^{13}C NMR (in CD_3OD) signals at δ_C 169.5 (C, C-11), 153.1 (CH, C-3), 113.0 (C, C-4), 100.6 (CH, Glc-1), and 99.1 (CH, C-1) suggested that compound **1** is an iridoid glucoside.[16,17] The observed HMBC correlation from the anomeric proton (Glc-1) of glucose to the acetal carbon at δ_C 99.1 (C-1) indicated that the location of the glucose unit is at C-1 in the molecule of **1**. The presence of a methyl

* To whom correspondence should be addressed. Tel: +1-614-247-8094. Fax: +1-614-247-8081. E-mail: kinghorn.4@osu.edu.
† Program for Collaborative Research in the Pharmaceutical Sciences.
‡ Present address: The Ohio State University.
§ The Ohio State University.
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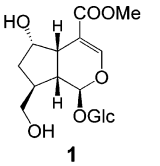

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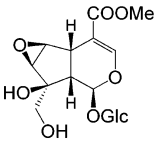

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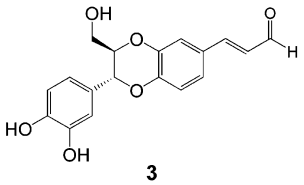

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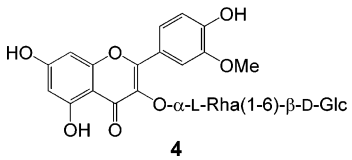

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ester group at C-4 was readily assigned on the basis of the chemical shifts of H-3, C-3, C-4, and C-11, as well as the observed correlations from both H-3 (δ_H 7.46) and the methoxy (δ_H 3.69) signals to the carbonyl carbon (δ_C 169.5) in the HMBC spectrum of **1**. Generally, the five- and six-membered rings of iridoids are *cis*-fused,[18] although a few *trans*-fused iridoids also have been reported.[16,19] The chemical shifts of C-1 are about 103 ppm and 96−100 ppm for *trans*- and *cis*-fused iridoids, respectively.[16] Therefore, the rings are *cis*-fused in the molecule of **1**, since the C-1 signal was detected at 99.1 ppm in the ^{13}C NMR spectrum of this isolate. Further analysis of the observed 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC) correlations indicated the gross structure of compound **1** to be 6-hydroxyadoxoside.[16,17] A literature survey revealed that 6β-hydroxyadoxoside has been previously isolated from *Bessaya plantaginea*, and a doublet of doublets with the coupling constants of 3.7 and 7.8 Hz was reported for H-6 of this compound.[17] However, in the ^1H NMR spectrum of **1**, the resonance signal of H-6 was displayed as a broad singlet at δ_H 4.29. This suggested that compound **1** is 6α-hydroxyadoxoside, which was confirmed by a ROESY NMR correlation from H-6 to H-9.

Compound **2** was isolated as a colorless gum, $[\alpha]^{23}_D$ −108.4° (*c* 0.17, MeOH). A molecular formula of $C_{17}H_{24}O_{12}$, indicating six degrees of unsaturation, was assigned to **2** on the basis of its HRFABMS (found *m/z* 443.1147, calcd for $C_{17}H_{24}O_{12}Na$, *m/z* 443.1166). Seventeen carbon signals, including one methoxy group, two methylenes, 11 methines, and three quaternary carbons, were evident from the ^{13}C NMR and DEPT data of **2**. Similar to **1**, the resonance signals of two doubly oxygenated methines were displayed at δ_C 99.9 (Glc-1) and 93.8 (C-1) in the ^{13}C NMR and DEPT spectra of **2**. This, in combination with the observed ^1H NMR signals at δ_H 5.76 (1H, s, H-1) and 4.56 (1H, d, J = 8.0 Hz, Glc-1), suggested that compound **2** is also an iridoid glucoside.[16−18] In the same manner as for **1**, the locations of the glucose unit and the methyl ester group in **2** were also determined at C-1 and C-4 on the basis of the observed HMBC correlations from the anomeric proton (Glc-1) of glucose to the acetal carbon at δ_C 93.8 (C-1) and from both H-3 (δ_H 7.48) and the methoxy (δ_H 3.73) signals to the carbonyl carbon (δ_C 168.5), respectively. The presence of an epoxy group between C-6 and C-7 and the hydroxyl groups at C-8 and C-10 was assigned by further analysis of the chemical shifts of the remaining protons and carbons, the observed 2D NMR correlations ($^1H-^1H$ COSY, HMQC, and HMBC), and the determined unsaturation value of **2**. In the NOESY NMR spectrum (Figure 1) of **2**, correlations from H-5 to H-9 and from H-6 to H-7 were evident, but there was no correlation from H-5 to H-6 or from H-9 to H-7, indicating the β-, α-, α-, and β-orientations of H-5, H-6, H-7, and H-9, respectively. Another key correlation observed in the NOESY spectrum (Figure 1) of **2** was observed between H-1 (δ_H 5.76, 1H, s) and H-10a (δ_H 3.68, 1H, d, J = 11.7 Hz), which was suggestive of an α-orientation of the hydroxymethylene group at C-8. This was consistent with absence of the NOESY correlation from H-9 to both H-10a and H-10b. Accordingly, the structure of this new iridoid glucoside was determined as 6β,7β-epoxy-8-*epi*-splendoside (**2**).

