http://dx.doi.org/10.5012/bkcs.2013.34.5.1555

Chemical Constituents of the *Morinda tomentosa* Leaves and their α-Glucosidase Inhibitory Activity

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Received December 10, 2012, Accepted February 21, 2013

Key Words : *Morinda tomentosa*, Morintoside A, Morintoside B, α-Glucosidase inhibitor

α-Glucosidase catalyzes the final step in the digestion of carbohydrates; hence α-glucosidase inhibitors could retard the digestion of dietary carbohydrates and suppress post-prandial hyperglycemia. Several α-glucosidase inhibitors (AGIs) are available, such as acarbose (Glucobay®) from *Actinoplane* sp.,[1] voglibose (Basen®) from *Streptomyces hygroscopicus* var. *limoneus*[2] and miglitol (Glyset®) from *S. roseochromogenus*.[3] Acarbose, when taken as directed, has been shown to reduce the intestinal absorption of sugars in humans.[4] Although several drugs that targeted for carbohydrate-hydrolyzing enzymes are in clinical use, it is necessary to have a large inhibitor pool, because diabetic patients can develop resistance to current regimens. Natural products have served as an important source of drugs since ancient times and about half of the useful drugs available today are derived from natural sources.[5] Medicinal plants still play an important role in drug discovery due to their chemical and pharmacological diversity. Moreover, their uses in traditional medicine guide sample selection. *Morinda tomentosa* is a known phytotherapeutic plant that is used for the treatment of various diseases in traditional oriental medicine.

Morinda tomentosa Heyne ex Roth., a small tree, belongs to the family Rubiaceae and is common in Vietnam. Its roots, barks, stems, leaves, and fruits have been used in folk medicine for the treatment of diabetes, hypertension, and cancer. A recent study reported that *M. tomentosa* extracts showed marked against human pathogens, such as *Staphylococcus aureus* and *Klebsiella pneumoniae*.[6] However, no phytochemical investigation of this plant has been reported to date. Herein, we report two new, morintoside A (**1**) and morintoside B (**5**) and ten known compounds from the leaves of *M. tomentosa* and their α-glucosidase inhibitory activity.

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{20}H_{26}O_{13}$ by HR-ESI-MS at *m/z* 509.1054 $[M + Cl]^-$ (Calcd $C_{20}H_{26}O_{13}Cl$ for 509.1054) and *m/z* 473.1292 $[M - H]^-$ (Calcd $C_{20}H_{25}O_{13}$ for 473.1295). The ^{1}H-NMR spectrum of **1** (CD_3OD) exhibited signals for two acetyl proton groups at δ_H 2.05 and 2.07, two olefinic protons at δ_H 5.82 and 7.31, and an anomeric proton at δ_H 4.69, as listed in Table 1. The ^{13}C-NMR and DEPT spectra revealed 20 carbon signals, of which, 10 were assigned to an iridoid moiety, 4 belonged to two acetyl moieties, and 6 contributed to a sugar moiety. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of 6-*O*-acetylscandoside[7] except for the addition of the acetyl group at C-10 of the iridoid. In the HMBC spectrum, the methyl

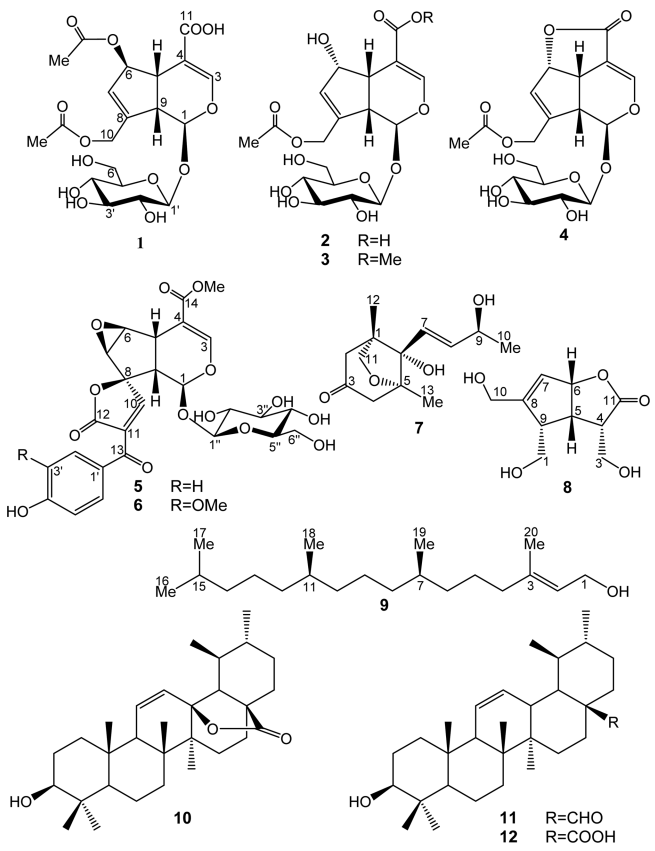

Figure 1. Structures of isolated compounds **1-12** from the leaves of *M. tomentosa*.

Table 1. NMR data for compounds **1-4**

Pos.	**1**				**2**	**3**	**4**
	δ_C[a,c]	δ_H[a,d] (mult., *J* in Hz)	δ_C[b,c]	δ_H[b,d] (mult., *J* in Hz)	δ_C[a,c]	δ_C[a,c]	δ_C[a,c]
Aglycone							
1	97.1	5.27 (d, 6.0)	95.5	5.17 (d, 5.5)	101.3	101.3	93.3
3	150.7	7.31 (s)	149.4	7.14 (s)	155.4	155.3	150.3
4	113.8	-	112.7	-	108.3	108.1	106.1
5	42.3	3.35*	40.6	3.11*	42.5	42.4	37.4
6	83.9	5.58 (br s)	81.8	5.51 (br s)	75.4	75.4	86.3
7	129.4	5.82 (s)	127.4	5.75 (br s)	131.9	131.8	128.9
8	145.1	-	163.6	-	145.9	146.0	144.2
9	47.6	3.09 (t, 6.0)	46.0	2.98*	46.2	46.2	45.2
10	62.8	4.82 (d, 15.0) 4.95*	61.1	4.65 (d, 15.0) 4.73 (d, 15.0)	63.8	63.7	61.9
11	173.0	-	170.3	-	171.0	169.3	172.2
6-$CH_3\underline{C}O$	172.5	-	169.8	-			
6-$\underline{CH_3}CO$	20.7	2.07 (s)	20.5	2.06 (s)			
10-$CH_3\underline{C}O$	172.7	-	169.9	-	172.5	172.5	172.5
10-$\underline{CH_3}CO$	21.3	2.05 (s)	21.0	1.97 (s)	20.7	20.7	20.6
11-OMe						51.8	
1-*O*-glc							
1'	100.3	4.69 (d, 7.5)	98.6	4.48 (d, 7.5)	100.6	100.6	100.0
2'	74.9	3.22 (dd, 7.5, 8.0)	73.2	2.98*	74.9	74.9	74.5
3'	77.9	3.38 (t, 8.0)	76.64	3.17*	78.5	78.6	78.3
4'	71.6	3.28 (m)	70.0	3.05 (t, 9.0)	71.6	71.5	71.6
5'	78.4	3.32*	77.2	3.12*	77.9	77.9	77.8
6'	62.9	3.67 (dd, 5.5, 12.0) 3.87 (dd, 2.0, 12.0)	61.1	3.42 (dd, 6.0, 12.0) 3.67 (br d, 12.0)	63.0	63.0	62.8

[a]Measured in CD_3OD. [b]measured in DMSO-d_6. [c]125 MHz. [d]500 MHz, *overlapped signals. Assignments were done by HMQC, HMBC, and NOESY experiments.

protons (δ_H 2.07) correlated with the carbonyl (δ_C 172.5), which in turn was correlated with the methine proton H-6 (δ_H 5.58); the remain methyl protons (δ_H 2.05) correlated with the carbonyl (δ_C 172.7) (see Figure 2), two methylene protons H-10 (δ_H 4.82 and 4.95) also correlated with this carbonyl (δ_C 172.7), which suggested the presence of two acetate groups at C-6 and C-10. The strong NOE correlations between H-1 (δ_H 5.27) and H-6 (δ_H 5.58) and between H-5 (δ_H 3.35) and H-9 (δ_H 3.09) (see Figure 3) confirmed the α configurations for both H-1 and H-6 and the β configuration for H-5 and H-9. Furthermore, the HMBC correlations between the glc H-1' (δ_H 4.69) and C-1 (δ_C 97.1) and the methine proton H-1 (δ_H 5.27) and glc C-1' (δ_C 100.3)

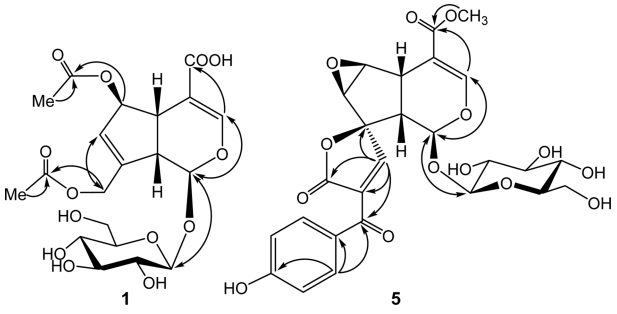

Figure 2. Important HMBC correlations of compounds **1** and **5**.

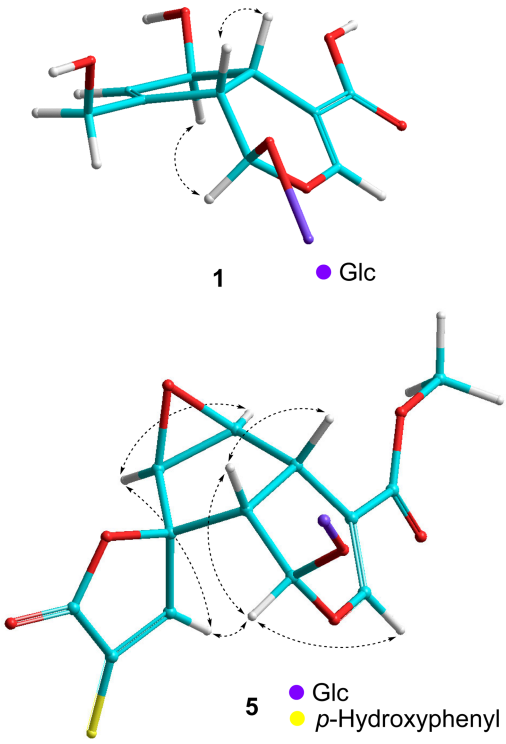

Figure 3. Important NOESY correlations of compounds **1** and **5**.

Table 2. ^{1}H and ^{13}C NMR data for compounds **5** and **6**

Pos.	**5**		**6**	
	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)
Aglycone				
1	92.8	5.60 (br s)	92.7	5.66 (br s)
3	153.8	7.58 (d, 1.5)	153.6	7.57 (s)
4	108.5	-	108.5	-
5	33.1	3.54 (br d, 8.0)	33.1	3.53 (br d, 8.5)
6	57.9	4.10 (d, 2.5)	58.0	4.09 (d, 2.5)
7	59.1	3.58 (d, 2.5)	59.2	3.57 (d, 2.5)
8	92.8	-	92.7	-
9	44.3	2.93 (br d, 8.0)	44.3	2.92 (br d, 8.5)
10	156.2	7.59 (s)	156.2	7.62 (s)
11	134.3	-	133.9	-
12	169.5	-	169.3	-
13	187.4	-	187.7	-
14	167.9	-	167.8	-
1'	127.5	-	129.2	-
2',	133.9	7.85 (d, 8.5)	113.1	7.48 (d, 1.5)
3'	117.5	6.85 (d, 8.5)	149.2	-
4'	167.9	-	154.7	-
5'	117.5	6.85 (d, 8.5)	149.2	6.91 (d, 8.0)
6'	133.9	7.85 (d, 8.5)	113.1	7.51 (dd, 1.5, 8.0)
1-*O*-Glc				
1"	99.8	4.62 (d, 8.0)	99.6	4.58 (d, 7.5)
2"	74.5	3.18 (dd, 8.0, 8.5)	74.4	3.18 (br t, 8.5)
3"	77.9	3.37 (t, 8.5)	77.4	3.37 (t, 8.5)
4"	71.5	3.26 (t, 8.5)	71.5	3.26 (t, 8.5)
5"	78.5	3.31 (m)	78.8	3.31 (m)
6"	62.6	3.66 (dd, 6.0, 12.0)	62.6	3.64 (dd, 6.0, 12.0)
		3.89 (dd, 2.0, 12.0)		3.89 (dd, 2.0, 12.0)
14-COOMe	52.0	3.77 (s)	52.1	3.76 (s)
3'-OMe			56.6	3.94 (s)

