

Article

Two New Bidesmoside Triterpenoid Saponins from the Seeds of *Momordica charantia* L.

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Abstract: Two new bidesmoside triterpenoid saponins which were identified as 28-*O*- β -D-xylopyranosyl(1 \rightarrow 3)- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamnopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl (1 \rightarrow 2)- β -D-glucopyranosiduronic acid (**C1**) and 28-*O*- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamnopyranosyl (1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)- β -D-glucopyranosiduronic acid (**C2**) were isolated together with two known compounds from the seeds of *Momordica charantia* L. Their structures were elucidated by the combination of mass spectrometry (MS), one and two-dimensional NMR experiments and chemical reactions.

Keywords: *Momordica charantia* L.; bidesmoside; triterpenoid saponin; NMR; MS

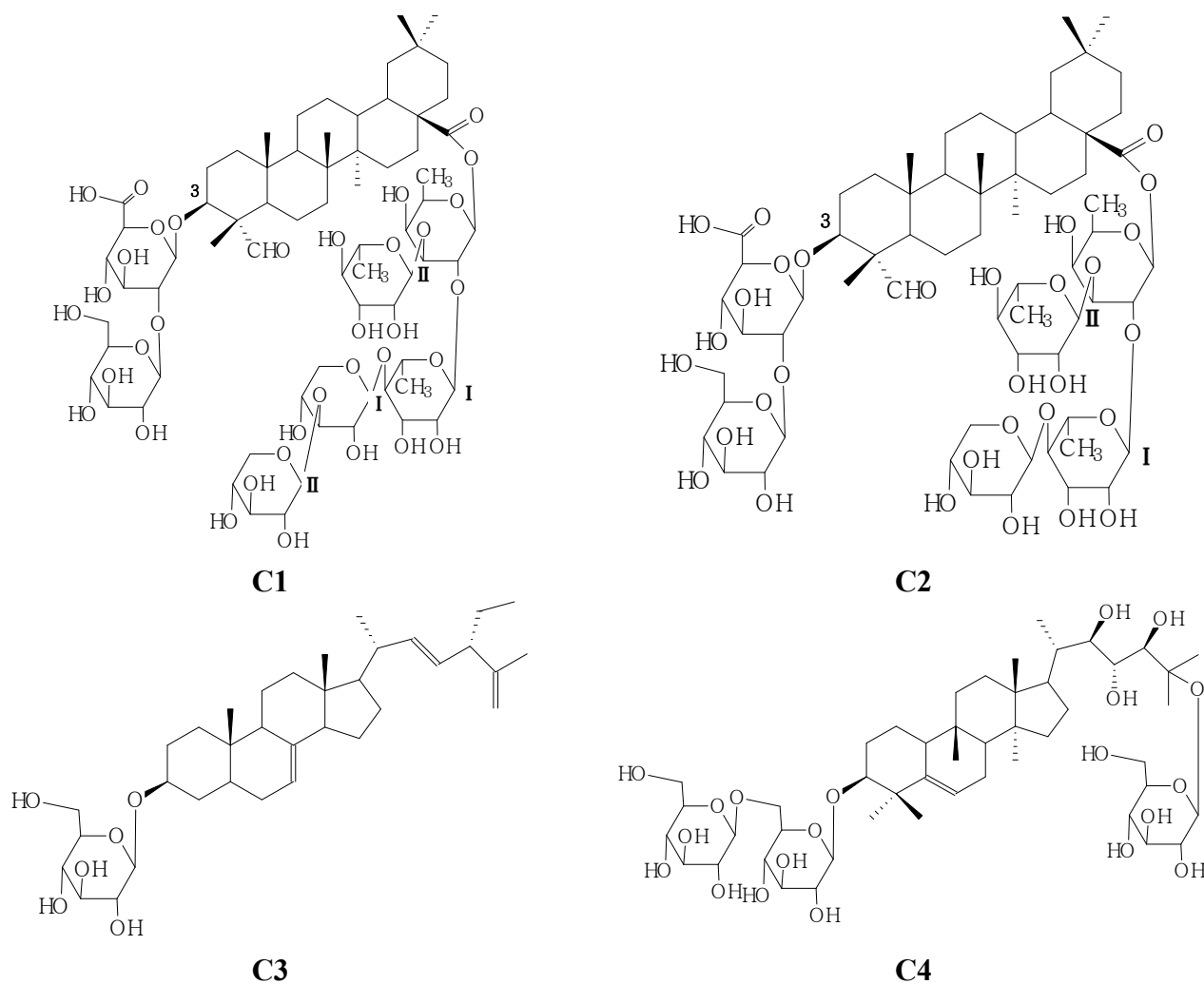
1. Introduction

The fruit, seeds, aerial parts and roots of *Momordica charantia* L. (Cucurbitaceae) have been used to treat diabetes. Over 100 compounds have been isolated from the fruits, seeds, leaves, canes and roots of this genus, mainly cucurbitane- and oleanene-type triterpenes, and recently studies have discovered that many new cucurbitane triterpenoids from the fruits and the roots of *M. charantia* L. [1–3], and

cucurbitane triterpenoids from the fruits of this genus showed a significant enhancement of glucose disposal and increases in fatty acid oxidation. The cucurbitane triterpenoids from *M. charantia* L. may therefore provide novel leads for the development of a new class of AMPK-activating agents [4]. Five cucurbitane-type triterpene glycosides from the seeds of *M. charantia* L. was reported [5,6].

In this paper, we now report the isolation and characterization of two new bidesmoside triterpenoid glycosides which were identified as 28-*O*- β -D-xylopyranosyl(1 \rightarrow 3)- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamnopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl (1 \rightarrow 2)- β -D-glucopyranosiduronic acid (**C1**) and 28-*O*- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamnopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)- β -D-glucopyranosiduronic acid (**C2**) and two known compounds identified as 3-*O*- β -D-glucopyranosyl-24 β -ethyl-5 α -cholesta-7,trans-22*E*,5(7)trien-3 β -ol (**C3**) and momordicoside S(**C4**) (Figure 1) on the basis of spectroscopic and chemical evidence.