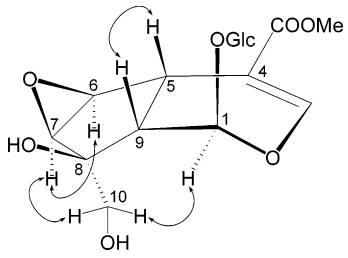

Figure 1. Selected NOESY correlations for 6β,7β-epoxy-8-*epi*-splendoside (**2**).

In addition to compounds **1** and **2**, 17 known compounds, americanin A (**3**),[20,21] narcissoside (**4**),[22] asperuloside,[23] asperulosidic acid,[15] borreriagenin,[24] citrifolinin B epimer a,[13] citrifolinin B epimer b,[13] cytidine,[25] deacetylasperuloside,[23] dehydromethoxygaertneroside,[26] *epi*-dihydrocornin,[27] D-glucose,[28] D-mannitol,[29] methyl α-D-fructofuranoside,[30] methyl β-D-fructofuranoside,[30] nicotifloroside,[31] and β-sitosterol 3-*O*-β-D-glucopyranoside, were also isolated in the present study. The structures of these known compounds were identified by comparing their physical and spectroscopic data ($[\alpha]_D$, ^1H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) with those of published values or by comparing with an authentic sample (β-sitosterol 3-*O*-β-D-glucopyranoside) directly. Americanin A (**3**) was initially isolated from *Phytolacca americana* in 1978,[20] and its structure was revised in 1986.[21] The structures of americanin A (**3**) and its regioisomer, isoamericannin A, were confirmed by total synthesis.[32] The ^1H and ^{13}C NMR data of these two neolignans are very similar.[32] Compound **3** obtained in the present study was assigned as americanin A on the basis of the observed key correlation from H-7 to C-4′ in the HMBC spectrum acquired on a 600 MHz NMR spectrometer using a cryoprobe (Supporting Information). Among these isolates, compound **4**, borreriagenin, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, *epi*-dihydrocornin, methyl α-D-fructofuranoside, and methyl β-D-fructofuranoside were isolated from *M. citrifolia* for the first time. One of the major compounds obtained in the present study is an iridoid glucoside, asperuloside (~0.08% w/w). The presence of this compound in the *n*-BuOH-soluble extract was confirmed by LC-MS analysis (Supporting Information).

The antioxidant ability of all isolates obtained in this study to scavenge DPPH, authentic $ONOO^-$, and 3-morpholinosydnonimine (SIN-1)-derived $ONOO^-$ was evaluated. As summarized in Table 1, the neolignan, americanin

Table 1. Antioxidant Activity of Compounds Isolated from *M. citrifolia* Fruits[a]

compound	DPPH (IC_{50} μM)	peroxynitrite: authentic $ONOO^-$ (IC_{50} μM)	peroxynitrite: SIN-1-derived $ONOO^-$ (IC_{50} μM)
3	16.9	3.3	25.8
4	ND[b]	3.8	9.6
gallic acid[c]	2.7		
penicillamine[c]		3.3	4.4

[a] All compounds obtained in this study were evaluated in both the DPPH and peroxynitrite free-radical scavenging assays. Except for compounds **3** and **4**, all other isolates were indicated to be inactive (IC_{50} values over 30 μM). [b] Not determined, since compound **4** was not active in the DPPH assay. [c] Positive controls used.

