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A New Unusual Iridoid with Inhibition of Activator Protein-1 (AP-1) from the Leaves of *Morinda citrifolia* L.

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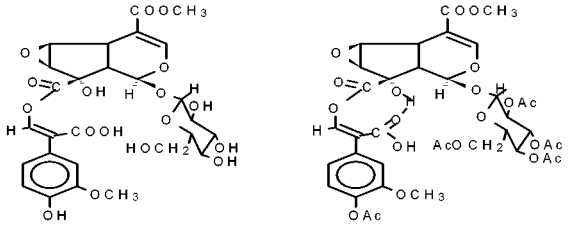
ABSTRACT



1 **1a**

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

The compounds of the family Rubiaceae are well-known as constituents of iridoids.[1−3] In our investigation for bioactive iridoids from plants, we studied the leaves of *Morinda citrifolia* L. (Rubiaceae), also known as noni, native to the Indian Ocean. The bark, stem, root, leaf, and fruit have been used traditionally as a folk remedies for many diseases including diabetes, hypertension, and cancer.[4,5] A new unusual iridoid, named citrifolinoside, which features the presence of a unique substituent, 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 *M. citrifolia*. 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 to Diaion HP-20, silica gel, and RP-18 silica gel to afford compound **1** (110 mg) (Figure 1).

The molecular formula of citrifolinoside (**1**) was determined to be $C_{27}H_{30}O_{17}$ by negative-ion APCI-MS ($[M - H]^-$ at m/z 625) 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 6β,7β-epoxysplendoside[6] except for the lack of

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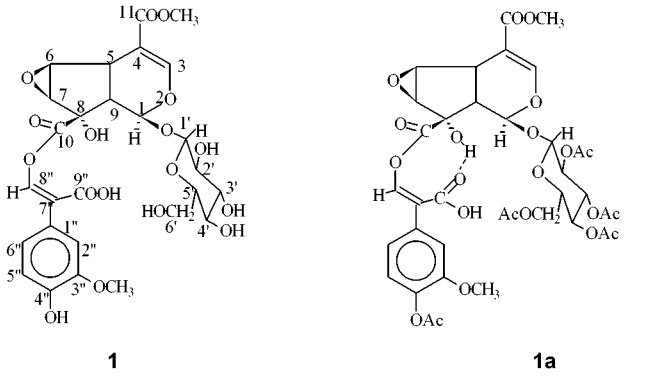

Figure 1. Structures of compounds **1** and **1a**.

signals of the hydroxymethyl group at C-8 and the downfield shift (0.69 ppm) of the H-9 resonance, both indicating the probable presence of a COOR group linked to the hydroxylated quaternary C-8. Therefore, the planar structure of compound **1** must be the epoxy derivative of mollugoside (8α-hydroxyapodanthoside).[7] It showed a singlet for the carbomethoxy group at δ 3.76, a singlet for the C-1 proton at δ 5.66, a singlet for the C-3 proton characteristic of iridoid at δ 7.57, two doublets ($J = 3.6$) for the epoxy protons at δ 4.08 and 3.58, and a doublet (J = 7.8 Hz) for the characteristic anomeric proton resonance of glucose at δ 4.59. In addition, the signals of three aromatic protons (δ 6.90, d, $J = 8.0$, H-5″; δ 7.52, d, $J = 8.0$, H-6″; δ 7.46, s, H-2″), one methoxyl group (δ 3.94, s), and one olefinic proton (δ 7.62, s, H-8″) indicated the presence of a rearranged ferulic acid moiety, and this was supported by the ^{13}C NMR spectrum (δ 132.6, s, C-1″; δ 111.9, d, C-2″; δ 154.0, s, C-3″; δ 148.0, s, C-4″; δ 115.6, d, C-5″; δ 125.7, d, C-6″; δ 127.9, s, C-7″; δ 155.6, d, C-8″; and δ 186.4, s, C-9″). The presence of the rearranged ferulic acid moiety was also confirmed by the HMBC and ROESY spectra. The HMBC spectral analysis (Figure 2) displayed correlation peaks

