α-Glucosidase Inhibition Properties of Cucurbitane-Type Triterpene Glycosides from the Fruits of *Momordica charantia*

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Fourteen cucurbitane-type triterpene glycosides (1—14) were isolated from a methanol extract of *Momordica charantia* fruits, including three new compounds, charantosides A—C (1, 5, 6). Their structures were elucidated by chemical and spectroscopic methods. All isolated compounds were evaluated for α-glucosidase inhibitory effect. Of which, 12 and 13 showed moderate inhibitory activity against α-glucosidase. Whereas, 2, 3, 6—11, and 14 showed weak inhibitory activity, and 1, 4, and 5 were inactive.

Key words *Momordica charantia*; Cucurbitaceae; cucurbitane-type triterpene glycoside; charantoside; α-glucosidase

The plant *Momordica charantia* L. (Cucurbitaceae) is widely cultivated in Asian countries, and the fruit of this plant has been used as a bitter stomachic, a laxative, an antidiabetic, and an anthelmintic agent for children in traditional Chinese, Indian, Vietnamese, and Indonesian medicines. The alcoholic extract of *M. charantia* fruits has been reported to inhibit the increase of serum glucose levels in glucose-loaded rats.[1)] In addition, many cucurbitane-type triterpenes and their glycosides were isolated from the roots,[2)] fruits,[3—13)] seeds,[14,15)] leaves, and vines[16—19)] of this plant.

α-Glucosidase inhibitors are oral anti-diabetic drugs used for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates. Carbohydrates are normally converted into simple sugars, which can be absorbed through the intestine. Hence, inhibition of α-glucosidase enzyme can significantly decrease the postprandial increase in blood glucose levels after a mixed carbohydrate diet and could be a key strategy in the control of diabetes mellitus. In fact, a potent α-glucosidase inhibitor, acarbose, has been shown to effectively reduce the intestinal absorption of sugars in humans.[20)] The main drawback of acarbose is its side effects, such as abdominal distention, flatulence, meteorism, and possibly diarrhea.[21)] Therefore, natural inhibitors from dietary plants that have minimal side effects and have been shown to have stronger inhibitory activity against α-glucosidase may be useful as effective therapies for postprandial hyperglycemia.[22)] Interestingly, in our search for bioactive metabolites, we have examined the constituents of the methanol extract of *M. charantia* fruits, and led to the isolation of fourteen cucurbitane-type triterpene glycosides, including three new compounds named charantosides A—C (**1**, **5**, **6**). For these reasons, the α-glucosidase inhibitory activity of all these compounds was tested. Herein, we report the isolation, structure elucidation, and biological activities of compounds **1**—**14** (Fig. 1).