A (**3**), was found to be a potent antioxidant in these bioassays. The flavonol glycoside, narcissoside (**4**), exhibited evident scavenging activity against both authentic $ONOO^-$ and SIN-1-derived $ONOO^-$. However, another structurally similar flavonol glycoside, nicotifloroside (unsubstituted instead of having a methoxy group at C-3′ as in **4**), was found to be inactive. The isolation and characterization of **3** as an antioxidant constituent of *M. citrifolia* fruits thus provides a basis for the previous literature reports of the antioxidant effects of extracts of this species.[6,33] Several lignans and neolignans including compound **3** were reported from *M. citrifolia* fruits very recently.[34] The copper-induced low-density lipoprotein oxidation inhibition activities of these isolates were determined.[34]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. The UV spectra were obtained with a Beckman DU-7 spectrometer, and the IR spectra run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature on a Bruker Advance DPX-300, 360, 400, or DRX-500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. The HMBC spectrum of americanin A (**3**) was acquired on a Bruker Advance DRX-600 NMR spectrometer with a TXI cryoprobe. Standard pulse sequences were employed for the measurement of 2D NMR spectra ($^1H-^1H$ COSY, HMQC, HMBC, and NOESY). FABMS was obtained on a VG 7070E-HF sector-field mass spectrometer, while CIMS, EIMS, and ESIMS were performed on a Finnigan/MAT 90/95 sector-field mass spectrometer. A YMC-pack ODC-AQ column (5 μm, 25 × 2 cm i.d., YMC Co., Wilmington, NC) was used for semipreparative HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual λ absorbance detector (Waters, Milford, MA). Column chromatography was carried out with silica gel G (Merck, 70−230 or 230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on 250 μm thickness Merck Si gel 60 F_{254} aluminum plates.

Plant Material. The freeze-dried fruit powder of *M. citrifolia* (lot number 229) used in this study was obtained from Nature's Sunshine Products, Inc. A representative sample (# N0001) was deposited in the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University. LC-MS traces of the *n*-BuOH-soluble extract of *M. citrifolia* fruits are included in the Supporting Information.

Extraction and Isolation. The freeze-dried fruit powder (1 kg) was extracted by maceration with MeOH three times (3 × 4.5 L) at room temperature, for 3 days each. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract was suspended in H_2O (800 mL), then partitioned in turn with petroleum ether (3 × 1000 mL), $CHCl_3$ (4 × 1000 mL), and *n*-BuOH (4 × 500 mL), to afford dried petroleum ether- (102 g), $CHCl_3$- (12.5 g), *n*-BuOH (41.2 g), and H_2O-soluble (~55 g) extracts. The *n*-BuOH-soluble partition part of the MeOH extract was found to be active (56.2% at 200 μg/mL) in a DPPH free-radical scavenging bioassay.

