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Iridoid Glycosides from the Leaves of *Morinda citrifolia*

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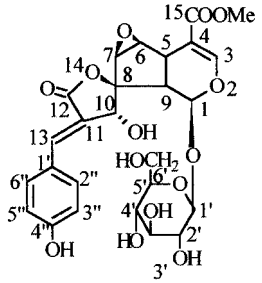
A new iridoid glucoside (**1**), named citrifolinoside A, was isolated from the leaves of *Morinda citrifolia* along with the known iridoids asperuloside and asperulosidic acid. The structure of **1** was established by interpretation and full assignments of NMR spectroscopic data.

In previous papers,[1,2] we have reported the isolation and structural elucidation of five new glycosides from the fruits of *Morinda citrifolia* (Rubiaceae), commonly known as noni, native to certain countries bordering the Indian Ocean. The bark, stem, roots, leaves, and fruits have been used traditionally as a folk remedy for many diseases including cancer, diabetes, and hypertension.[3,4] However, only two compounds, β-sitosterol and ursolic acid, have been isolated previously from the leaves of this plant.[5] There are no reports in the literature on the chemistry of the polar fraction of leaves. In this report, we describe the isolation and structure elucidation of a new iridoid glucoside (**1**), named citrifolinoside **A**, together with two known iridoid glucosides, asperuloside and asperulosidic acid, from the *n*-butanol fraction of *Morinda citrifolia* leaf extract.



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This fraction was chromatographed successively on Diaion HP-20, Si gel, Sephadex LH-20, and RP-18 Si gel columns to afford three compounds.

Compound **1**, an amorphous solid, was assigned a molecular formula of $C_{26}H_{28}O_{14}$ determined by negative-ion APCIMS ($[M - H]^-$ at *m/z* 563), as well as from its ^{13}C and DEPT NMR data. Its IR spectrum indicated the presence of hydroxyl groups (3410 cm^{-1}), an α,β-unsaturated γ-lactone (1750 cm^{-1}), an iridoid enol ether system conjugated with an ester carbonyl group (1710, 1640 cm^{-1}), and a *p*-substituted phenyl group (1606, 1515, 817 cm^{-1}). The ^1H NMR spectrum of **1** showed a singlet for a carbomethoxy group at δ 3.73, a singlet for the C-1 proton at δ 5.35, a doublet (J = 2.0 Hz) for the characteristic C-3 proton of iridoids at δ 7.43, two doublets (J = 2.5 Hz) for the C-6,C-7-epoxy protons at δ 4.04 and 3.83, and a doublet (J = 8.0 Hz) for the C-1′ proton at δ 4.41, suggesting that the cyclopentanopyran ring system and the sugar moiety of **1** were identical to those of 6β,7β-epoxysplendoside.[6] In addition, a pair of two-proton doublets (J = 8.4 Hz) at δ 6.84 and 7.62 indicated the presence of a *p*-hydroxyphenyl group, and this was supported by resonances in the ^{13}C NMR spectrum (δ 126.3, C-1″; 134.8, C-2″ and C-6″; 117.0, C-3″ and C-5″; 162.2, C-4″).

Table 1. ^{13}C (150 MHz) and ^1H (600 MHz) NMR Spectral Data for Compound **1** (CD_3OD) (δ in ppm, J in Hz)

position	δ_C	δ_H	position	δ_C	δ_H
1	92.6 d	5.35 brs	1′	99.3 d	4.41 d (8.0)
3	153.3 s	7.43 d (2.0)	2′	74.4 d	3.09 m
4	108.1 s		3′	78.1 d	3.26 m
5	33.3 d	3.39 dd (2.0, 8.8)	4′	71.2 d	3.26 m
6	58.2 d	4.04 d (2.5)	5′	77.6 d	3.21 m
7	58.2 d	3.83 d (2.5)	6′	62.3 t	3.80 m
8	92.8 s				3.62 m
9	45.2 d	2.44 d (8.8)	1″	126.3 s	
10	69.1 d	5.12 s	2″	134.8 d	7.62 d (8.4)
11	124.0 s		3″	117.0 d	6.84 d (8.4)
12	172.9 s		4″	162.2 s	
13	144.0 d	7.58 s	5″	117.0 d	6.84 d (8.4)
15	168.1 s		6″	134.8 d	7.62 d (8.4)
			OCH_3	51.9 q	3.73 s

Altogether, the ^{13}C NMR spectrum of **1** exhibited 26 carbon signals (Table 1), with 10 representing the aglycon, one methoxy group (δ 51.9), and six for the glucopyranose unit (δ 99.3, d, C-1′; δ 74.4, d, C-2′; δ 78.1, d, C-3′; δ 71.2, d, C-4′; δ 77.6, d, C-5′; and δ 62.3, t, C-6′). The β-anomeric configuration for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants (J = 7.6 Hz).[7] HMBC and ROESY correlations between C-1/H-1′, H-1/C-1′, and H-1/H-1′ suggested that the β-glucopyranose unit was attached at the C-1 position of the aglycon. The remaining ^{13}C NMR resonances of **1** showed two quaternary carbons at δ 124.0 (C-11) and 172.9 (C-12), an oxygenated methine carbon at δ 69.1 (C-10), and one olefinic carbon atom at δ 144.0 (C-13), indicating a characteristic iridoid with a five-membered spiro-lactone ring at C-8.[8,9] These assignments were confirmed by HMBC and NOESY spectra (Figure 1). On the basis of the HMQC spectrum, the corresponding proton signals were found as a singlet at δ 5.12 for H-10 and a singlet for the trisubstituted olefinic proton (H-13) at δ 7.58.

The configuration of the C-6,C-7-epoxide group was confirmed from the NMR spectra of **1** as β ($J_{H1,9}$ < 1 Hz, $\Delta\delta$ C_3-C_4 = 45.2 < 47 ppm, and δ_{C1} = 92.6 < 99 ppm).[10,13] To determine the stereochemistry at C-8 and C-10, ROESY measurements were carried out on **1**. In the ROESY

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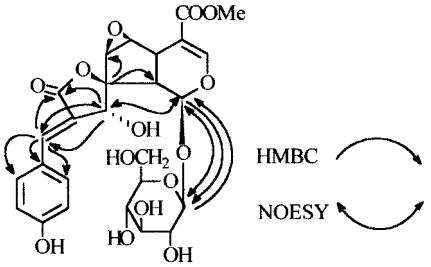

Figure 1. Significant HMBC (H→C) and ROESY correlations of **1**.

spectrum, the presence of strong cross-peaks between H-1 and H-10 indicated that the linkage between C-8 and C-10 was α-oriented and H-10 was *β*. Thus, compound **1** was determined as citrifolinoside **A**. Full assignments of the ^{1}H and ^{13}C NMR signals were accomplished using HMBC, HMQC, ^{1}H−^{1}H COSY, TOCSY, and NOESY experiments (Table 1). It is noted that a similar compound, oruwacin, was obtained from the same genus (*Morinda*).[9]

In addition to the new iridoid glycoside, two known iridoid glycosides, asperuloside and asperulosidic acid, have also been isolated in this study. Their structures were identified by comparison of their NMR and MS data with those reported in the literature.[11−13] The isolation of these two compounds has been reported from the fruits of *M. citrifolia* previously.[1,14] However, both are being reported from the leaves of this plant for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO DIP-181 polarimeter. UV spectra were recorded on a Varian Cary 1E UV−visible spectrophotometer. FT-IR spectra were obtained on a Perkin-Elmer 1600 apparatus. ^{1}H (600 Hz), ^{13}C (150 Hz), and all 2D NMR spectra were run on a Varian AM-600 NMR spectrometer, with TMS as internal standard. The APCIMS was performed on a Fisons/VG Platform II mass spectrometer. Elemental analysis was performed on a Carlo Erba 1106 instrument. Thin-layer chromatography was performed on Sigma-Aldrich TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution.

Plant Material. The dried leaves of *Morinda citrifolia* were collected from Bengal, India, in 1999 and were identified by Dr. Vladimir Badmaev at the Sarbinsa Corporation (Piscataway, NJ). A voucher specimen (HS16) was deposited in the Department of Food Science, Cook College, Rutgers University.

Extraction and Isolation. The dried leaves (5 kg) were extracted with 95% ethanol (4 L) at 50 °C for 1 day. The extract was concentrated to dryness under reduced pressure, and the residue was suspended in water (500 mL) and partitioned successively with hexane (3 × 500 mL), ethyl acetate (3 × 500 mL), and *n*-butanol (3 × 500 mL). The butanol fraction was subjected to a Diaion HP-20 column, eluted with a water−ethanol (water, 30% EtOH, 70% EtOH, 95% EtOH) solvent system. The fraction (5 g) eluted by 30% EtOH was subjected to Si gel column chromatography with an ethyl acetate−MeOH−H_2O−hexane solvent system (10:1:1:0.5 → 5:1:1:0). A fraction eluted by ethyl acetate−MeOH−H_2O (5:1:1) was subjected to a Sephadex LH-20 column eluted by 95% EtOH, and then RP-18 Si gel column chromatography with 10−20% MeOH to give compounds **1** (14 mg), asperuloside (110 mg), and asperulosidic acid (30 mg).

Citrifolinoside A: amorphous solid; $[\alpha]^{24}_D$ +65.2° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.75), 233 (4.03), 327 (4.14); IR (KBr) ν_{max} 3410, 1750, 1710, 1640, 1600, 1515, 1435, 1276, 1000−1100, 817 cm^{-1}; ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) of **1**, see Table 1; APCIMS *m*/*z* 563 [M−H]$^-$; *anal*. C 55.28%, H 4.93%, calcd for $C_{26}H_{28}O_{14}$, C 55.32%, H 4.96%.

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