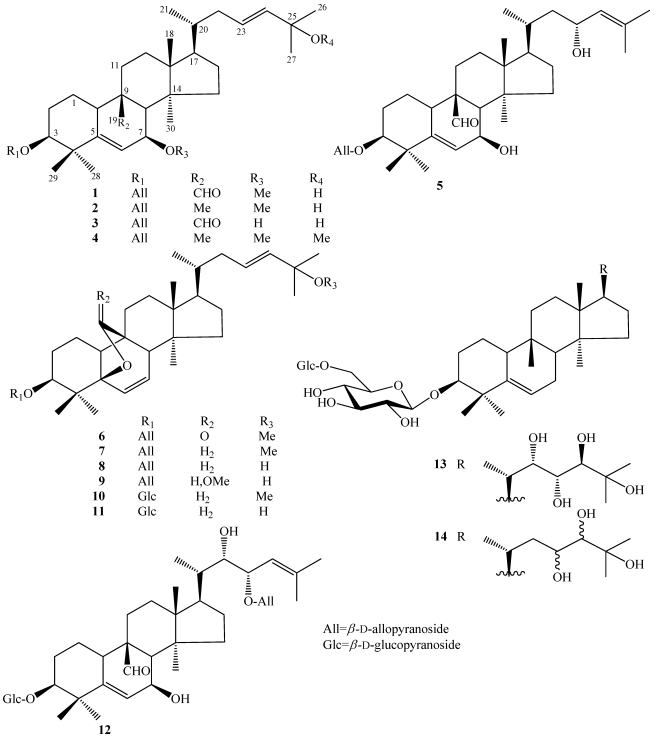

Fig. 1. Structures of **1**—**14**

Results and Discussion

The methanol extract of *M. charantia* fruits yielded three new compounds (**1**, **5**, **6**). Compound **1** was obtained as a white powder. The absorption bands at 3407 cm^{-1} and 1728 cm^{-1} in the IR spectrum indicated hydroxyl and carbonyl functionalities, respectively. A $[M+H]^+$ pseudomolecular ion peak observed at *m*/*z*: 649.4337 (Calcd for $C_{37}H_{61}O_9$: 649.4316) in positive HR-electrospray ionization (ESI)-MS, and the ^{13}C-NMR spectroscopic data indicated a molecular formula of $C_{37}H_{60}O_9$. The ^{13}C-NMR spectrum of **1** showed 37 carbon signals, of which were attributed to a methoxy group, a sugar moiety, and a triterpene moiety. The distortionless enhancement by polarization transfer (DEPT) experiment permitted differentiation of the 37 carbon signals including eight methyl, eight methylene, fifteen methine, and six quaternary carbons. The ^{1}H- and ^{13}C-NMR data assign-

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Table 1. The NMR Spectroscopic Data for **1**, **5**, and **6**

Position	**1**		**5**		**6**	
	δ_C [a,c]	δ_H [a,d] (J in Hz)	δ_C [a,c]	δ_H [a,d] (J in Hz)	δ_C [b,c]	δ_H [b,d] (J in Hz)
Aglycone						
1	23.4	1.41 (d, 5.4)	23.3	1.41 (d, 5.4)	19.1	1.54[e]
		2.36[e]		2.38[e]		
2	28.7	2.10 (dd, 3.6, 13.8)	28.7	1.88 (m)	26.4	1.75 (m)
3	87.4	3.54 (br s)	87.2	3.50 (br s)	82.8	3.48 (br s)
4	42.6	—	42.5	—	37.8	—
5	149.2	—	147.7	—	84.3	—
6	121.5	5.92 (d, 4.2)	123.3	5.83 (d, 4.2)	131.6	6.20 (d, 9.6)
7	77.0	3.53 (d, 4.2)	66.7	3.98 (d, 4.2)	133.0	5.69 (dd, 3.6, 9.6)
8	51.1	1.55 (br s)	51.3	1.90 (br s)	49.5	2.04 (br s)
9	51.2	—	50.5	—	50.5	—
10	37.6	1.56[e]	37.3	2.56 (dd, 4.8, 12.6)	40.3	2.62 (dd, 6.6, 13.2)
11	22.8	1.56[e]	23.0	1.55[e]	21.4	1.70[e]
12	30.2	1.68 (m)	30.3	1.22 (d, 2.4)	29.8	1.30[e]
				1.67[e]		1.69[e]
13	46.8	—	46.7	—	45.0	—
14	48.9	—	48.6	—	47.7	—
15	35.9	1.39 (m)	35.6	1.35 (m)	33.3	1.35 (m)
16	28.5	2.05[e]	28.7	2.05[e]	27.4	1.05[e]
17	52.1	1.52 (m)	52.0	1.49 (m)	50.2	1.49 (m)
18	15.3	0.96 (s)	15.3	0.90 (s)	14.6	0.92 (s)
19	209.6	9.72 (s)	210.0	9.80 (s)	181.8	—
20	37.3	2.60 (m)	33.7	1.71 (m)	36.1	1.55 (m)
21	19.2	0.95 (d, 6.6)	19.3	0.96 (d, 6.6)	18.6	0.91 (d, 6.6)
22	40.2	1.73[e]	44.6	1.61 (m)	39.3	1.79[e]
		2.15 (dd, 3.6, 13.8)				2.18 (dd, 3.6, 13.8)
23	125.8	5.58[e]	66.5	4.39 (dt, 2.4, 8.4)	128.2	5.51 (m)
24	140.9	5.58[e]	130.4	5.13 (d, 8.4)	136.9	5.40 (d, 16.2)
25	71.2	—	133.4	—	74.9	—
26	30.0	1.26 (s)	25.9	1.66 (s)	26.1	1.24 (s)
27	30.0	1.26 (s)	18.1	1.63 (s)	25.8	1.24 (s)
28	27.7	1.09 (s)	27.6	1.05 (s)	23.6	0.92 (s)
29	26.0	1.32 (s)	26.0	1.27 (s)	20.6	1.24 (s)
30	18.7	0.82 (s)	18.7	0.78 (s)	19.2	0.83 (s)
7-OMe	56.4	3.26 (s)				
25-OMe					50.3	3.15 (s)
3-O-All						
1′	103.9	4.66 (d, 7.8)	103.8	4.61 (d, 7.8)	101.7	4.72 (d, 7.8)
2′	72.6	3.29 (dd, 3.0, 7.8)	72.7	3.22 (dd, 3.0, 7.8)	71.5	3.42 (d, 3.0, 7.8)
3′	73.2	4.03 (t, 3.0)	73.1	4.10 (t, 3.0)	70.4	4.23 (t, 3.0)
4′	69.0	3.46 (dd, 3.0, 9.6)	69.0	3.42 (dd, 3.0, 9.6)	68.3	3.57 (dd, 3.0, 9.6)
5′	75.1	3.64 (m)	75.1	3.60 (m)	73.9	3.67 (m)
6′	63.3	3.63 (m)	63.2	3.61 (m)	63.2	3.75 (dd, 5.4, 10.8)
		3.80 (d, 8.4)		3.78 (d, 8.4)		3.89 (dd, 4.2, 10.8)