[a]Measured in CD_3OD. [b]125 MHz. [c]500 MHz. Assignments were done by HMQC, HMBC, and NOESY experiments.

suggested the presence of a glucose moiety at C-1 of the iridoid. The sugar in **1** was proved to be D-glucose by acid hydrolysis of **1** (identified as TMS derivative). In addition, the β-orientation glucose was confirmed by the coupling constant $J_{1\text{-}2}$ = 7.5 Hz. Consequently, compound **1** was determined to be 6,10-*O*-diacetylscandoside, a new compound named as morintoside A.

Compound **6** was isolated as a white amorphous powder. The ^{1}H- and ^{13}C-NMR data (CD_3OD, Table 2) of **6** were found to be identical to those of dehydroepoxymethoxygaertneroside, previously reported incorrectly to be citrifolinoside and yopaaoside A.[8] The configuration at C-8 of the plumeria iridoid skeleton was compared to those reported by Cimanga *et al*.,[9] and further confirmed by NOESY correlation between H-1 (δ_H 5.66) and H-10 (δ_H 7.62). However, the chemical shifts of C-11 and C-1' in the report of Schripsema *et al*.,[8] are interchanged based on the HMBC cross peaks from H-10 (δ_H 7.62) to C-11 (δ_C 133.9) and from H-2' (δ_H 7.48) and H-6' (δ_H 7.51) to C-1' (δ_C 129.2).

Compound **5** was also isolated as a white amorphous powder and determined to be $C_{26}H_{26}O_{14}$ by HR-ESI-MS at *m/z* 597.1008 $[M + Cl]^-$ (Calcd $C_{26}H_{26}O_{14}Cl$ for 597.1011) and *m/z* 561.1247 $[M - H]^-$ (Calcd $C_{26}H_{25}O_{14}$ for 561.1244). The ^{1}H-NMR spectrum of **5** (CD_3OD) showed signals for methoxy protons at δ_H 3.77, two olefinic protons at δ_H 7.58 and 7.59, an anomeric proton at δ_H 4.62, and a coupling pattern protons of an AA'BB' aromatic ring system at δ_H 6.85 and 7.85 (each 2H, d, *J* = 8.5 Hz), as listed in Table 2. The ^{13}C-NMR spectrum of **5** showed the presence of a plumeria iridoid skeleton and a sugar moiety. The ^{1}H- and ^{13}C-NMR data of **5** were similar to those of dehydroepoxymethoxygaertneroside[7] except for disappearance of a methoxy group at C-3'. The coupling constant $J_{5\text{-}6}$ = 0 Hz between H-5 and H-6 indicated a β-orientation of the epoxide ring.[9] The chemical shift of C-8 (δ_C 92.8), C-10 (δ_C 156.2), C-11 (δ_C 134.3), and C-12 (δ_C 169.5) were very characteristic for a spiro-lactone ring, connected at C-8,[8,9] and was further confirmed by HMBC correlations between H-10 (δ_H 7.59) and C-8 (δ_C 92.8), C-11 (δ_C 134.3), C-12 (δ_C 169.5), and C-13 (δ_C 187.4). Moreover, the spirolactone configuration was confirmed based on plumieride,[10] epoxygaertneroside, and epoxymethoxygaertneroside,[9] as well as dehydroepoxymethoxygaertneroside and citrifolinoside A.[8] The chemical shift of C-14 moved to upfield (δ_C 167.9) and the HMBC correlation between the methoxy group (δ_H 3.77) and the carbonyl C-14 (δ_C 167.9) confirmed that the methoxy group was at C-14. In addition, the HMBC cross peaks from the glc H-1" (δ_H 4.62) to C-1 (δ_C 92.8) and also from the methine proton H-1 (δ_H 5.60) to glc C-1" (δ_C 99.8) suggested the glucose moiety was at C-1 of the plumeria iridoid. The sugar in **5** was determined to be D-glucose by acid hydrolysis of **5** (identified as the TMS derivative). Although the NMR data of compound **5** were identical to those of morinipticoside,[11] the configuration at C-8 of morinipticoside should be corrected based on the NMR data of compound **6**, NOESY correlations between H-10 (δ_H 7.59) and H-1 (δ_H 5.60) and H-7 (δ_H 3.58) (see Figure 3), and reference compound, dehydroepoxymethoxygaertneroside and citrifolinoside A.[8] Consequently, we named compound **5** as morintoside B.

