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New anti-malarial phenylpropanoid conjugated iridoids from *Morinda morindoides*

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ABSTRACT

A new phenylpropanoid conjugated iridoid together with four known congeners was isolated from *Morinda morindoides*, used for the therapy of malaria traditionally in some African countries, as anti-malarial principles through bioassay-guided separation. Furthermore, their absolute stereostructures were unambiguously established by a combination of modified Mosher's method and chemical correlation.

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Malaria remains endemic in more than 90 countries, principally in the tropical world. There are between 300 and 500 million infected persons and more than 1 million deaths, mostly of children, are attributable to the disease. For instance, in every 40 second a child dies of malaria, resulting in a daily loss of more than 2000 young lives worldwide. Not only lack of vaccine but also emerging resistance of a deadly form of malaria parasite, *Plasmodium falciparum,* to the commercially available anti-malarial drugs, has urged search for new anti-malarials as a global demand.[1,2] This circumstance prompted us to be devoted to exploring some new anti-malarial candidates with high selectivities against the mammalian host cells from natural medicinal plant resources.[3–6]

In some African countries, the leaves of *Morinda morindoides* have been utilized for treatment of malaria.[7] Recently, the extract of the plant was found to show not only inhibition of proliferation of *P. falciparum* in vitro but also in vivo anti-malarial potency against mice infected by *P. berghei*.[8,9] On the basis of this finding as well as the traditional reputation, we undertook to reveal the principles responsible for this bioactivity of the medicinal plant. Herein, we describe a new anti-malarial phenylpropanoid conjugated iridoid together with the four congeners and elucidation of their absolute stereostructures.

The MeOH extract of *M. morindoides*, which was collected at Democratic Republic of the Congo at 2006, was suspended with water, then the suspension was partitioned between *n*-hexane, EtOAc, *n*-BuOH successively. The EtOAc layer with the most potent activity was further separated by a combination of SiO_2 column, normal, and reversed phase-HPLC with monitoring anti-malarial potency to furnish a new phenylpropanoid conjugated iridoid glycoside **1**[10] along with the four congeners (**2**–**5**) as active constituents.

The molecular formula of **1** was established as $C_{29}H_{32}O_{15}$ by HR FAB-MS indicative of a quasimolecular ion peak at *m/z* 619.1669 $[M-H]^-$ (calcd: 619.1663). The IR spectrum of **1** showed the absorption bands due to a hydroxyl (3391 cm^{-1}), an α,β-unsaturated γ-lactone (1756 cm^{-1}), an ester carbonyl (1744 cm^{-1}), an α, β-unsaturated ester (1707 cm^{-1}), and an ester conjugated olefin (1642 cm^{-1}) functions, and an aromatic ring (1605 cm^{-1}). The ^1H NMR spectrum of **1** showed the signals ascribable to an anomeric proton [δ 4.54 (1H, d, J = 7.9 Hz, H-1″)], an acetal proton [δ 4.97 (1H, d, J = 3.1 Hz, H-1)], an oxymethine [δ 5.35 (1H, br s, H-7′)], two methoxyl [δ 3.89 (3H, s, 3′-OCH_3), 3.74 (3H, s, 4-$COOCH_3$)], and an acetyl [δ 1.83 (3H, s, 6″-OAc)] groups. Additionally, the presence of a 1,3,4-trisubstituted aromatic ring, two α-substituted α,β-unsaturated enones, and a nonconjugated olefin was suggested by the following ^1H NMR data; δ 7.01 (1H, br s, H-2′), 6.79 (1H, d,

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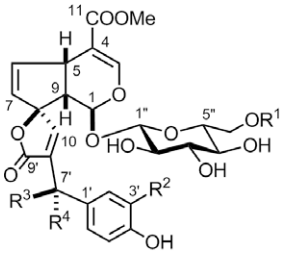
J = 7.9 Hz, H-5′), 6.89 (1H, br d, J = 7.9 Hz, H-6′), 7.51 (1H, br s, H-3), 7.28 (1H, br s, H-10), 6.48 (1H, dd, J = 5.5, 2.4 Hz, H-6), 5.59 (1H, dd, J = 5.5, 1.8 Hz, H-7).