Figure 1. Structures of compounds **C1**–**4**.



2. Results and Discussion

The *n*-BuOH-soluble portions of the 95% EtOH extract of seeds of *M. charantia* L. was subjected to silica gel, RP-18, and Sephadex LH-20 column chromatographies and semipreparative HPLC to yield two new oleanane-type triterpenoids **C1** and **C2** and two known compounds identified as 3-*O*- β -D-glucopyranosyl-24 β -ethyl-5 α -cholesta-7-*trans*-22 E ,5(7)trien-3 β -ol (**C3**) and momordicoside S (**C4**).

Compound **C1** was an amorphous white powder, which gave a positive result in the Liebermann-Burchard test. Acid hydrolysis of compound **C1** with 2 mol/L HCl–1,4-dioxane (1:1, *v/v*) furnished D-xylose, L-rhamnose, D-glucose, and D-fucose in the ratio of 2:2:1:1 by HPLC analysis of the corresponding thiazolidine derivatives following conversion to the 1-[(*S*)-N-acetyl-(*R*)-methylbenzylamino]-1-deoxyalditol acetate derivatives [7]. In the (–)- and (+)-ESI-MS of **C1**, quasimolecular ion peaks were observed at *m/z* 1,509 [M–H][–] and *m/z* 1,533 [M+Na]⁺ respectively, HR-ESI-MS (*m/z* 1,533.7190 [M+Na]⁺) analysis revealed the molecular formula of **C1** to be C₆₉H₁₁₂O₃₃Na (calcd. 1,533.7168). The five fragment ions at *m/z* 1,377 [M–132–H], 1,329 [M–162–H], 1,171 [M–162–176–H], 807 [M–146 × 3–132 × 2–H] and 469 [M–146 × 3–132 × 2–162–176–H], indicated the sequential losses of seven sugar moieties (five hexoses and two pentoses).

The ¹H (pyridine-*d*₅) spectra of **C1** revealed the presence of nine methyl proton signals at δ_H 0.76 (Me-25), 0.89 (Me-29), 0.92 (Me-26), 0.96 (Me-30), 1.18 (Me-27), 1.36 (Me-24), 9.89 (H-29) and included signals due to a gynosogenin skeleton [8], 1.54, 3H, d, *J* = 5.5 Hz (Me-Fuc), 1.61, 3H, d, *J* = 5.5 Hz (Me-RhaII), 1.67, 3H, d, *J* = 5.5 Hz, (Me-RhaI), as well as an olefinic proton at δ_H 5.37, 1H, s (H-12); The signals at δ_C 122.4, 143.8 in the ