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Anti-inflammatory and Quinone Reductase Inducing Compounds from Fermented Noni (*Morinda citrifolia*) Juice Exudates

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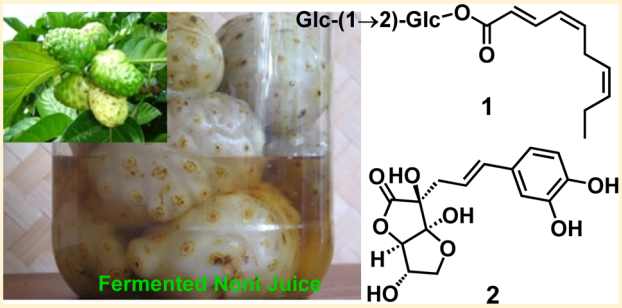
Supporting Information

ABSTRACT: A new fatty acid ester disaccharide, 2-*O*-(*β*-D-glucopyranosyl)-1-*O*-(2*E*,4*Z*,7*Z*)-deca-2,4,7-trienoyl-*β*-D-glucopyranose (**1**), a new ascorbic acid derivative, 2-caffeoyl-3-ketohexulofuranosonic acid *γ*-lactone (**2**), and a new iridoid glycoside, 10-dimethoxyfermiloside (**3**), were isolated along with 13 known compounds (**4**−**16**) from fermented noni fruit juice (*Morinda citrifolia*). The structures of the new compounds, together with **4** and **5**, were determined by 1D and 2D NMR experiments, as well as comparison with published values. Compounds **2** and **7** showed moderate inhibitory activities in a TNF-*α*-induced NF-*κ*B assay, and compounds **4** and **6** exhibited considerable quinone reductase-1 (QR1) inducing effects.



Morinda citrifolia L. (Rubiaceae), commonly known as noni, is native to certain countries bordering the Indian Ocean and is cultivated in tropical areas such as Hawai'i, as well as Tahiti and Samoa. The stem bark, roots, leaves, and fruits have been used traditionally as a folk remedy for many diseases including diabetes, hypertension, skin inflammation, and cancer.[1,2] In previous chemical studies, anthraquinones, including damnacanthal, 7-hydroxy-8-methoxy-2-methylanthraquinone, morenone 1, and morenone 2, have been found in the roots of noni.[3−5] From the heartwood, two known anthraquinones (morindone and physcion) and an anthraquinone glycoside have been isolated.[6] Anthraquinone glycoside and flavone glycosides have been isolated from the flowers of this plant.[7] In studies of noni fruits, iridoid glycosides, flavonoid glycosides, anthraquinones, and fatty acid glycosides have been reported.[8−12]

Noni juice, which is commonly prepared as a drip/exudate from senescing fruits held in fermentation vessels, has been consumed widely as a dietary supplement or medicinal food for the purported prevention of several diseases such as diabetes, high blood pressure, and arteriosclerosis.[13] However, chemical components and biological activities of noni juice fermented from the fruits have been less well studied. In the course of a search for biologically active constituents from tropical plants, a butanol partition of fermented noni juice of *M. citrifolia* fruits was found to exhibit inhibitory activity in vitro against the tumor necrosis factor alpha (TNF-*α*) activated nuclear factor-kappa B (NF-*κ*B) pathway in transfected human embryonic kidney cells 293 and also induced quinone reductase-1 (QR-1) in Hepa 1c1c7 cells.

This report describes the isolation and structure elucidation of the new compounds **1**−**3**, which were obtained from fermented noni fruit juice along with 13 known compounds, as well as their biological activities.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white, amorphous powder. The molecular formula was determined as $C_{22}H_{34}O_{12}$ by the positive-ion HRESIMS (*m*/*z* 513.1759 $[M + Na]^+$, calcd for $C_{22}H_{34}O_{12}Na$, 513.1743). The UV absorption band at λ_{max} 310 nm was indicative of the presence of a dienone system. The IR spectrum showed one or more hydroxy groups and an ester carbonyl at 3319 and 1732 cm^{-1}, respectively. The ^1H and ^{13}C NMR (Table 1) and HSQC spectra of **1** displayed signals for an ester carbonyl carbon at δ_C 165.5, a *trans*-olefinic group at δ_H 6.03 (d, J = 15.2 Hz)/δ_C 120.5 (C-2) and δ_H 7.77 (dd, J = 15.2, 11.2 Hz)/δ_C 140.5 (C-3), two *cis*-olefinic groups at δ_H 6.25 (t, J = 11.2 Hz)/δ_C 125.9 (C-4) and δ_H 5.92 (q, J = 8.0 Hz)/δ_C 140.0 (C-5) and at δ_H 5.34 (q, J = 7.2 Hz)/δ_C 125.0 (C-7) and

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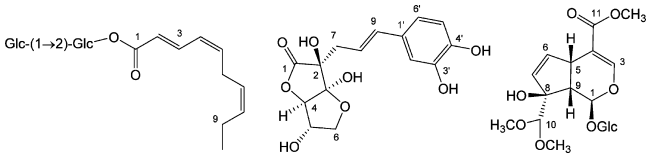
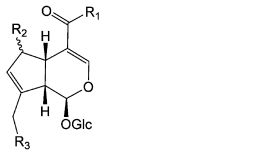
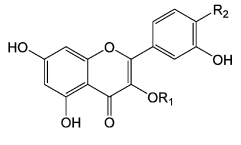