a) Measured in CD_3OD, *b*) measured in $CDCl_3$, *c*) 150 MHz. *d*) 600 MHz. *e*) Overlapped signals, assignments were done by HMQC, HMBC, ^1H–^1H COSY, and ROESY experiments.

ments of **1** (Table 1) were based on the heteronuclear multiple quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC) (Fig. 2), ^1H–^1H correlation spectroscopy (COSY) spectra, and in comparison with published data.[13] The NMR data of **1** were similar to that of 7β,25-dimethoxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-allopyranoside[7] except for the disappearance of the methoxy group and instead of the hydroxyl group at C-25. The HMBC cross peaks from H-7 (δ_H 3.53) to OCH_3 (δ_C 56.4), C-5 (δ_C 149.2), C-6 (δ_C 121.5), and C-9 (δ_C 51.2), from H-23 (δ_H 5.58), H-24 (δ_H 5.58), H_3-26 (δ_H 1.26), and H_3-27 (δ_H 1.26) to C-25 (δ_C 71.2) confirmed the methoxy and hydroxyl groups at C-7 and C-25, respectively, and the olefinic bonds at C-5 and C-23. The methoxy group at C-7 was confirmed to be β-oriented as in 7β,25-dimethoxycucurbita-5(6),23(*E*)-dien-19-al 3-*O*-β-D-allopyranoside[13] by the significant rotating frame Overhauser effect spectroscopy (ROESY) correlations between H-7α (δ_H 3.53) and biogenetically α-oriented C-30 methyl (δ_H 0.82) as well as OCH_3 (δ_H 3.26) at C-7 and β-oriented H-19 (δ_H 9.72). The sugar linked to C-3 confirmed by the HMBC correlations between H-3 (δ_H 3.54) and C-1′ (δ_C 103.9) as well as those between the anomeric proton H-1′ (δ_H 4.66) and C-3 (δ_C 87.4). Its sugar was identified as an allose by comparing the NMR data with that of the sugar moieties of charantosides II, IV, and VI,[9] and by acid hydrolysis (see Experimental). Based on the above evidence, **1** was elucidated as 25-hydroxy-7β-methoxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-allopyranoside, named charantoside A.