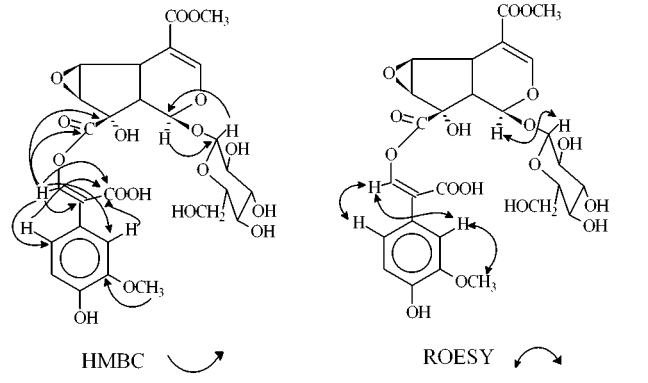

Figure 2. Significant HMBC (H → C) and ROESY correlations of compound **1**.

between H-8″ and C-7″, H-8″ and C-9″, H-2″ and C-9″, and H-6″ and C-9″. The ROESY spectrum showed crosses between H-8″ and H-2″ and H-8″ and H-6″. In the HMBC, long-range connectivity ^3J was observed between H-8″ (δ 7.89) and C-10 (δ 169.0), clearly indicating the location of the rearranged ferulic acid moiety. The ^{13}C NMR chemical shift of δ 186.4 seems unusually high for an unsaturated carboxylic group, and also δ_{C-8} (91.9 ppm) is slightly higher than the normal value (<90 ppm). These observations can be explained mainly by the existence of a nine-membered intramolecular hydrogen bond which can deshield the carboxyl carbon and the carbon which directly connected to the hydroxyl group by as much as +10 ppm,[8] between the carbonyl group of C-9″ (δ 186.4) and the hydroxyl group of C-8 (δ 91.9) 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] (Figure 1). Both the ^{1}H NMR and ^{13}C NMR spectra of **1a** displayed 5 acetyl groups (δ_H 1.86, 1.96, 1.98, 2.05, and 2.32 ppm; δ_C 20.3, 20.7, 20.8, 20.9, 20.9 ppm and 166.9, 168.9, 169.5, 170.4, 170.9 ppm). Among them, four belong to the glucose and one to the hydroxyl group of the aryl. Therefore, there must be an intramolecular hydrogen bond between the carbonyl group of C-9″ and the hydroxyl group of C-8. To observe this hydrogen directly, we recorded an ^{1}H NMR spectrum of **1a** using DMSO as solvent. At δ 3.21, an additional singlet peak was observed compared to the previous ^{1}H NMR spectrum (dissolved in $CDCl_3$) of **1a**.

The ^{13}C NMR spectrum of compound **1** exhibited 27 carbon signals (Table 1), 10 corresponding to the aglycone,

Table 1. δ_H (400 MHz) and δ_C (100 MHz) NMR Spectral Data of Compounds **1** ($CDOD_3$) and **1a** ($CDCl_3$) (δ in ppm, J in Hz)