4 R_1 = OH, R_2 = α*O*-*n*-butyl, R_3 = OH
5 R_1 = OH, R_2 = β*O*-*n*-butyl, R_3 = OH
6 R_1 = OCH_3, R_2 = βOH, R_3 = OH



7 R_1 = Glc-(1→6)-Rha, R_2 = H

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1 and 2

	1[a]		2[b]	
position	δ_H	δ_C	δ_H	δ_C
1		165.5		175.8
2	6.03 (d, 15.2)	120.5		78.9
3	7.77 (dd, 15.2, 11.2)	140.5		107.5
4	6.25 (t, 11.2)	125.9	4.33 (s)	87.8
5	5.92 (q, 8.0)	140.0	4.23 (br t, 5.2)	74.5
6α	3.10 (t, 7.6)	25.9	4.13 (dd, 9.4, 6.2)	74.4
6β			3.86 (dd, 9.4, 4.2)	
7	5.34 (q, 7.2)	125.0	2.51 (m)[c]	39.5
8	5.49 (q, 7.2)	132.7	5.92 (dt, 16.0, 15.6)	129.0
9	2.15 (q, 7.4)	20.1	6.22 (d, 16.0)	133.7
10	1.01 (t, 7.6)	13.1		
1′	5.71 (d, 6.8)	93.0		129.0
2′	3.66 (m)[c]	81.6	6.78 (d, 1.4)	113.0
3′	3.37 (m)[c]	76.4		145.7
4′	3.41 (m)[c]	69.3		145.5
5′	3.41 (m)[c]	77.3	6.66 (d, 8.0)	116.0
6′a	3.87 (d, 12.0)	60.80	6.59 (dd, 8.0, 1.4)	118.4
6′b	3.65 (m)[c]			
1″	4.56 (d, 7.6)	104.2		
2″	3.21 (m)[c]	74.4		
3″	3.37 (m)[c]	76.2		
4″	3.41 (m)[c]	69.5		
5″	3.41 (m)[c]	76.3		
6″a	3.72 (m)[c]	60.81		
6″b	3.65 (m)[c]			

[a,b]Spectra were recorded at ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) in CD_3OD and DMSO-d_6, respectively. Chemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses. The assignments are based on DEPT, COSY, NOESY, HSQC, and HMBC experiments. [c]Overlapping signals.

δ_H 5.49 (q, J = 7.2 Hz)/δ_C 132.7 (C-8), two methylene groups at δ_H 3.10 (t, J = 7.6 Hz)/δ_C 25.9 (C-6) and δ_H 2.15 (q, J = 7.4 Hz)/δ_C 20.1 (C-9), and a methyl group at δ_H 1.01 (t, J = 7.6 Hz)/δ_C 13.1 (C-10), indicating the presence of a highly conjugated alkyl acetate unit, deca-2,4,7-trienoate,[14] as supported by two- and three-bond ^{1}H−^{13}C HMBC and ^{1}H−^{1}H COSY correlations (Figure 1). The geometry of the olefinic double bonds was proposed as 2*E*, 4*Z*, and 7*Z*, by NOESY correlations of H-3 with H-6 and of H-4 with H-5 (Figure 2), as well as the magnitude of their coupling constants, as shown in Table 1. The NMR spectra revealed two additional anomeric proton signals at δ_H 5.71 (d, J = 6.8 Hz, H-1′) and at δ_H 4.56 (d, J = 7.6 Hz, H-1″), along with 12 carbon signals for the inner β-D-glucose moiety at δ_C 93.0, 81.6, 77.3, 76.4, 69.3, and 60.80 and for the terminal β-D-glucose group at δ_C 104.2, 76.3, 76.2, 74.4, 69.5, and 60.81, indicating that C-1 of the terminal glucose is connected to C-2 of the inner glucose.[15,16] The NOESY correlations of H-2′ with H-1′/H-1″ and the HMBC correlations of H-1″/C-2′ and H-2′/C-1″ further supported the connectivity proposed for the two glucose units (Figures 1 and 2). The linkage of the fatty acid and the glucose moieties was confirmed on the basis of the HMBC correlation between H-1′ and C-1. Accordingly, compound **1** was established as the new fatty acid ester disaccharide 2-*O*-(β-D-glucopyranosyl)-1-*O*-(2*E*,4*Z*,7*Z*)-deca-2,4,7-trienoyl-β-D-glucopyranose.

Compound **2** was obtained as a white, amorphous powder, $[\alpha]_D^{25}$ +24 (*c* 0.01, MeOH). The molecular formula was determined as $C_{15}H_{16}O_8$ from the positive-ion HRESIMS (*m*/*z* 324.0731 $[M + Na]^+$, calcd for $C_{15}H_{16}O_8Na$, 324.0737). The IR spectrum showed one or more hydroxy groups and an olefinic double bond at 3323 and 1687 cm^{-1}, respectively. The ^{1}H NMR spectrum of **2** displayed a set of ABX-type aromatic signals at δ_H 6.78 (d, J = 1.4 Hz, H-2′), 6.66 (d, J = 8.0 Hz, H-5′), and 6.59 (dd, J = 8.0, 1.4 Hz, H-6′), a *trans*-olefinic proton signal at δ_H 6.22 (d, J = 16.0 Hz, H-9) and 5.92 (dd, J = 16.0, 15.6 Hz, H-8), and a methylene proton at δ_H 2.51 (2H, m, H-7), indicating a caffeoyl group,[17] as confirmed by HMBC correlations of H-9 with C-7/C-2′/C-6′. In addition, the HSQC and HMBC spectra (Figure 1) revealed two kinds of oxygenated methine groups at δ_H 4.33 (s)/δ_C 87.8 (C-4) and at δ_H 4.23 (br t, J = 5.2 Hz)/δ_C 74.5 (C-5), an oxygenated methylene group at δ_H 4.13 (1H, dd, J = 9.4, 6.2 Hz) and 3.86 (1H, dd, J = 9.4, 4.2 Hz)/δ_C 74.4 (C-6), an ester carbonyl carbon at δ_C 175.8 (C-1), a hemiketal carbon at δ_C 107.5 (C-3), and an oxygenated quaternary carbon at δ_C 78.9 (C-2), indicating a 3-ketohexulofuranosonic acid γ-lactone derived from ascorbic acid.[18] This was supported by HMBC correlations of H-4 with C-1/C-2/C-6 and H-6 with C-3/C-4. The HMBC spectrum showed additional three-bond correlations between H-7 and C-1/C-3, and between H-8 and C-2, consistent with the methylene group (C-7) of the caffeoyl moiety being connected to C-2 of a 3-ketohexulofuranosonic acid γ-lactone unit (Figure 1), which is similar to that of delesserine isolated from marine alga, *Delesseria sanguinea*.[18] The relative configuration of **2** was determined to be the same as that of delesserine by comparison of its optical rotation with a published value, $[\alpha]_D^{25}$ + 41 (*c* 0.01, MeOH),[18] and by the observed NOESY correlation of H-4 with H-8 (Figure 2). On the basis of the above data, **2** was determined as the new compound 2-caffeoyl-3-ketohexulofuranosonic acid γ-lactone.

