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# Citrifolinin A, a new unusual iridoid with inhibition of Activator Protein-1 (AP-1) from the leaves of noni (*Morinda citrifolia* L.)

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**Abstract**—A new unusual iridoid, named citrifolinin **A**, showing significant inhibition of UVB-induced Activator Protein-1 (AP-1) activity in cell cultures, has been isolated from the leaves of *Morinda citrifolia*. Its structure was elucidated based on a detailed high-field 1D and 2D spectral analysis. 

Iridoids are of biogenetic and chemotaxonomic importance and are found mainly as glycosides in higher plants. This class of compounds[1] displays various biological activities. The family Rubiaceae is well known for the constituents of iridoid.[2–4] In our investigation for bioactive iridoids from plants, we studied the leaves of *Morinda citrifolia* L. (Rubiaceae), also known as noni, native of the Indian Ocean, which grows in the open coastal regions and in forest areas up to about 1300 feet above the sea level. The plant is a small evergreen tree. The bark, stem, root, leaf, and fruit have been used traditionally as a folk remedy for many diseases including diabetes, hypertension, and cancer.[5,6] A new unusual iridoid, named citrifolinin **A**, which features the presence of a unique substitute, a rearranged ferulic acid moiety, and has shown significant inhibition of UVB-induced Activator Protein-1 (AP-1) activity, has been isolated from the leaves of *Morinda citrifolia*. To our knowledge, this is the first example of an iridoid possessing a rearranged ferulic acid moiety. We herein describe the isolation, structural elucidation and inhibitory effect on UVB-induced AP-1 of this compound.

The butanol fraction of the ethanol extract of the dried noni leaves (5 kg) was subjected successively on Diaion HP-20, silica gel, and RP-18 silica gel to afford compound **1** (200 mg).

Citrifolinin **A** (**1**), a yellow powder, $[\alpha]_D^{20}$ −23.4 (*c* 0.26, MeOH), was assigned a molecular formula of $C_{27}H_{30}O_{16}$ determined by negative-ion APCI-MS ($[M-H]^-$ at $m/z$ 609) as well as from its $^{13}C$ and DEPT NMR data. The $^1H$ NMR spectrum of **1** displayed a signal pattern similar to that of mollugoside (8α-hydroxyapodanthoside).[7] It showed a singlet for the carbomethoxy group at $\delta$ 3.78 ppm, a doublet ($J=4.5$ Hz) for the C-1 proton at $\delta$ 5.57, a singlet for the C-3 proton characteristic of iridoids at $\delta$ 7.55, two double doublets ($J=2.6$, 6.2 and $J=6.2$, 1.7) for disubstituted olefinic protons at $\delta$ 5.70 and 6.78, and a doublet ($J=7.6$ Hz) for the characteristic anomeric proton resonance of glucose at $\delta$ 4.86. In addition, the signals of three aromatic protons ($\delta$ 6.96, d, $J=8.0$, H-5″; $\delta$ 7.52, dd, $J=8.0$, 1.3, H-6″; $\delta$ 7.55, d, $J=1.3$, H-2″), one methoxyl group ($\delta$ 3.92, s), along with one olefinic proton ($\delta$ 7.89, s, H-8″) indicated the presence of a rearranged ferulic acid moiety, and this was supported by the $^{13}C$ NMR spectrum ($\delta$ 130.5, s, C-1″; $\delta$ 111.6, d, C-2″; $\delta$ 153.8, s, C-3″; $\delta$ 146.1, s, C-4″; $\delta$ 115.2, d, C-5″; $\delta$ 125.9, d, C-6″; $\delta$ 128.1, s, C-7″; $\delta$ 158.1, d, C-8″; and $\delta$ 187.0, s, C-9″). In the meantime, the presence of the rearranged ferulic acid moiety was also confirmed by the HMBC and ROESY spectrum. The HMBC spectral analysis (Fig. 2) displayed correlation peaks between H-8″ and C-7″, H-8″ and C-9″, H-2″ and C-9″, H-6″ and C-9″. The ROESY spectrum showed the crosses between H-8″ and H-2″, H-8″ and H-6″. In the HMBC, long-range connectivity $^3J$ was observed between H-8″ ($\delta$ 7.89) and C-10 ($\delta$ 169.0), clearly indicating the

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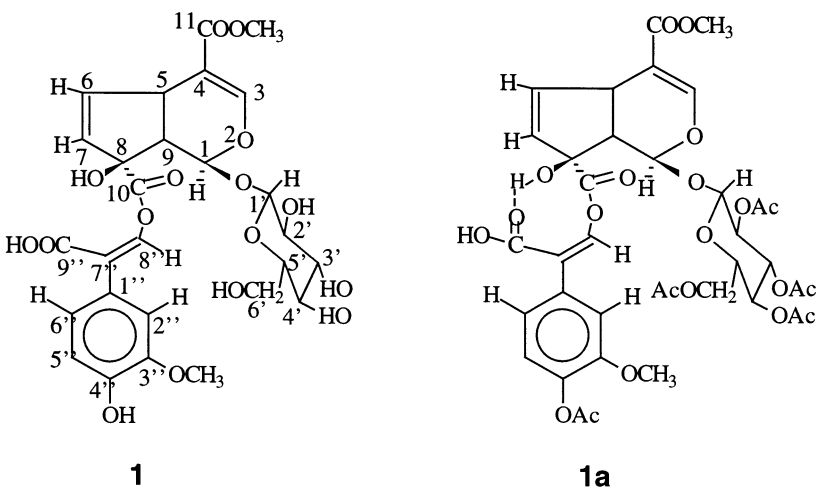
**Table 1.** $\delta_H$ (400 MHz) and $\delta_C$ (100 MHz) NMR spectra data of compounds **1** ($CDOD_3$) and **1a** ($CDCl_3$) ($\delta$ in ppm, $J$ in Hz)

| | **1** | | **1a** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 5.51 d, 4.5 | 92.6 (CH) | 5.26 s | 92.2 (CH) |
| 3 | 7.55 s | 151.2 (CH) | 7.36 s | 149.4 (CH) |
| 4 | | 109.9 (C) | | 111.8 (C) |
| 5 | 3.99 dd, 2.6, 9.0 | 39.2 (CH) | 3.82 d, 8.4 | 37.9 (CH) |
| 6 | 6.78 dd, 6.2, 2.6 | 141.8 (CH) | 6.56 d, 6.0 | 140.0 (CH) |
| 7 | 5.70 dd, 6.2, 1.7 | 128.3 (CH) | 5.57 d, 6.0 | 128.6 (CH) |
| 8 | | 96.7 (C) | | 96.1 (C) |
| 9 | 3.10 dd, 4.5, 9.0 | 50.2 (CH) | 3.30 d, 8.4 | 49.6 (CH) |
| 10 | | 169.0 (C) | | 168.5 (C) |
| 11 | | 167.0 (C) | | 166.1 (C) |
| 1′ | 4.68 d. 7.6 | 98.9 (CH) | 4.82 d, 8.4 | 95.7 (CH) |
| 2′ | 3.20 m | 73.8 (CH) | 4.98 t, 8.4 | 70.7 (CH) |
| 3′ | 3.38 m | 77.8 (CH) | 5.21 t, 9.6 | 72.5 (CH) |
| 4′ | 3.10 m | 70.7 (CH) | 5.08 t, 9.6 | 67.9 (CH) |
| 5′ | 3.40 m | 76.8 (CH) | 3.72 m | 72.4 (CH) |
| 6′ | 3.40 m<br>3.82 dd | 61.9 (CH) | 4.11 d, 12.0<br>4.29 d, 12.0 | 61.5 (CH) |
| 1″ | | 130.5 (C) | | 134.5 (C) |
| 2″ | 7.55 d, 1.3 | 111.6 (CH) | 7.60 s | 112.5 (CH) |
| 3″ | | 153.8 (C) | | 152.0 (C) |
| 4″ | | 146.1 (C) | | 145.1 (C) |
| 5″ | 6.96 d, 8.0 | 115.2 (CH) | 7.18 d, 8.4 | 123.2 (CH) |
| 6″ | 7.52 dd, 8.0, 1.3 | 125.9 (CH) | 7.42 d, 8.4 | 123.6 (CH) |
| 7″ | | 128.1 (C) | | 131.1 (C) |
| 8″ | 7.89 s | 158.1 (CH) | 7.51 s | 158.0 (CH) |
| 9″ | | 187.0 (C) | | 186.2 (C) |
| 3″-$OCH_3$ | 3.92 s | 55.4 ($CH_3$) | 3.91 s | 56.3 ($CH_3$) |
| 11-$OCH_3$ | 3.78 s | 51.0 ($CH_3$) | 3.75 s | 51.9 ($CH_3$) |