Therefore, the *n*-BuOH-soluble extract was subjected to chromatography over a silica gel column (9 × 45 cm), eluted with $CHCl_3$−MeOH (15:1 to 1:1, then pure MeOH), to give seven fractions (F01−F07). Compound **3** (6.5 mg; 0.00065% w/w) was obtained as a white amorphous powder from a $CHCl_3$−MeOH mixture (ca. 10:1) of fraction F01, eluted with $CHCl_3$−MeOH (15:1). Fraction F04 (9.17 g), eluted with $CHCl_3$−MeOH (8:1), was chromatographed over a silica gel column (5 × 45 cm), eluted with EtOAc−MeOH (10:1 to 2:1), and five combined subfractions were obtained (F0401−F0405). F0401 was further purified over a Sephadex LH-20 column (2.8 × 45 cm), using pure MeOH for elution, and afforded borreriagenin (200 mg; 0.02% w/w) and cytidine (58 mg; 0.0058% w/w), and a mixture. This mixture was purified by semipreparative HPLC, by eluting with MeOH−H_2O (40:60; 8 mL/min), to afford pure asperuloside (t_R = 6.5 min, 64 mg; 0.0064% w/w) and dehydromethoxygaertneroside (t_R = 28.5 min, 1.6 mg; 0.00016% w/w). Asperuloside (750 mg; 0.075% w/w) was obtained as a white amorphous powder from a solution (EtOAc−MeOH, ca. 8:1) of F0402 as a major component. Fraction F05 (4.0 g), eluted with $CHCl_3$−MeOH (6:1), was chromatographed over a silica gel column (5 × 40 cm), eluted with EtOAc−MeOH (10:1 to 2:1), to give pure asperulosidic acid (400 mg; total yield 0.0435% w/w; an additional 35 mg was isolated from F0602) and four subfractions (F0501−F0504). F0502 was further fractionated over a Sephadex LH-20 column (2.8 × 45 cm), eluted using pure MeOH, and gave three fractions (F050201−F050203). F050201 was finally purified by preparative TLC (500 μM; 20 × 20 cm), with $CHCl_3$−MeOH (4:1) as developing solvent, to give *epi*-dihydrocornin (R_f = 0.54, 4.5 mg; 0.00045% w/w) and a mixture of citrifolinin B epimer a and citrifolinin B epimer b (R_f = 0.50, 6.0 mg; 0.0006% w/w) in a ratio of 1:1 (integration of ^1H NMR signals). F0503 was purified by HPLC using MeOH−H_2O (40:60; 8 mL/min) as solvent, to afford two flavonol glycosides, nicotifloroside (t_R = 35.0 min, 16 mg; 0.0016% w/w) and compound **4** (t_R = 39 min, 11 mg; 0.0011% w/w). β-Sitosterol 3-*O*-β-D-glucopyranoside (58 mg; 0.0058% w/w) was obtained as an amorphous solid from a $CHCl_3$−MeOH (~6:1) solution of F0504. Fraction F06, eluted with $CHCl_3$−MeOH (4:1), was separated over a Sephadex LH-20 column (2.8 × 45 cm), eluted with MeOH, and afforded three subfractions (F0601−F0603). F0602 was then purified by semipreparative HPLC using MeOH−H_2O (30:70; 7 mL/min) as eluent, to afford compound **1** (t_R = 16.7 min, 7.5 mg; 0.00075% w/w) and asperulosidic acid (t_R = 22.0 min, 35 mg; total yield 0.0435% w/w; an additional 400 mg was isolated from F05). F0603 was chromatographed over a silica gel column (5.0 × 40 cm) using $CHCl_3$−MeOH−H_2O (from 8:1:0.05 to 2:1:0.1) as solvent system, to give methyl α-D-fructofuranoside (35 mg; 0.0035% w/w) and methyl β-D-fructofuranoside (105 mg; 0.011% w/w), five subfractions (F060301−F060305), and the mixture of α- and β-D-glucopyranoses (250 mg; 0.025% w/w) in a ratio of 10:3 (based on the integrations of ^1H NMR spectra in DMSO-d_6), in order of polarity. F060302 was finally purified by semipreparative HPLC using MeOH−H_2O (30:70; 6 mL/min) as solvent, to give deacetylasperuloside (t_R = 19.5 min, 13 mg; 0.0013% w/w) and compound **2** (t_R = 21.5 min, 1.8 mg; 0.00018% w/w). D-Mannitol (180 mg; 0.018% w/w) was obtained as colorless needles from a $CHCl_3$−MeOH (ca. 2:1) solution of fraction F07.

6α-Hydroxyadoxoside (1): colorless gum; $[\alpha]^{23}_D$ −50.7° (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (3.55) nm; IR (dried film) ν_{max} 3355, 1692, 1635, 1294, 1077, 807 cm^{-1}; ^1H NMR (360 MHz, in CD_3OD, TMS) δ 7.46 (1H, d, J = 1.2 Hz, H-3), 5.15 (1H, d, J = 6.0 Hz, H-1), 4.65 (1H, d, J = 7.9 Hz, Glc-1), 4.29 (1H, brs, H-6), 3.87 (1H, dd, J = 12.2, 1.5 Hz, Glc-6a), 3.79 (2H, brd, J = 5.4 Hz, H_2-10), 3.69 (3H, s, −COOMe), 3.66 (1H, dd, J = 12.2, 5.6 Hz, Glc-6b), 3.35 (1H, m, Glc-5), 3.27−3.31 (Glc-3 and Glc-4, overlapped with solvent signal), 3.19 (1H, dd, J = 9.0, 7.9 Hz, Glc-2), 3.13 (1H, m, H-8), 2.26 (1H,

m, H-7a), 2.04−2.09 (2H, m, H-5, and H-9), 1.54 (1H, m, H-7b); ^{13}C NMR (90 MHz, in CD_3OD, TMS) δ 169.5 (C, C-11), 153.1 (CH, C-3), 113.0 (C, C-4), 100.6 (CH, Glc-1), 99.1 (CH, C-1), 78.3 (CH, Glc-3), 78.0 (CH, Glc-5), 74.7 (CH, Glc-2), 73.2 (CH, C-6), 71.5 (CH, Glc-4), 62.7 (CH_2, Glc-6), 62.3 (CH_2, C-10), 51.7 (CH_3, −COO*Me*), 49.9 (CH, C-9), 43.1 (CH_2, C-7), 42.4 (CH, C-8), 35.5 (CH, C-5); LRESIMS *m/z* 835 (100) $[2M + Na]^+$, 429 (55) $[M + Na]^+$, 360 (15), 339 (13); HRESIMS *m/z* 429.1378 $[M + Na]^+$ (calcd for $C_{17}H_{26}O_{11}Na$, 429.1373).

6*β*,7*β*-Epoxy-8-*epi*-splendoside (2): colorless gum; $[\alpha]^{23}_D$ −108.4° (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 232 (4.00), 280 (3.05) nm; IR (dried film) ν_{max} 3397, 1700, 1639, 1440, 1293, 1178 cm^{-1}; ^{1}H NMR (400 MHz, in CD_3OD, TMS) δ 7.48 (1H, d, J = 1.5 Hz, H-3), 5.76 (1H, s, H-1), 4.56 (1H, d, J = 8.0 Hz, Glc-1), 3.87 (1H, dd, J = 11.9, 1.8 Hz, Glc-6a), 3.80 (1H, d, J = 2.5 Hz, H-6), 3.73 (3H, s, −COOMe), 3.68 (1H, d, J = 11.7 Hz, H-10a), 3.65 (1H, dd, J = 11.9, 5.8 Hz, Glc-6b), 3.50 (1H, d, J = 2.6 Hz, H-7), 3.47 (1H, d, J = 11.7 Hz, H-10b), 3.23−3.33 (H-5, Glc-3, Glc-4, and Glc-5, overlapped with solvent signal), 3.13 (1H, dd, J = 8.9, 8.1 Hz, Glc-2), 2.32 (1H, d, J = 8.8 Hz, H-9); ^{13}C NMR (100 MHz, in CD_3OD, TMS) δ 168.5 (C, C-11), 154.2 (CH, C-3), 107.7 (C, C-4), 99.9 (CH, Glc-1), 93.8 (CH, C-1), 80.7 (C, C-8), 78.4 (CH, Glc-3), 78.0 (CH, Glc-5), 74.6 (CH, Glc-2), 71.6 (CH, Glc-4), 65.2 (CH_2, C-10), 62.8 (CH_2, Glc-6), 60.8 (CH, C-7), 57.9 (CH, C-6), 51.9 (CH_3, −COO*Me*), 46.4 (CH, C-9), 33.3 (CH, C-5); LRCIMS *m/z* 438 (80) $[M + NH_4]^+$, 258 (55), 241 (15), 198 (35), 180 (40), 75 (100); HRFABMS *m/z* 443.1147 $[M + Na]^+$ (calcd for $C_{17}H_{24}O_{12}Na$, 443.1166).

Measurement of DPPH Free-Radical Scavenging Activity. This assay used the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) to determine the potential scavenging activity of extracts and pure compounds, as previously described.[35] Briefly, extracts and pure compounds, in DMSO, were plated in triplicate to give a final concentration of 200 μg/mL and incubated in 200 μM DPPH in EtOH at 37 °C for 30 min in the dark. DMSO was used as the negative control and gallic acid as the positive control. Absorbance was measured at 515 nm, and the percent scavenging activity was determined by comparison with the DMSO negative control. Active compounds were tested in triplicate in the same manner at final concentrations of 2.5, 5, 10, and 20 μg/mL. The IC_{50} values were obtained through extrapolation from linear regression analysis.[35]

Measurement of ONOO$^-$-Scavenging Activity. ONOO$^-$ used in this study was purchased from Cayman Chemical Co. or derived by 3-morpholinosydnonimine (SIN-1). ONOO$^-$-scavenging ability was measured by monitoring the oxidation of DHR 123 using the modified method of Kooy et al.[36] Briefly, a stock solution of DHR 123 (5 mM) purged with nitrogen was prepared in advance and stored at −20 °C. A working solution of DHR 123 (5 μM) diluted from the stock solution was placed on ice in the dark immediately prior to each experiment. The rhodamine buffer (sodium phosphate dibasic, 50 mM; sodium phosphate monobasic, 50 mM; sodium chloride, 90 mM; potassium chloride, 5 mM) including diethylenetriaminepentaacetic acid (DTPA, 5 mM) was purged with nitrogen and placed on ice before use. The ONOO$^-$-scavenging ability was determined at room temperature by a microplate fluorescence spectrophotometer FL500 (Bio-Tek Instruments) with excitation and emission wavelengths of 485 and 530 nm, respectively. The background and final fluorescent intensities were measured 5 min after treatment with or without native ONOO$^-$ (10 μM) in 0.3 N sodium hydroxide or SIN-1 (10 μM). Oxidation of DHR 123 by decomposition of SIN-1 gradually increased, whereas native ONOO$^-$ rapidly oxidized DHR 123 with its final fluorescent intensity being stable over time on the inhibition of DHR 123 oxidation by ONOO$^-$. Penicillamine was used as a positive control.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**3**, partial HMBC NMR spectrum of compound **3** acquired at 600 MHz with a TXI cryoprobe, and the LC-MS analysis profiles of asperuloside and a *n*-BuOH-soluble extract of *M. citrifolia*. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) McClatchey, W. *Integr. Cancer Ther.* **2002**, *1*, 110−120.
(2) Nelson, S. C. *Fruits Nuts* **2001**, *4*, 1−4.
(3) Tabrah, F. L.; Eveleth, B. M. *Hawaii Med.* **1966**, *25*, 223−230.
(4) Morton, J. F. *Econ. Bot.* **1992**, *46*, 241−256.
(5) Hornick, C. A.; Myers, A.; Sadowska-Krowicka, H.; Anthony, C. T.; Woltering, E. A. *Angiogenesis* **2003**, *6*, 143−149.
(6) Wang, M. Y.; Su, C. *Ann. NY Acad. Sci.* **2001**, *952*, 161−168.
(7) Li, R. W.; Myers, S. P.; Leach, D. N.; Lin, G. D.; Leach, G. *J. Ethnopharmacol.* **2003**, *85*, 25−32.
(8) Wang, M.-Y.; West, B. J.; Jensen, C. J.; Nowicki, D.; Chen, S.; Palu, A. K.; Anderson, G. *Acta Pharmacol. Sin.* **2002**, *23*, 1127−1141.