Compound **3** was obtained as a white, amorphous powder, $[\alpha]_D^{25}$ −476 (*c* 0.01, MeOH). The molecular formula was determined as $C_{19}H_{28}O_{12}$ from the positive-ion HRESIMS (*m*/*z* 471.1085 $[M + Na]^+$, calcd for $C_{19}H_{28}O_{12}Na$, 471.1084). The UV, IR, and NMR spectra of **3** were supportive of the compound being an iridoid glycoside, including a 4-substituted enol-ether system.[19,20] The ^{1}H, ^{13}C (Table 2), and HSQC NMR spectra of **3** showed signals for a methoxy group (δ_H 3.72/δ_C 48.2) connected to a carbonyl group (C-11), two upfield shifted methoxy groups at δ_H 3.48/δ_C 55.5 (OCH_3-10a) and δ_H 3.45/δ_C 56.0 (OCH_3-10b), a disubstituted olefinic group at δ_H 5.66 (dd, J = 5.6, 1.6 Hz)/δ_C 133.6 (C-7) and 6.25 (dd, J = 5.6, 2.8 Hz)/δ_C 134.5 (C-6), a dioxygenated methine group at δ_H 4.14 (s)/δ_C 106.1 (C-10), and an oxygenated

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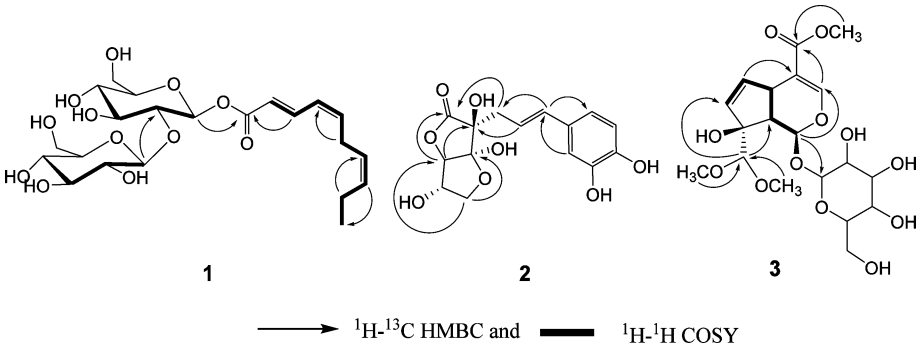

Figure 1. Key ^{1}H−^{13}C HMBC and ^{1}H−^{1}H COSY correlations for compounds **1**−**3**.

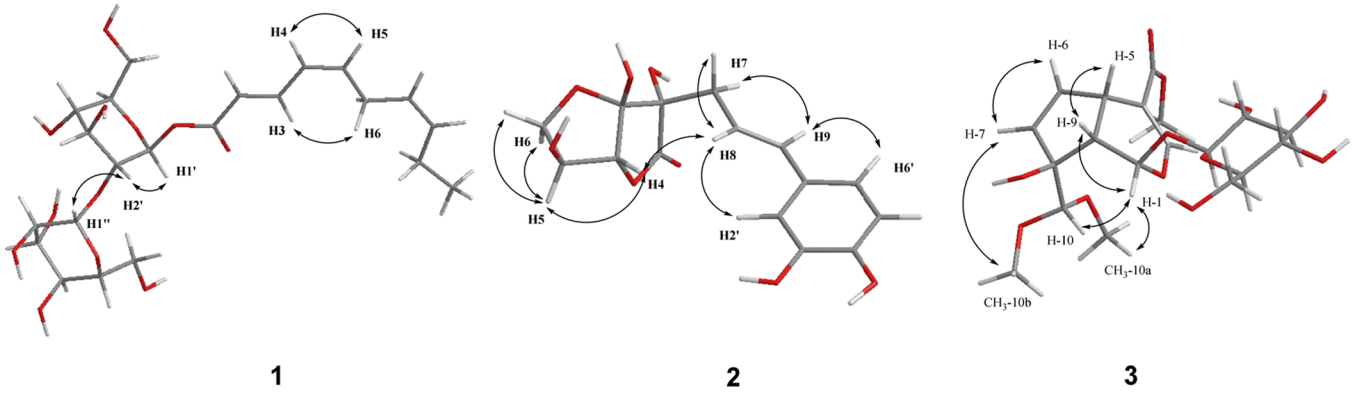

Figure 2. Key NOESY correlations for compounds **1**−**3**.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 3−5