	1		**1a**	
	δ_H	δ_C	δ_H	δ_C
1	5.66 s	91.9 (CH)	5.20 s	91.1 (CH)
3	7.57 s	152.4 (CH)	7.42 s	152.1 (CH)
4		107.2 (CH)		108.4 (CH)
5	3.51 d, 9.8	31.9 (CH)	3.40 d, 8.4	31.9 (CH)
6	4.08 d, 3.6	58.1 (CH)	4.09 s	57.9 (CH)
7	3.58 d, 3.6	57.0 (CH)	3.39 s	56.6 (CH)
8		91.9 (C)		91.8 (C)
9	2.91 d, 9.6	43.6 (CH)	3.02 d, 8.4	42.7 (CH)
10		169.2 (C)		168.9 (C)
11		167.0 (C)		165.7 (C)
1′	4.59 d. 7.8	98.2 (CH)	4.83 d, 7.8	96.1 (CH)
2′	3.20 t, 8.4	73.9 (CH)	4.91 t, 8.4	70.8 (CH)
3′	3.35 m	77.7 (CH)	5.15 t, 9.6	72.5 (CH)
4′	3.28 t, 10.2	70.2 (CH)	5.06 t, 9.6	67.9 (CH)
5′	3.38 t, 9.6	76.9 (CH)	3.68 d, 8.4	72.4 (CH)
6′	3.60 m	61.9 (CH)	4.07 d, 12.6	61.5 (CH)
	3.88 d, 9.6		4.28 dd, 4.2, 12.6	
1″		132.6 (C)		134.2 (C)
2″	7.46 s	111.9 (CH)	7.56 s	112.6 (CH)
3″		154.0 (C)		151.5 (C)
4″		148.0 (C)		145.3 (C)
5″	6.90 d, 8.4	115.6 (CH)	7.14 d, 8.4	123.2 (CH)
6″	7.52 d, 8.4	125.7 (CH)	7.37 d, 8.4	123.7 (CH)
7″		127.9 (C)		133.5 (C)
8″	7.62 s	155.6 (CH)	7.42 s	156.1 (CH)
9″		186.4 (C)		185.7 (C)
3″-OCH_3	3.94 s	55.3 (CH_3)	3.88 s	56.3 (CH_3)
11-OCH_3	3.76 s	51.0 (CH_3)	3.75 s	52.1 (CH_3)

9 to the rearranged ferulic acid residue, 2 to the methoxy group (δ 55.4 and 51.0), and 6 to the glucopyranose unit (δ 98.2, d, C-1′; δ 73.9, d, C-2′; δ 77.7, d, C-3′; δ 70.2, d, C-4′; δ 76.9, d, C-5′; and δ 61.9, t, C-6′). The β anomeric configurations for the glucose were judged from its large $^3J_{H1,H2}$ coupling constants (J = 7.8 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 the C-1 position of the aglycone.

According to the literature,[11,12] if $J_{H1,9} < 1$ Hz, $\Delta\delta$ $C_3-C_4 < 47$ ppm, and $\delta_{C1} < 99$ ppm, the configuration of C6, C7-epoxy is β, otherwise it should be α. For compound **1**, $J_{H1,9} = 0 < 1$ Hz, $\Delta\delta$ $C_3-C_4 = 45.2 < 47$ ppm, and $\delta_{C1} = 91.9 < 99$ ppm, so the configuration of the C6,C7-epoxy was confirmed as β. 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 " [13,14] (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** (δ 43.6) with that of the corresponding carbon in mollugoside (δ 47.5) indicated that the configuration at the C-8 center of **1** was the same as that of mollugoside. This stereochemistry was further supported by the magnitude of the $J_{1,9}$ coupling constant[11] (the magnitude of the $J_{1,9}$ coupling constant is significantly smaller in the α-OH series than in the β-OH series). Since H-1 is a singlet, the stereochemistry at the C-8 center should be α-hydroxyl and β-carboxyl. Thus, the structure of compound **1** was deduced as shown (Figure 1) and named citrifolinoside. The complete interpretation of the NMR data was based on the results of COSY, TOCSY, HMQC, HMBC, and ROESY experiments (Table 1).

It is well-known that UVB irradiation plays a major role in the development of human skin cancer,[15,16] acting both as a tumor initiator and tumor promoter. Transaction of AP-1 plays a key role in tumor promotion,[17,18] so we investigated the inhibitory activity of UVB-induced AP-1 for citrifolinoside. It was shown that citrifolinoside displayed a significant inhibitory effect with an IC_{50} of 29.0 μM. Citrifolinoside is a unique example of iridoids that shows inhibitory activity of UVB-induced AP-1.

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