location of the rearranged ferulic acid moiety. A $^{13}C$ NMR chemical shift of $\delta$ 187.0 seems unusually high for an unsaturated carboxylic group. This can be explained mainly by the existence of a nine-membered intramolecular hydrogen bond which can deshield the carboxyl carbon by as much as +10 ppm,[8] between the carbonyl group of C-9″ ($\delta$ 187.0) and the hydroxyl group of C-8 ($\delta$ 96.7) according to the three-dimensional structure model. The existence of the intramolecular hydrogen bond was also proved by the acetylation product **1a** of compound **1**.[9] Both the $^1H$ NMR and $^{13}C$ NMR spectrum of **1a** displayed five acetyl groups ($\delta_H$ 1.93, 2.01, 2.02, 2.08, and 2.35 ppm; $\delta_C$ 20.3, 20.7, 20.7, 20.8, 20.9 ppm and 168.5, 169.1, 169.4, 170.3, 170.8 ppm). Among them, four belong to the glucose, one for the hydroxyl group of the aryl. However, compound **1** has six hydroxyl groups, so there must has a intramolecular hydrogen bond between the carbonyl group of C-9″ and the hydroxyl group of C-8. In the HMBC of **1a**, $\delta$ 186.2 (C-9″) also showed the correlation peaks with H-2″ ($\delta$ 7.60, s), H-6″ ($\delta$ 7.42, d, 8.4) and H-8″ ($\delta$ 7.51, s).

The $^{13}C$ NMR of compound **1** exhibited 27 carbon signals (Table 1), ten corresponding to the aglycone, nine for the rearranged ferulic acid residue, two methoxy group ($\delta$ 55.4 and 51.0), six for the glucopyranose unit ($\delta$ 98.9, d, C-1′; $\delta$ 73.8, d, C-2′; $\delta$ 77.8, d, C-3′; $\delta$ 70.7, d, C-4′; $\delta$ 76.8, d, C-5′; and $\delta$ 61.9, t, C-6′). The β anomeric configurations for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants ($J=7.6$ Hz).[10] HMBC and ROESY correlations between C-1/H-1, H-1/C-1′ and H-1/H-1′ suggested that the β-glucopyranose unit attached at C-1 position of the aglycone.

The stereochemistry at the C-8 center of **1** was demonstrated to be of the 'monoterpein-type', on the basis of the 'C-8 epimers rule'[11,12] (an α-hydroxyl group at C-8 exerts on C-9 a shielding of 5–7 ppm with respect to its β counterpart). Comparison of the chemical shift value of C-9 of 1 ($\delta$ 50.2) with that of the corresponding carbon in mollugoside ($\delta$ 47.5), indicated that the configuration at the C-8 center of **1** was β-hydroxyl and α-carboxyl. This stereochemistry was further supported by the magnitude of the $J_{1,9}$ coupling constant[13] (the magnitude of the $J_{1,9}$ coupling constant is significantly




**1** **1a**

**Figure 1.** Structure of compounds **1** and **1a**.

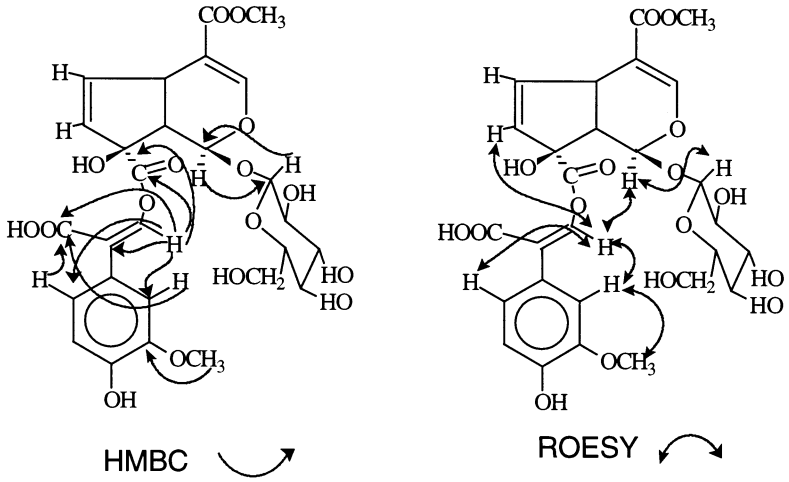



**Figure 2.** Significant HMBC (H→C) and ROESY correlations of compound **1**.

smaller in the α-OH series than in the β-OH series). The $J_{1,9}$ value of compound **1** is 4.5 Hz, which is significantly bigger than that of mollugoside ($J_{1,9}$=1.3 Hz). Furthermore, in the ROESY spectra, the presence of cross peaks between H-8″ and H-1, and H-8″ and H-7 also indicated that an α linkage between C-8 and C-10. Thus, the structure of compound **1** was deduced as shown (Fig. 1) and named citrifolinin **A**. The complete interpretation of the NMR data was based on the results of COSY, TOCSY, HMQC, HMBC, and ROESY experiments (Table 1).

Citrifolinin **A** was tested for suppressing UVB-induced AP-1 activity. It was shown that it displayed significant inhibitory effect with $IC_{50}$ of 69.6 μM. The inhibition on UVB-induced AP-1 activity of iridoid is reported for the first time.

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