	3[a]		4[a]		5[a]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.94 (br s)	92.0	5.05 (d, 8.8)	100.4	5.64 (d, 3.2)	93.5
3	7.41 (s)	150.5	7.62 (d, 1.2)	153.6	7.42 (s)	150.9
4		110.4		107.3		110.3
5	3.70 (m)[b]	37.0	3.07 (t, 6.4)	40.9	3.21 (t, 8.8)[b]	38.4
6	6.25 (dd, 5.6, 2.8)	134.5	4.46 (d, 1.6)	82.0	4.31 (br s)[b]	87.1
7	5.66 (dd, 5.6, 1.6)	133.6	6.13 (d, 1.6)	126.9	5.84 (br s)	126.4
8		86.7		150.8		147.9
9	2.60 (d, 9.2)	50.2	2.53 (t, 8.0)	44.5	3.29 (m)[b]	46.0
10a	4.14 (s)	106.1	4.48 (d, 15.6)	60.3	4.30 (d, 14.8)	59.2
10b			4.22 (d, 15.6)		4.21 (d, 14.8)	
11		167.4		169.5		166.0
1′a			3.48 (m)[b]	69.0	3.71 (m)[b]	68.6
1′b			3.40 (m)[b]		3.61 (m)[b]	
2′			1.45 (m)	31.8	1.55 (m)	31.8
3′			1.32 (m)	19.0	1.40 (m)	18.9
4′			0.89 (t, 6.8)	12.7	0.95 (t, 6.8)	12.9
1″	4.63 (d, 8.2)	98.7	4.73 (d, 7.6)	99.4	4.62 (d, 7.6)	98.5
2″	3.18 (t, 8.2)	73.2	3.26 (t, 8.0)	73.6	3.21 (t, 8.0)[b]	73.3
3″	3.26−3.40 (m)[b]	76.8	3.25−3.39 (m)[b]	76.9	3.35 (m)[b]	77.0
4″	3.26−3.40 (m)[b]	76.6	3.25−3.39 (m)[b]	76.5	3.35 (m)[b]	76.5
5″	3.26−3.40 (m)[b]	70.2	3.25−3.39 (m)[b]	69.9	3.37 (m)[b]	70.3
6″a	3.91 (d, 12.0)	61.4	3.91 (d, 12.0)	61.1	3.91 (d, 10.8)	61.4
6″b	3.67[b]		3.66 (d, 12.0)		3.66 (d, 10.8)	
OCH_3-10a	3.48 (s)	55.5				
OCH_3-10b	3.45 (s)	56.0				
OCH_3-11	3.72 (s)	48.2				

[a]Spectra were recorded at ^{1}H (400 MHz) and ^{13}C (100 MHz) in CD_3OD. Chemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses. The assignments are based on DEPT, COSY, NOESY, HSQC, and HMBC experiments. [b]Overlapping signals.

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quaternary carbon at δ_C 86.7 (C-8), which were similar to those of a hemiacetal derivative from tudoside, except for two methoxy groups at C-10 and an olefinic group (C_6−C_7) instead of the 6β,7β-epoxy group of tudoside.[21] These observations were supported by the HMBC correlations from two methoxy protons (δ_H 3.48 and 3.45) to C-10 and from H-10 to C-7/C-8/C-9 and by ^{1}H−^{1}H COSY correlations (Figure 1). The orientation of the hydroxy group at C-8 was determined as β by comparison of the chemical shift value at C-9 (δ_C 50.2) with the published data.[22−24] In addition, the NOESY spectrum revealed correlations from H-10/OCH$_3$ (δ_H 3.48) to H-1, suggestive of an α-orientation of the hemiacetal group at C-8 (Figure 2). The conformation of the pyran ring in **3** was comparable to that of **5**, based on analyses of the NMR data and NOESY correlations. Accordingly, the structure of the new iridoid glycoside **3** (10-dimethoxyfermiloside) was confirmed as shown in Figures 1 and 2.

Compound **3**, possessing two methoxy groups at the C-10 position, appears to have been converted from **3a** (Scheme S1, Supporting Information). However, when the methanol-d_4 NMR solvent was dried thoroughly, **3a** was not detected. Although LC-MS analysis did not show the presence of **3** or its derivatives (**3a** and **3b**) in the crude extract, compound **3** is anticipated to occur in the form of **3b** in noni juice (Scheme S1, Supporting Information).[21]

Compound **4**, $[\alpha]_D^{25}$ +148 (*c* 0.01, MeOH), was obtained as white, amorphous powder, and the molecular formula was determined as $C_{20}H_{30}O_{11}$ from the positive-ion HRESIMS (*m*/*z* 469.1664 [M + Na]$^+$, calcd for $C_{20}H_{30}O_{11}Na$, 469.1646). The UV, IR, and ^{1}H and ^{13}C NMR spectra of **4** indicated an iridoid glycoside and were in good agreement with those of rhodolatouside A.[25] The orientation of the oxygenated butyl group at C-6 was confirmed as the α form, based on chemical shift values in the NMR spectra [δ_H 5.05 ($^3J_{H1,9}$ = 8.8 Hz, H-1), δ C_1 = 100.4, $\Delta\delta$ C_3−C_4 = 46.3 ppm] (Table 2).[22] The NOESY correlations observed from H-6 to H-5 and from H-9 to H-5/H-10 suggested that the protons are positioned on the same side (β form) in the molecule. In addition, compound **4** showed a weak NOESY correlation between H-1 and H-3, while no correlation was observed between H-1 and H-9/H-10. The NOESY analysis and the coupling constant value ($^3J_{H1,9}$ = 8.8 Hz) indicated that the dihedral angle between H-1 and H-9 is around 170° and that the C−H bond and the glucopyranosyl group at C-1 are oriented as α-axial and β-equatorial, respectively (Figure S31, Supporting Information).

Compound **5** was obtained as a white, amorphous powder, $[\alpha]_D^{25}$ −186 (*c* 0.01, MeOH), and the molecular formula was determined as $C_{20}H_{30}O_{11}$ from the positive-ion HRESIMS (*m*/*z* 469.1656 [M + Na]$^+$, calcd for $C_{20}H_{30}O_{11}Na$, 469.1646). The ^{1}H and ^{13}C NMR spectra (Table 2) were quite similar to those of **4**, except for a smaller coupling constant value with chemical signals shifted [δ_H 5.64 ($^3J_{H1,9}$ = 3.2 Hz, H-1), δ C_1 = 93.5, $\Delta\delta$ C_3−C_4 = 40.6 ppm] (Table 2), suggesting that the oxygenated butyl group at C-6 is oriented in the β form.[22] The orientation of H-1 was confirmed as α by the NOESY correlations of H-1 with H-9/H-10 and by the coupling constant value ($^3J_{H1,9}$ = 3.2 Hz) with the β-pseudoequatorial H-9, where the dihedral angle is close to 60°, compared to those of **4** (Figure S31, Supporting Information).[26] Accordingly, the iridoid glucoside **5** was confirmed as rhodolatouside B.[25]

In the previous study, two iridoid glycosides, rhodoratousides A and B, were isolated from *Rhododendron latoucheae*.[25] However, in the present study it was found that **4** and **5** each possess a different conformation and relative configuration, through the rationalization of their 1D and 2D NMR data.

The other 11 compounds isolated were identified as the known compounds scandoside methyl ester (**6**),[22] kaemperol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**7**),[27] deacetylasperulosidic acid,[28] scandoside,[28] asperulosidic acid,[28] 6-methoxy-9-epigeniposidic acid,[22] scopoletin,[29] magnolioside,[29] 7-*O*-(β-D-apiofuranosyl)-(1→6)-β-D-glucopyranosyl-6-methoxycoumarin,[30] quercetin,[31] and rutin,[27] by comparison of their physical and spectroscopic data with published values.

NF-κB is an inducible transcription factor that plays a vital role in the regulation of apoptosis, cell proliferation, and cell survival.[32] NF-κB also has an essential role in inflammation and innate immunity.[33] Recent studies have indicated aberrantly active NF-κB pathways in different types of human cancers. Consequently, inhibitors of NF-κB signaling might be useful as anticancer therapeutics. Genetic polymorphisms of the QR-1 gene have shown that the loss or reduction of QR-1 function is related to increased vulnerability to a variety of cancers and chemical toxicity.[34,35] QR-1, in having a protective function for cells against the toxicity of electrophiles and reactive oxygen species, is classified as an important phase 2 detoxifying enzyme.

The isolated compounds were evaluated for their anti-inflammatory and quinone reductase-1 activities. Among the isolates, the ascorbic acid derivative **2** and the flavonoid glycoside **7** showed inhibitory activities against the TNF-α-induced NF-κB (inhibition rates of 70% and 62%, respectively), without or with low toxicity. Here, compounds **4** and **6** exhibited considerable QR-1 inducing activities, with IR values of 2.3 and 2.5, respectively. The coumarin glycoside magnolioside also showed a similar inducing activity, while the other compounds showed no inducing activity (Table S1, Supporting Information). Previous phytochemical studies of noni fruits (*M. citrifolia*) have identified anthraquinones, flavonoid glycosides, and iridoid glycosides with cancer chemopreventive activity.[10,36,37] In particular, an anthraquinone 2-methoxy-1,3,6-trihydroxyanthraquinone exhibited pronounced quinone reductase (QR) inducing activity.[37] However, anthraquinones have been reported as possible hepatotoxic components in noni juice.[38,39] In contrast, iridoid glycosides activated crucial antioxidant enzymes, such as glutathione, catalase, and superoxide dismutase, and inhibited malondialdehyde, which occurs naturally as a biomarker for oxidative stress in hepatic cells.[40] In this study, iridoid glycosides were obtained as major constituents in fermented noni juice, and some exhibited induction activity on QR-1. These results suggest that fermented noni juice may possess cancer chemopreventive activity without or with low hepatotoxicity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol IV multiwavelength polarimeter. UV spectra were scanned on a Shimadzu PharmaSpec-1700 UV−visible spectrophotometer. IR spectra were obtained on a Bruker Tensor-27 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AVANCE (400 MHz) NMR spectrometer. Low- and high-resolution mass spectra were obtained with an Agilent 6530 LC-qTOF high mass accuracy mass spectrometer operated in both the positive- and negative-ion modes. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany). Silica gel (230−400 mesh, Merck) and RP-18 (YMC·GEL ODS-A, 12 nm, S-150 μm, YMC, Tokyo, Japan) were used for column

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chromatography. Semipreparative HPLC was conducted on a Beckman Coulter Gold-168 system equipped with a DAD detector, utilizing an Alltech reversed-phase Econosil C_{18} column (10 μm, 10 × 250 mm) with a flow rate of 2 mL/min.

Plant Material. The fruits of *M. citrifolia* were harvested from a commercial orchard in Kalapana, Hawai'i, USA, from 2012 through 2014. The plant was identified by Dr. Marisa M. Wall, United States Department of Agriculture, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, Hilo, USA. Fruits harvested were at the firm, yellow-white maturity stage. The fruits were washed with soft-bristled brushes in soapy water, rinsed in tap water, air-dried, sorted, placed into sterile 3.8 L wide-mouth glass jars (~1.5 to 2.0 kg fruit per jar) with lids, and stored in the dark at 22 °C in a walk-in chamber for 16 days. During this time, juice exuded from the fruit and the naturally fermented juice were drained from containers by pouring through a sterile plastic sieve into a metal pot and pasteurized at 85 °C for 3 min; the pasteurized juice was poured immediately into 3 L food-grade sterile buckets with lids and frozen at −10 °C. A voucher specimen (no. FNJ 005) was deposited at the Natural Product Chemistry Laboratory, Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo.