Compound **5** was also obtained as a white powder. Its molecular formula was determined as $C_{36}H_{58}O_9$ due to a $[M+H]^+$ ion in the positive HR-ESI-MS at *m*/*z*: 635.4144 (Calcd for $C_{36}H_{59}O_9$: 635.4159). The DEPT experiment re-

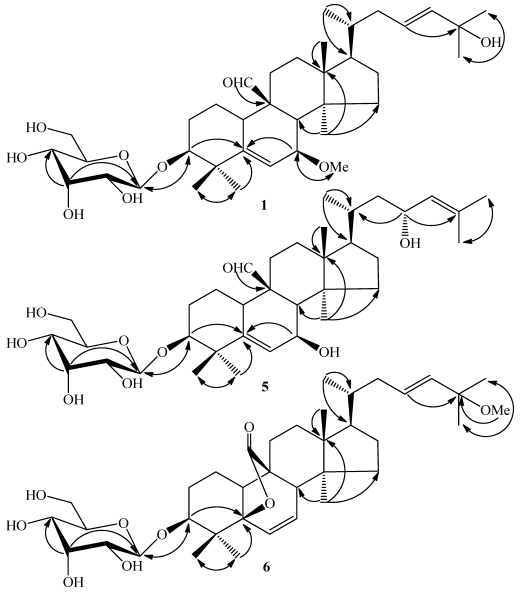

Fig. 2. Key HMBC Correlations of **1**, **5**, and **6**

Table 2. Rat Intestinal α-Glucosidase Inhibitory Activity of Compounds **1**—**14**

Compounds	% Enzyme inhibition
1	5.50±1.10
2	15.85±3.14
3	12.86±3.47
4	4.37±1.22
5	5.95±1.00
6	13.61±2.90
7	13.14±2.17
8	12.50±1.25
9	10.88±0.83
10	11.51±2.73
11	10.11±3.47
12	18.63±2.34
13	21.71±2.08
14	12.98±2.26
Acarbose[a)]	50.96±2.97

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

solved the 36 carbon signals including seven methyl, eight methylene, fifteen methine, and six quaternary resonances. The ^{1}H- and ^{13}C-NMR data of **5** (Table 1) was similar to that of 7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-allopyranoside,[11)] except for the signals due to a side chain. In the HMBC spectrum, the protons of two methyl groups H-26 (δ_H 1.66) and H-27 (δ_H 1.63) correlated with carbons C-24 (δ_C 130.4) and C-25 (δ_C 133.4); proton signal H-23 (δ_H 4.39) correlated with C-20 (δ_C 33.7), C-22 (δ_C 44.6), C-24 (δ_C 130.4), and C-25 (δ_C 133.4), indicating that the double bond was at C-24 and the hydroxyl group at C-23 in the side chain, similar to that of karavilagenin.[8)] The stereochemistry at C-23 of **5** was deduced to be *R* by comparing the ^{13}C-NMR chemical shifts of side-chain [δ_C: 33.7 (C-20), 44.6 (C-22), 66.5 (C-23), 130.4 (C-24), and 133.4 (C-25)] with those of (23*R*)-cycloart-24-ene-3β,23-diol [δ_C 33.0 (C-20), 44.5 (C-22), 66.1 (C-23), 129.1 (C-24), and 133.8 (C-25)] and (23*S*)-cycloart-24-ene-3β,23-diol [δ_C 33.5 (C-20), 44.5 (C-22), 67.3 (C-23), 128.4 (C-24), and 135.6 (C-25)].[23)] On the other hand, the HMBC correlations observed between the aldehydic proton H-19 (δ_H 9.80) and C-8 (δ_C 51.3), C-9 (δ_C 50.5), and C-11 (δ_C 23.0), and between H-1′ (δ 4.61) and C-3 (δ 87.2) confirmed that the aldehydic group was located at C-9 and the sugar was linked to C-3. Similar to **1**, the sugar was identified as D-allose. Accordingly, **5** was identified as (23*R*) 7β,23-dihydroxycucurbita-5,24-dien-19-al 3-*O*-β-D-allopyranoside, a new compound named charantoside B (**5**).