On the other hand, two acetals [δ_c 94.0 (C-1), 100.4 (C-1″)] and three carbonyl [δ_c 172.3 (C-9′), 168.3 (C-11), 172.8 (6″-OAc)] carbon signals appeared in the ^{13}C NMR spectrum of **1**. Furthermore, the ^{13}C NMR spectrum showed the presence of one oxymethine carbon [δ_c 70.0 (C-7′)] in the aglycone moiety. Intense analysis of the NMR data between **1** and **3** revealed that both data resemble each other except for those around C-6″ (Table 1). Namely, the definite acylation shift[11] around C-6″ was observed with respect to **1**. In addition, the HMBC correlation appeared from H-6″ to the carbonyl carbon in the acetyl moiety. Consequently, the anti-malarial principle **1** was elucidated to be the 6″-*O*-acetyl congener of **3**. The known compounds **2**–**5** were identified by direct comparison of their spectral data with those reported in the literature.[12] Although the relative configuration of **2**–**5** except for C-8 and C-7′ were determined by the coupling constants of protons signals in the ^{1}H NMR spectra, no obvious establishment for absolute configurations of **2**–**5** were conducted.

Determination of the absolute configurations at position C-1 and C-7′ of **1**–**5** was achieved in the following manner. In the NOESY spectra of **3** and **4**, definite correlations between H-1 and H-10 appeared. Thus, the relative configurations at C-8 of **3** and **4** were determined as shown in Figure 1. Treatment of **1** and **2** with NaOMe in MeOH, respectively, afforded **3** and **4**, this indicating that the above two pairs of congeners possessed the same absolute configuration. Accordingly, the absolute configurations of aglycone moieties of **3** and **4** were at first elucidated by modified Mosher's method.[13] Methylation of the aromatic hydroxyl groups in **3** and **4** with trimethylsilyldiazomethane followed by enzymatic hydrolysis with naringinase provided the two corresponding hemiacetals **8** and **9**. Condensation of (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) with **8** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) and 4-dimethylaminopyridine (DMAP) furnished di-(*S*)-MTPA ester **10a** in 88% yield. In the same manner, di-(*R*)-MTPA ester **10b** was also provided in 98% yield. Distribution of difference in the chemical shifts of **10a** and **10b** established 1*R*,7′*S* configurations of **8** as depicted in Figure 2. According to the same protocol, the aglycone **9** was found to possess the same absolute configurations as **8**. By a combination of the above described findings, the absolute stereostructures of the aglycone moieties in **3** and **4** were unambiguously established.



	R^1	R^2	R^3	R^4
1	Ac	OMe	H	OH
2	Ac	H	H	OH
3	H	OMe	H	OH
4	H	H	H	OH
5	H	OMe	=O	=O

Figure 1. Anti-malarial phenylpropanoid conjugated iridoids from *M. morindoides*.

Next, the stereochemistry of sugar portion was determined by application of the ^{13}C NMR glycosylation shift rule of 1,1″-disaccharides. In this rule, *RR* and *SS* disaccharides showed smaller shifts on two hemiacetal carbon signals by less than 3.5 ppm, while *SR* disaccharide induced larger $\Delta\delta_c$ values by more than 4 ppm in pyridine-d_5.[14] With regard to the acetal glucoside **6** ($\delta_{C\text{-}1}$:94.0, $\delta_{C\text{-}1''}$:101.1) analogous to the disaccharide, both glycosylation shifts for **8** ($\delta_{C\text{-}1}$:92.5) and glucose ($\delta_{C\text{-}1''}$:98.9) were less than 3.5 ppm. In the case of **7** ($\delta_{C\text{-}1}$:94.4, $\delta_{C\text{-}1''}$:101.3), nearly similar behavior was observed for **9** ($\delta_{C\text{-}1}$:92.5) and glucose. These outcomes are characteristic of the *RR*- or *SS*-dihemiacetal combinations. Taking 1*R* configurations of the aglycone moieties of **6** and **7** into account, each sugar residue was determined to be D-glucose with 1*R* configuration. Consequently, the absolute stereostructures of **1**–**4** were definitely elucidated as depicted in Figure 1. On the other hand, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of **4** furnished **5** in 98% yield. On the basis of this chemical correlation, the absolute stereostructure of **5** was unambiguously constructed (Scheme 1).

Table 2 summarizes anti-malarial potency against *P. falciparum*[15] together with cytotoxicity against the mammalian host KB 3-1 cells[16] with regard to **1**–**5**. All the isolates exhibited little cytotoxicity against KB 3-1 cells even at the concentration of 150 μM. In particular, the 7′-ketocongener (**5**) displayed the most potent inhibitory effect for proliferation of the malaria parasite (IC_{50} = 0.04 μM) without showing any cytotoxicity. From the structure activity relationship analysis of the five congeners, the 6″-acetyl, 3′-methoxyl, and

Table 1
^{1}H and ^{13}C NMR data for **1**[a]

Position	^{1}H (600 MHz)	^{13}C (150 MHz)
1	4.97 (d, 3.1 Hz)	94.0
3	7.51 (br s)	152.1
4		111.5
5	3.86 (br d, 7.9 Hz)	39.4
6	6.48 (dd, 5.5, 2.4 Hz)	140.7
7	5.59 (dd, 5.5, 1.8 Hz)	130.3
8		97.8
9	2.98 (dd, 7.9, 3.1 Hz)	51.0
10	7.28 (br s)	149.8
11		168.3
1′		134.0
2′	7.01 (br s)	111.6
3′		149.1
4′		147.7
5′	6.79 (d, 7.9 Hz)	116.2
6′	6.89 (br d, 7.9 Hz)	121.3
7′	5.35 (br s)	70.0
8′		138.3
9′		172.3
1″	4.54 (d, 7.9 Hz)	100.4
2″	3.13 (dd, 7.9, 8.6 Hz)	74.2
3″	3.42 (m)	77.7
4″	3.33 (m)	71.4
5″	3.24 (dd, 9.8, 6.1 Hz)	75.6
6″	4.20 (dd, 12.2, 6.1 Hz) 4.15 (br d, 12.2 Hz)	64.6
4-$COOCH_3$	3.74 (s)	52.0
3′-OCH_3	3.89 (s)	56.6
6″-OAc	1.83 (s)	172.8 20.6

[a] The spectra were taken in CD_3OD.

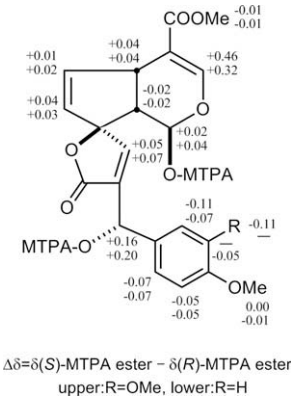
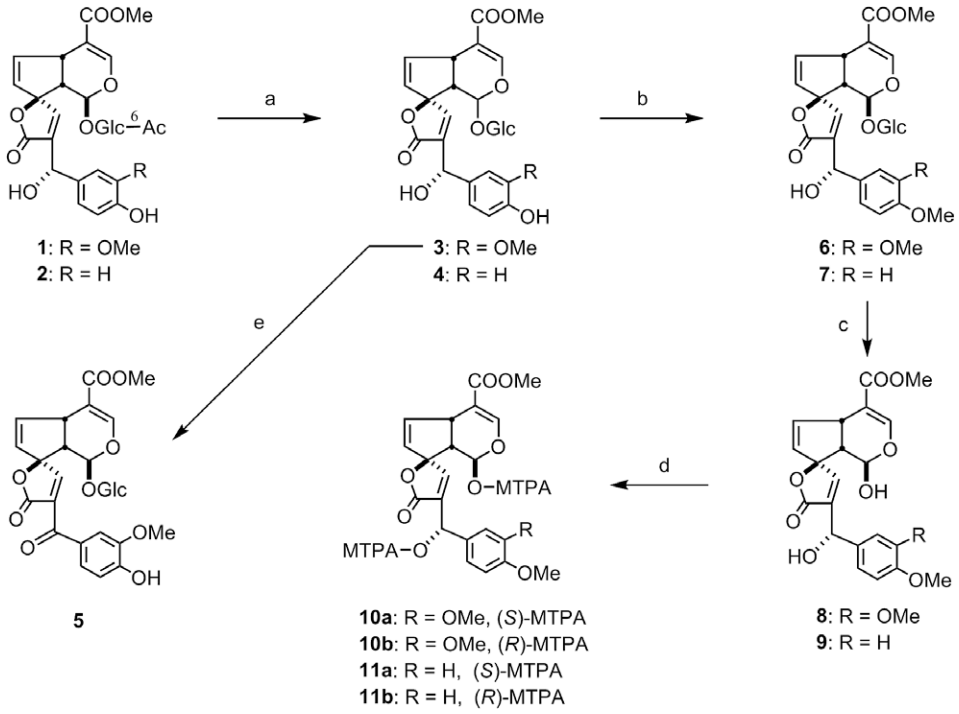