The remaining compounds were identified as asperulosidic acid (**2**),[12] daphylloside (**3**),[13] asperuloside (**4**),[13] (1*R*, 6*R*,9*R*) 6,9,11-trihydroxy-4-megastigmen-3-one (**7**), 4-*epi*-borreriagenin (**8**),[14] *trans*-phytol (**9**),[15] ursolic acid lactone (**10**),[17] 3β-hydroxyurs-12-en-28-al (**11**), and ursolic acid (**12**)[16] (see Figure 1). Their structures were established on the basis of spectral and chemical evidence, which were in good agreement with those reported in literature.

Compounds **1-12** were then evaluated for α-glucosidase inhibitory activity at a concentration of 50 μM. Acarbose was used as the positive control, with inhibition of 51.0% at a concentration of 50 μM. As shown in Table 3, three ursolic derivatives **10-12** exhibited strong inhibitory activities of 20.4%-23.8% at the same concentration. The iridoid glycosides **1-6** exhibited inhibition values of 15.5%-19.4%.

Table 3. Rat intestinal α-glucosidase inhibitory activities of compounds **1-12**

Compounds	% Enzyme inhibition
1	14.5 1.2
2	17.1 1.8
3	17.8 1.7
4	19.4 2.0
5	18.0 1.3
6	18.2 2.1
7	10.0 1.6
8	13.4 2.2
9	6.7 6.9
10	23.8 5.4
11	22.7 1.00
12	20.4 2.6
Positive control*	51.0 ± 3.0

Percentage of enzyme inhibition at the concentration of 50 μM. *Acarbose was used as positive controls. Data presented is the mean ± SD of samples run in triplicate.

Our results suggested that *M. tomentosa* may be useful for the treatment of diabetes mellitus.

Experimental

Plant Material. The leaves of *M. tomentosa* were collected in Tam Dao, Vinh Phuc province, Vietnam in June, 2010, and identified by one of the authors, Dr. Ninh Khac Ban. A voucher specimen (MT1006) was deposited at the Herbarium of Institute of Marine Biochemistry.

Morintoside A (1): A white amorphous powder, $[\alpha]_D^{25}$: –60 (c = 0.5, MeOH), negative ESI-MS: *m/z* 473 $[M - H]^-$, HR-ESI-MS found *m/z* 509.1054 $[M + Cl]^-$ (Calcd $C_{20}H_{26}O_{13}Cl$ for 509.1054), *m/z* 473.1292 $[M - H]^-$ (Calcd $C_{20}H_{25}O_{13}$ for 473.1295), ^{1}H- and ^{13}C-NMR: see Table 1.

Morintoside B (5): A white amorphous powder, $[\alpha]_D^{25}$: –42 (c = 0.5, MeOH), negative ESI-MS: *m/z* 561 $[M - H]^-$, HR-ESI-MS found *m/z* 597.1008 $[M + Cl]^-$ (Calcd $C_{26}H_{26}O_{14}Cl$ for 597.1011), *m/z* 561.1247 $[M - H]^-$ (Calcd $C_{26}H_{25}O_{14}$ for 561.1244), ^{1}H- and ^{13}C-NMR: see Table 2.

Supporting Information. General procedures, extraction, isolation, hydrolysis procedure, α-glucosidase assays, NMR, and MS spectra of **1** and **5** are available as Supporting information.

Acknowledgments. This study was financially supported by Vietnam National Foundation for Science & Technology Development (Project No: 104.01-2010.53). This research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011) and in part by the Yonsei University Research Fund of 2011.

Declaration of Interest. The authors report no conflicts of interest.

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