Compound **6** was purified as a white powder. The absorption bands at 3430 cm^{-1} and 1749 cm^{-1} in the IR spectrum indicated hydroxyl and carbonyl functionalities, respectively. The $[M+H]^+$ pseudomolecular ion observed at *m*/*z*: 641.4179 (Calcd for $C_{37}H_{59}O_9$: 647.4159) in positive HR-ESI-MS, and the ^{13}C-NMR spectroscopic data indicated a molecular formula of $C_{37}H_{58}O_8$. The ^{13}C-NMR spectrum of **6** exhibited 37 carbon signals that were attributed to a methoxy group, a sugar moiety, and a triterpene moiety. The DEPT experiment revealed differentiation of the 37 carbon signals comprising eight methyl, eight methylene, fourteen methine, and seven quaternary carbons. The ^{1}H- and ^{13}C-NMR data assignments of **6** (Table 1) were based on HMQC, HMBC (Fig. 2), ^{1}H–^{1}H COSY spectra, and compared to published data.[8)] The sugar was located at C-3 confirmed by the HMBC correlations between H-3 (δ_H 3.48) and C-1′ (δ_C 101.7), as well as between the anomeric proton H-1′ (δ_H 4.72) and C-3 (δ_C 82.8). In addition, the methoxy protons (δ_H 3.15), H-23 (δ_H 5.51), H-24 (δ_H 5.40), H$_3$-26 (δ_H 1.24), and H$_3$-27 (δ_H 1.24) correlated to C-25 (δ_C 74.9), and proton H-10 (δ_H 2.62) correlated to carbon C-19 (δ_C 181.8) in the HMBC spectrum, confirming that the methoxy group was at C-25, the olefinic bond was at C-23, and the carbonyl group was at C-19. The aglycone of **6** was found to be the same as that of karaviloside VI,[8)] and its sugar moiety was also identified as D-allose, similar to **1**. Base on the above evidence, **6** was determined to be 25-methoxy-5β,19-epoxycucurbita-6,23-dien-19-on 3-*O*-β-D-allopyranoside, and has been named charantoside C.

The other compounds were characterized as karaviloside III (**2**),[7)] 7β,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-β-D-allopyranosyl (**3**),[11)] karaviloside II (**4**),[7)] momordicoside G (**7**),[12)] momordicoside F$_2$ (**8**),[12)] goyaglycoside-b (**9**),[5)] momordicoside F$_1$ (**10**),[12)] momordicoside I (**11**),[12)] momordicoside M (**12**),[10)] momordicoside A (**13**),[14)] and momordicoside C (**14**),[15)] (Fig. 1) by comparing their NMR spectroscopic data with the literature values.

The fruit of *M. charantia* is one of the most popular dietary botanicals for the treatment of diabetes mellitus. The beneficial effects of the *M. charantia* extract and the mechanism with which it treats diabetes mellitus have been extensively studied.[24,25)] However, the α-glucosidase inhibitory properties of the extract and its isolated compounds have not been evaluated. Thus, we evaluated the isolated compounds for their inhibitory activity against α-glucosidase (Table 2). The most polar compound **13** which has two sugar moieties at the C-3 position and four hydroxyl groups at C-22, C-23, C-24, and C-25 and compound **12**, which also has two sugar moieties at C-3 and C-23, showed stronger α-glucosidase inhibitory activity (21.71% and 18.63% inhibition at the concentration of 50 μM, respectively) than the other compounds. Compounds **6**—**11** all had a 5,19-epoxy bridge, displayed weak α-glucosidase inhibitory activity. Other than that,

changes in the functional groups at C-3, C-19, and C-25 positions of **6**—**11** did not alter the degree of inhibitory activity against α-glucosidase. Of the three new compounds, **1** and **5** displayed very weak inhibitory activity against α-glucosidase. To the best of our knowledge, this is the first time α-glucosidase inhibitory activity by cucurbitane-type triterpene glycosides from the fruits of bitter melon has been observed. It was also noticed that the most polar compound displayed the strongest enzyme inhibition against α-glucosidase. This may provide a new point for semi-synthetic chemists to create new α-glucosidase inhibitors, which may be useful for the development of new therapies for the treatment of diabetes mellitus.