Extraction and Isolation. The freeze-dried fermented juice from noni fruits (10 L) was extracted with ethyl acetate (EtOAc) and *n*-butanol (3 × 4 L), successively. The butanol-soluble partition (80.0 g) was subjected to silica gel column chromatography (CC; ⌀ 20 cm; 230−400 mesh, 5 kg) eluted by $CHCl_3$−MeOH (100:1 to 50:50) to afford 12 fractions (BS1−BS12). Fraction BS1 (0.8 g) was chromatographed on a silica gel (200 g) column using a gradient solvent system of $CHCl_3$−MeOH, (20:1 to 5:1) to give five major subfractions (BS1S1 to BS1S5). Scopoletin (25.0 mg) and quercetin (10.0 mg) were recrystallized using the solvent mixture $CHCl_3$−MeOH (1:1) from subfractions BS1S1 and BS5, respectively. Fraction BS9 (1.0 g) was chromatographed on a silica gel (300 g) column using a gradient solvent system of $CHCl_3$−MeOH (20:1 to 1:1) to afford 10 subfractions (BS9S1 to BS9S10). Fraction BS9S5 was subjected to semipreparative HPLC [MeOH−H_2O (0:100 to 20:80)] to yield 6-methoxy-9-epigeniposidic acid (3 mg, t_R 97 min). Fraction BS12 (1.0 g) was subjected to passage over a column containing RP-18 gel (⌀ 2 × 100 cm; 40−63 mesh, 200 g), with H_2O−MeOH (100:0 to 70:30) as the solvent system, yielding seven subfractions (BS12R1 to BS12R7). Two major iridoid glycosides, deacetylasperulosidic acid (48 mg, t_R 73 min) and scandoside (25 mg, t_R 90 min), were purified by repeated silica gel (400 g) CC and semipreparative HPLC [MeOH−H_2O (0:100 to 20:80)] from fraction BS12R1 (1.0 g). Fraction BS12R2 was subjected to semipreparative HPLC [MeOH−H_2O (10:90 to 40:60)] to yield **6** (5.6 mg, t_R 87 min), **3** (1.5 mg, t_R 90 min), and asperulosidic acid (10 mg, t_R 95 min). Compounds **5** (5.5 mg, t_R 77 min) and **4** (6.5 mg, t_R 80 min) were obtained by semipreparative HPLC [MeOH−H_2O (5:95 to 20:80)] from subfraction BS12R4. Subfraction BS12R5 was purified by semi-preparative HPLC [MeOH−H_2O (5:95 to 20:80)] to yield **1** (5.5 mg, t_R 85 min). Compound **7** (1.5 mg), magnolioside (1.5 mg), 7-*O*-(β-D-apiophyranosyl)-(1→6)-β-D-glucopyranosyl-6-methoxycoumarin (2.0 mg), and rutin (3.0 mg) were obtained by repeated semipreparative HPLC from subfraction BS12R7.

*2-O-(β-D-Glucopyranosyl)-1-O-(2E,4Z,7Z)-deca-2,4,7-trienoyl-β-D-glucopyranoside (**1**):* white, amorphous powder; UV (MeOH) λ_{max} (log ε) 310 (4.2) nm; IR ν_{max} (KBr) 3319, 2924, 1732, 1633, 1076 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1; HRESIMS *m/z* 513.1759 $[M + Na]^+$, calcd for $C_{22}H_{34}O_{12}Na$, 513.1743.

*2-Caffeoyl-3-ketohexulofuranosonic acid γ-lactone (**2**):* white, amorphous powder; $[\alpha]_D^{25}$ +24 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 278 (4.2) nm; IR ν_{max} (KBr) 3323, 2919, 1687, 1632, 1439, 1038 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1; HRESIMS *m/z* 324.0731 $[M + Na]^+$, calcd for $C_{15}H_{16}O_8Na$, 324.0737.

*10-Dimethoxyfermiloside (**3**):* white, amorphous powder; $[\alpha]_D^{25}$ −476 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 242 (4.2) nm; IR ν_{max} (KBr) 3319, 2933, 2846, 1674, 1621, 1076 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 2; HRESIMS *m/z* 471.1085 $[M + Na]^+$, calcd for $C_{19}H_{28}O_{12}Na$, 471.1084.

*Rhodolatouside A (**4**):* white, amorphous powder; $[\alpha]_D^{25}$ +148 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.2) nm; IR ν_{max} (KBr) 3331, 2930, 1678, 1634, 1035 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 2; HRESIMS *m/z* 469.1664 $[M + Na]^+$, calcd for $C_{20}H_{30}O_{11}Na$, 469.1646.

*Rhodolatouside B (**5**):* white, amorphous powder; $[\alpha]_D^{25}$ −186 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.0) nm; IR ν_{max} (KBr) 3292, 2925, 1674, 1626, 1014 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 2; HRESIMS *m/z* 469.1656 $[M + Na]^+$, calcd for $C_{20}H_{30}O_{11}Na$, 469.1646.