Figure 2. Distribution of Δδ in the MTPA esters of **8** and **9**.



Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, 89% for **3**, 91% for **4**; (b) $TMSCHN_2$, MeOH–Et_2O, quant. for **6**, quant. for **7**; (c) naringinase, acetate buffer (pH 5), 79% for **8**, 77% for **9**; (d) (*S*)- or (*R*)-MTPA, EDCI·HCl, DMAP, CH_2Cl_2, 79% for **10a**, 91% for **10b**, 88% for **11a**, 98% for **11b**; (e) DDQ, 1,4-dioxane, 98%.

Table 2
Antimalarial and cytotoxic activity of **1**–**5**

Compound	Antimalarial activity (IC_{50}, μM)	Cytotoxicity (%, 150 μM)
1	0.1	9.3
2	4.1	4.3
3	21.9	13.1
4	0.8	13.4
5	0.04	6.1

7′-ketonic carbonyl functionalities enhanced anti-malarial potency independent of cytotoxicity against the host cells.

In conclusion, bioassay-guided separation of the MeOH extract of *M. morindoides* disclosed the new phenylpropanoid conjugated iridoid **1** together with the known four congeners **2**–**5** as the anti-malarial candidates. To date, the two anti-malarial iridoids, showing IC_{50} of about 40 μg/mL, have been found from natural resources.[17,18] In comparison with the two predecessors, it is worthwhile that all the isolates except for **3** potently inhibited proliferation of the parasites with little cytotoxicity against the host mammalian cells. Furthermore, it should be noted that the most potent congener **5** is directly prepared in high yield by DDQ oxidation from the most abundant constituent **2** without prior protection of the hydroxyl groups. Exploration for more potent derivatives accompanied by efficacy in mouse model is currently under investigation by use of the five principles as the scaffolds.

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16. Cytotoxicity against human epidermoid carcinoma KB cells (KB-3-1) was evaluated by means of MTT colorimetric assay performed in 96-well plates. KB-3-1 cells were cultured in RPMI 1640 medium supplemented with 10% newborn calf serum, 0.44 mg/mL of glutamine, and 50 μg/mL of kanamycin sulfate under a 5% CO_2 atmosphere at 37 °C. Equal numbers of cells

(1.0×10^4 cells) were inoculated into each well with 100 μL of the culture medium, then 100 μL solution of the tested sample in 2% DMSO-contained medium was added to each well. After incubation for 72 h, 25 μL of MTT solution (2 mg/mL in PBS) was added to each well and the whole was treated at 37 °C for further 3 h. The medium was removed by aspiration, thereafter the resulting formazan was dissolved with 200 μL of dimethylsulfoxide. The percentage of cell growth inhibition was calculated from the absorbance at 540 nm. In this assay, mitomycin C showed IC_{50} of 0.1 μg/mL as a positive control.

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