Experimental

General Experimental Procedures The optical rotation was determined on a Jasco DIP-370 digital polarimeter. Electrospray ionization (ESI) mass spectra were obtained using an Agilent 1200 LC-MSD Trap spectrometer. HR-ESI mass spectra were obtained using a JEOL JMS-T100LC spectrometer. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were recorded on a Jeol ECA 600 spectrometer and TMS was used as an internal standard. GC was performed on a Shidmazu-2010 instrument. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70—230 mesh and 230—400 mesh, Merck) and YMC RP-18 resins.

Plant Material The fruits of *M. charantia* were collected in Vuthu, Thaibinh province, Vietnam on June, 2009, and identified by Dr. Ninh Khac Ban. A voucher specimen (INPC MC0609) was deposited at the herbarium of Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and Isolation The dried fruits of *M. charantia* (5.0 kg) were powdered and extracted with methanol (MeOH) (3×10 l) to afford the MeOH extract (400 g), which was then suspended in water (5 l) and then extracted with chloroform ($CHCl_3$) and ethyl acetate (EtOAc) (3×5 l each), yielding $CHCl_3$ (MC1, 250 g), ethyl acetate (MC2, 80 g), and water layer (MC3) extracts. The $CHCl_3$ fraction (250 g) was passed through a silica gel column using a solvent mixture of *n*-hexane and EtOAc with increasing polarity as the eluent. Six fractions were collected as follows: MC1A [4 l, *n*-hexane–EtOAc (40 : 1, v/v)], MC1B [4 l, *n*-hexane–EtOAc (20 : 1, v/v)], MC1C [4 l, *n*-hexane–EtOAc (10 : 1, v/v)], MC1D [4 l, *n*-hexane–EtOAc (5 : 1, v/v)], MC1E [4 l, *n*-hexane–EtOAc (2.5 : 1, v/v)], and MC1F [1 l, EtOAc]. Fraction MC1C (20 g) was separated using silica gel CC eluting with $CHCl_3$–MeOH (20 : 1, v/v) to obtain four sub-fractions, MC1C1—MC1C4. Sub-fraction MC1C2 (4 g) was further separated by silica gel CC using CH_2Cl_2–acetone (5 : 1, v/v) as the eluent to afford four smaller fractions, MC1C2A—MC1C2D. Compounds **2** (80 mg) and **4** (100 mg) were isolated from fraction MC1C2A (850 mg) by YMC RP-18 CC eluting with acetone–H_2O (3 : 1, v/v). In a similar manner, compounds **1** (30 mg) and **3** (11 mg) were purified from fraction MC1C2C (600 mg). The fraction MC1D (15 g) was separated using silica gel CC eluting with $CHCl_3$–MeOH (14 : 1, v/v) to afford four fractions, MC1D1—MC1D4. Fraction MC1D2 (2 g) afforded compounds **6** (25 mg) and **8** (13 mg) after subjecting it to silica gel CC eluting with $CHCl_3$–acetone (2 : 1, v/v) followed by YMC RP-18 CC with acetone–H_2O (2.5 : 1, v/v). Fraction MC1D3 (5 g) was separated into five sub-fractions, MC1D3A—MC1D3E, by silica gel CC eluting with $CHCl_3$–*n*-hexane–MeOH (6 : 1 : 1, v/v). Compounds **5** (40 mg) and **9** (35 mg) were obtained from sub-fraction MC1D3B after subjecting it to silica gel CC eluting with CH_2Cl_2–acetone–H_2O (1.4 : 1 : 0.2, v/v/v) followed by YMC RP-18 CC eluting with MeOH–H_2O (2 : 1, v/v). Sub-fraction MC1D3C (1.5 g) was treated in a similar manner to MC1D3B to furnish compounds **10** (35 mg) and **11** (20 mg). Fraction MC1E (19 g) was separated into four sub-fractions, MC1E1—MC1E4, by silica gel CC eluting with $CHCl_3$–MeOH–H_2O (5 : 1 : 0.1, v/v/v). Compounds **7** (8 mg) and **12** (15 mg) was isolated from fraction MC1E2 (2 g) by YMC RP-18 CC eluting with acetone–H_2O (0.8 : 1, v/v). Compounds **13** (17 mg) and **14** (25 mg) were purified from sub-fraction MC1E3 (1.9 g) using YMC RP-18 CC and eluting with MeOH–H_2O (1.1 : 1, v/v).