Enzymatic Hydrolysis of 5. A solution of compound **5** (1.5 mg) in H_2O (1 mL) was treated with β-glucosidase from almonds (Fluka, Buches, Switzerland) (3 mg) at 37 °C for 24 h. The reaction mixture was extracted with EtOAc (2 × 3 mL). The H_2O layer was concentrated, and the residue was subjected to column chromatography over C_{18} gel, eluting with MeOH−H_2O (1:9 to 5:5), to yield D-glucose (0.3 mg). It gave a positive specific rotation, $[\alpha]_D^{25}$ +40.0 (*c* 0.02, MeOH).[41] The solvent system CH_3CN−H_2O (6:1) was used for TLC identification of glucose (R_f = 0.34).

Tumor Necrosis Factor-α Activated Nuclear Factor-Kappa B Assay. Human embryonic kidney cells 293 (Panomic, Fremont, CA, USA) were employed for monitoring changes occurring in the NF-κB pathway.[42] Stable constructed cells were seeded into 96-well plates at 20 × 10^3 cells per well. Cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen Co., Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. After 48 h incubation, the medium was replaced and the cells were treated with various concentrations of the test substances. TNF-α (recombinant, human, *E. coli*; Calbiochem, Gibbstown, NJ, USA) was used as an activator at a concentration of 2 ng/mL (0.14 nM). The plate was incubated for 6 h. Spent medium was discarded, and the cells were washed once with PBS. Cells were lysed using 50 μL (for each 96-well plate) of Reporter Lysis Buffer from Promega (Madison, WI, USA), which was added and incubated for 5 min on a shaker and stored at −80 °C. The luciferase assay was performed using the Luc assay system (Promega). The gene product, luciferase enzyme, reacted with the luciferase substrate, emitting light that was detected using a luminometer (LUMIstar Galaxy BMG, Ortenberg, Germany). Data for NF-κB constructs are expressed as IC_{50} values (i.e., the concentration required to inhibit TNF-activated NF-κB activity by 50%). As a positive control, two known NF-κB inhibitors were used, namely, TPCK, IC_{50} 3.8 μM, and BAY-11, IC_{50} 2.0 μM.

Quinone Reductase-1 Induction Assay.[43] Murine hepatoma (Hepa-1c1c7) (CRL-2026 ATCC, Manassas, VA, USA) cells were used in this assay. Cells were plated at 200 μL per well with 0.5 × 10^4 cells/mL in α-MEM (minimum essential medium) without ribonucleosides or deoxyribonucleosides, supplemented with 100 units penicillin and 100 μg/mL streptomycin, and 10% FBS (Gibco). Cells were incubated for 24 h in a CO_2 incubator. After 24 h, the medium was replaced with 190 μL of fresh medium, and 10 μL of test samples was added for a final concentration of 50 μM. After incubation for 48 h, digitonin was used to permeabilize cell membranes, and enzyme activity was measured by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a blue formazan.[44] Production was measured by absorption at 595 nm. A total protein assay using crystal violet staining was run in parallel. Specific activity is defined as nmol of formazan formed/mg of protein. The induction ratio of QR activity represents the specific enzyme activity of agent-treated cells compared with dimethyl sulfoxide-treated control. The concentration to double activity (CD) was determined through a dose−response assay. 4′-Bromoflavone (CD = 0.01 μM) was used as a positive control.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00970.

Spectroscopic data including ^{1}H and ^{13}C NMR, 2D NMR, and HRESIMS of new compounds **1**−**3**, along with **4** and **5** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Youngken, H. W.; Jenkis, H. J.; Butler, C. L. *J. Am. Pharm. Assoc., Sci. Ed.* **1960**, *40*, 271−273.

(2) Hirazumi, A.; Furusawa, E.; Chou, S. C.; Hokama, Y. *Proc. West Pharmacol. Soc.* **1996**, *39*, 25−27.

(3) Rusia, K.; Srivastava, S. K. *Curr. Sci.* **1989**, *58*, 249−252.

(4) Jain, R. K.; Srivastava, S. D. *Proc. Nat. Acad. Sci. India* **1992**, *62A*, 11−13.

(5) Deng, Y.; Chin, Y.-W.; Chai, H.; Keller, W. J.; Kinghorn, A. D. *J. Nat. Prod.* **2007**, *70*, 2049−2052.

(6) Srivastava, M.; Singh, J. *Pharm. Biol.* **1993**, *31*, 182−184.

(7) Tiwari, R. D.; Singh, J. *J. Indian Chem. Soc.* **1977**, *54*, 429−430.

(8) Levand, O.; Larson, H. *Planta Med.* **1979**, *36*, 186−187.

(9) Liu, G.; Bode, A.; Ma, W. Y.; Sang, S.; Ho, C. T.; Dong, Z. *Cancer Res.* **2001**, *61*, 5749−5756.

(10) Su, B. N.; Pawlus, A. D.; Jung, H. A.; Keller, W. J.; MaLaughlin, J. L.; Kinghorn, A. D. *J. Nat. Prod.* **2005**, *68*, 592−595.

(11) Kanchanapoom, T.; Kasai, R.; Yamasaki, K. *Phytochemistry* **2002**, *59*, 551−556.

(12) Sang, S.; Cheng, X.; Zhu, N.; Wang, M.; Jhoo, J.-W.; Stark, R. E.; Badmaev, V.; Ghai, G.; Rosen, R. T.; Ho, C.-T. *J. Nat. Prod.* **2001**, *64*, 799−800.

(13) Wang, M.-Y.; West, B. J.; Jensen, J. C.; Nowicki, D.; Su, C.; Palu, A. K.; Anderson, G. *Acta Pharmacol. Sin.* **2002**, *23*, 1127−1141.

(14) Hu, M.-X.; Zhang, H.-C.; Wang, Y.; Liu, S.-M.; Liu, L. *Molecules* **2012**, *17*, 12651−12656.