(9) Hiwasa, T.; Arase, Y.; Chen, Z.; Kita, K.; Umezawa, K.; Ito, H.; Suzuki, N. *FEBS Lett.* **1999**, *444*, 173−176.
(10) Hirazumi, A.; Furusawa, E.; Chou, S. C.; Hokama, Y. *Proc. West. Pharmacol. Soc.* **1994**, *37*, 145−146.
(11) http://www.crch.org/CenStudyNoni.htm
(12) Perkin, A. G. *Proc. Chem. Soc.* **1908**, *24*, 149.
(13) Sang, S.; Cheng, X.; Zhu, N.; Stark, R. E.; Badmaev, V.; Ghai, G.; Rosen, R. T.; Ho, C.-T. *J. Agric. Food Chem.* **2001**, *49*, 4478−4481.
(14) Sang, S.; Wang, M.; He, K.; Liu, G.; Dong, Z.; Badmaev, V.; Zheng, Q. Y.; Ghai, G.; Rosen, R. T.; Ho, C.-T. In *Quality Management of Nutraceuticals*; Ho, C.-T., Zheng, Q. Y., Eds.; American Chemical Society: Washington, DC.; Symposium Series 803; 2002; pp 134−150.
(15) Wang, M.; Kikuzaki, H.; Csiszar, K.; Boyd, C. D.; Maunakea, A.; Fong, S. F. T.; Ghai, G.; Rosen, R. T.; Nakatani, N.; Ho, C.-T. *J. Agric. Food Chem.* **1999**, *47*, 4880−4882.
(16) Krull, R. E.; Stermitz, F. R.; Franzyk, H.; Jensen, S. R. *Phytochemistry* **1998**, *49*, 1605−1608.
(17) Gardner, D. R.; Narum, J.; Zook, D.; Stermitz, F. R. *J. Nat. Prod.* **1987**, *50*, 485−489.
(18) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod.* **1980**, *43*, 649−707.
(19) Foderaro, T. A.; Stermitz, F. R.; Hope, H. *Tetrahedron Lett.* **1992**, *33*, 2953−2954.
(20) Woo, W. S.; Kang, S. S.; Wagner, H.; Chari, V. M. *Tetrahedron Lett.* **1978**, 3239−3242.
(21) Antus, S.; Seligmann, O.; Wagner, H. *Justus Liebigs Ann. Chem.* **1986**, 647−654.
(22) Beck, M.-A.; Häberlein, H. *Phytochemistry* **1999**, *50*, 329−332.
(23) Otsuka, H.; Yoshimura, K.; Yamasaki, K.; Cantoria, M. C. *Chem. Pharm. Bull.* **1991**, *39*, 2049−2052.
(24) Vieira, I. J. C.; Mathias, L.; Braz-Filho, R.; Schripsema, J. *Org. Lett.* **1999**, *1*, 1169−1171.
(25) Shaw, A. A.; Shetlar, M. D. *J. Am. Chem. Soc.* **1990**, *112*, 7736−7742.
(26) Cimanga, K.; Hermans, N.; Apers, S.; Van Miert, S.; Van den Heuvel, H.; Claeys, M.; Pieters, L.; Vlietinck, A. *J. Nat. Prod.* **2003**, *66*, 97−102.
(27) Bianco, A.; Passacantilli, P.; Polidori, G. *Org. Magn. Reson.* **1983**, *21*, 460−461.
(28) Kitao, S.; Sekine, H. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 2011−2014.
(29) Grindley, T. B.; McKinnon, M. S.; Wasylishen, R. E. *Carbohydr. Res.* **1990**, *197*, 41−52.
(30) Haraguchi, Y.; Yagi, A.; Koda, A.; Inagaki, N.; Noda, K.; Nishioka, I. *J. Med. Chem.* **1982**, *25*, 1495−1499.
(31) Sang, S.; Lapsley, K.; Jeong, W.-S.; Lachance, P. A.; Ho, C.-T.; Rosen, R. T. *J. Agric. Food Chem.* **2002**, *50*, 2459−2463.
(32) Tanka, H.; Kato, I.; Ito, K. *Chem. Pharm. Bull.* **1987**, *35*, 3603−3608.
(33) Zin, Z. M.; Abdul-Hamid, A.; Osman, A. *Food Chem.* **2002**, *78*, 227−231.
(34) Kamiya, K.; Tanaka, Y.; Endang, H.; Umar, M.; Satake, T. *J. Agric. Food Chem.* **2004**, *52*, 5843−5848.
(35) Gu, J.-Q.; Park, E. J.; Luyengi, L.; Hawthorne, M. E.; Mehta, R. G.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **2001**, *58*, 121−127.
(36) Kooy, N. W.; Royall, J. A.; Ischiropulos, H.; Beckman, J. S. *Free Radical Biol. Med.* **1994**, *16*, 149−156.

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