Charantoside A (**1**): White powder; $[\alpha]_D^{25}$ −18 (c=0.1, MeOH); IR (KBr) ν_{max} 3407 (OH), 1728 (C=O), 1085 (C–O–C) cm^{-1}; ^{1}H- and ^{13}C-NMR are given in Table 1; ESI-MS *m/z*: 649 $[M+H]^+$, 647 $[M-H]^-$; HR-ESI-MS *m/z*: 649.4337 $[M+H]^+$ (Calcd for $C_{37}H_{61}O_9$, 649.4316).

Charantoside B (**5**): White powder; $[\alpha]_D^{25}$ −14 (c=0.1, MeOH); IR (KBr) ν_{max} 3411 (OH), 1727 (C=O), 1086 (C–O–C) cm^{-1}; ^{1}H- and ^{13}C-NMR are given in Table 1; ESI-MS *m/z*: 635 $[M+H]^+$, 633 $[M-H]^-$; HR-ESI-MS *m/z*: 635.4144 $[M+H]^+$ (Calcd for $C_{36}H_{59}O_9$, 635.4159).

Charantoside C (**6**): White powder; $[\alpha]_D^{25}$ −30 (c=0.1, MeOH); IR (KBr) ν_{max} 3430 (OH), 1749 (C=O), 1081 (C–O–C) cm^{-1}; ^{1}H- and ^{13}C-NMR are given in Table 1; ESI-MS *m/z*: 647 $[M+H]^+$, 645 $[M-H]^-$; HR-ESI-MS *m/z*: 647.4179 $[M+H]^+$ (Calcd for $C_{37}H_{59}O_9$, 647.4159).

Acid Hydrolysis of 1, 5, and 6 Compounds **1**, **5**, and **6** (2 mg, each) were dissolved in HCl 1 N (dioxane–H_2O, 1 : 1, 1 ml) and heated for 3 h at 80 °C in a water bath. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven off by a stream of N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 ml of dry pyridine, and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 ml) was added to the solution. The reaction mixture was heated at 60 °C for 2 h, and 0.1 ml of trimethylsilylimidazole was added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 ml of each), and the organic layer was analyzed by gas chromatography (GC): column SPB-1 (0.25 mm×30 m); detector FID, column temp. 210 °C, injector temp. 270 °C, detector temp. 300 °C, carrier gas He (2 ml/min). Under these conditions, standard sugars gave peaks at t_R (min) 5.17 and 8.24 for D- and L-allose, respectively. A peak at t_R (min) 5.17 corresponding to D-allose was observed for all three compounds.

Glucosidase Inhibition Assay Rat intestinal acetone powders were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). A slightly modified version of the rat intestinal α-glucosidase assay method developed by Kwon *et al.*[26] was used. A total of 1 g of rat-intestinal acetone powder was suspended in 3 ml of 0.9% saline, and the suspension was sonicated twelve times for 30 s at 4 °C. After centrifugation (10000×***g***, 30 min, 4 °C), the resulting supernatant was used for the assay. The sample solution (50 μl) and 0.1 M phosphate buffer (pH 6.9, 100 μl) containing α-glucosidase solution (1.0 U/ml) was incubated at 25 °C for 10 min. After pre-incubation, 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution (50 μl) in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, the absorbance was read at 405 nm by a micro-plate reader Sunrise (Tecan, Salzburg, Austria) and compared to a control which had 50 μl of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\%\ \text{inhibition} = \left(\left[\frac{\Delta A_{405}^{\text{Control}} - \Delta A_{405}^{\text{Extract}}}{[\Delta A_{405}^{\text{Control}}]} \right] \right) \times 100$$

Statistical Analysis All experiments were performed in triplicate. Data is presented as the means±S.D. The results were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $p<0.05$.

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