(15) Akihisa, T.; Matsumoto, K.; Tokuda, H.; Yasukawa, K.; Seino, K.; Nakamoto, K.; Kuninaga, H.; Suzuki, T.; Kimura, Y. *J. Nat. Prod.* **2007**, *70*, 754−757.

(16) Wang, C. Z.; Yu, D. Q. *Phytochemistry* **1998**, *48*, 711−717.

(17) Prevost, M. S.; Delarue-Cochin, S.; Marteaux, J.; Colas, C.; Van, R. C.; Blondel, A.; Malliavin, T.; Corringer, P.-J.; Joseph, D. *J. Med. Chem.* **2013**, *56*, 4619−4630.

(18) Poss, A. J.; Belter, R. K. *J. Org. Chem.* **1988**, *53*, 1535−1540.

(19) Ling, S.-K.; Komorita, A.; Tanaka, T.; Fujioka, T.; Mihashi, K.; Kouno, I. *J. Nat. Prod.* **2002**, *65*, 656−660.

(20) Calis, I.; Kirmizibekmez, H.; Sticher, O. *J. Nat. Prod.* **2001**, *64*, 60−64.

(21) Schripsema, J.; Caprini, G. P.; van der Heijden, R.; Bino, R.; de Vos, R.; Dagnino, D. *J. Nat. Prod.* **2007**, *70*, 1495−1498.

(22) Damtoft, S.; Jensen, S. R.; Nielsen, B. J. *Phytochemistry* **1981**, *20*, 2717−2732.

(23) 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.

(24) Schripsema, J.; Caprini, G. P.; Dagnino, D. *Org. Lett.* **2006**, *8*, 5337−5340.

(25) Fan, C. Q.; Zhao, W. M.; Ding, B. Y.; Qin, G. W. *Fitoterapia* **2001**, *72*, 449−452.

(26) Podanyi, B.; Reid, R. S.; Kocsis, A.; Szabo, L. *J. Nat. Prod.* **1989**, *52*, 135−142.

(27) 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.

(28) Kim, D.-H.; Lee, H.-J.; Oh, Y.-J.; Kim, M.-J.; Kim, S.-H.; Jeong, T.-S.; Baek, N.-I. *Arch. Pharmacal Res.* **2005**, *28*, 1156−1160.

(29) Tsukamoto, H.; Hisada, S.; Nishibe, S. *Chem. Pharm. Bull.* **1985**, *33*, 396−399.

(30) Sati, S. P.; Chaukiyal, D. C.; Sati, O. P.; Yamada, F.; Ono, M. *J. Nat. Prod.* **1989**, *52*, 376−379.

(31) Nitteranon, V.; Zhang, G.; Darien, B. J.; Parkin, K. *Food Res. Int.* **2011**, *44*, 2271−2277.

(32) Karin, M. *Nature* **2006**, *441*, 431−436.

(33) Hoesel, B.; Schmid, J. A. *Mol. Cancer* **2013**, *12*, 1−15.

(34) Takagi, S.; Kinouchi, Y.; Hiwatashi, N.; Hirai, M.; Suzuki, S.; Takahashi, S.; Negoro, K.; Obana, N.; Shimosegawa, T. *Anticancer Res.* **2002**, *22*, 2749−2752.

(35) Fleming, R. A.; Dress, J.; Loggie, B. W.; Russell, G. B.; Geisinger, K. R.; Morris, R. T.; Sachs, D.; McQuellon, R. P. *Pharmacogenetics* **2002**, *12*, 31−37.

(36) Nitteranon, V.; Zhang, G.; Darien, B. J.; Parkin, K. *Food Res. Int.* **2011**, *44*, 2271−2277.

(37) Pawlus, A. D.; Su, B.-N.; Keller, W. J.; Kinghorn, A. D. *J. Nat. Prod.* **2005**, *68*, 1720−1722.

(38) Millonig, G.; Stadlmann, S.; Vogel, W. *Eur. J. Gastroenterol. Hepatol.* **2005**, *17*, 445−447.

(39) Stadlbauer, V.; Fickert, P.; Lackner, C.; Schmerlaib, J.; Krisper, P.; Trauner, M.; Stauber, R. E. *World J. Gastroenterol.* **2005**, *11*, 4758−4760.

(40) Peng, W.; Qiu, X.-Q.; Shu, Z.-H.; Liu, Q.-C.; Hu, M.-B.; Han, T.; Rahman, K.; Qin, L.-P.; Zheng, C.-J. *J. Ethnopharmacol.* **2015**, *174*, 317−321.

(41) Yu, Y.; Song, W.; Zhu, C.; Lin, S.; Zhao, F.; Wu, X.; Yue, Z.; Liu, B.; Wang, S.; Yuan, S.; Hou, Q.; Shi, J. *J. Nat. Prod.* **2011**, *74*, 2151−2160.

(42) Kondratyuk, T. P.; Park, E.-J.; Yu, R.; van Breemen, R. B.; Asolkar, R. A.; Murphy, B. T.; Fenical, W.; Pezzuto, J. M. *Mar. Drugs* **2012**, *1*, 451−464.

(43) Song, L. L.; Kosmeder, J. W., II; Lee, S. K.; Gerhauser, C.; Lantvit, D.; Moon, R. C.; Moriarty, R. M.; Pezzuto, J. M. *Cancer Res.* **1999**, *59*, 578−585.

(44) Nam, S.-J.; Gaudencio, S. P.; Kauffman, C. A.; Jensen, P. R.; Kondratyuk, T. P.; Marler, L. E.; Pezzuto, J. M.; Fenical, W. *J. Nat. Prod.* **2010**, *73*, 1080−1086.

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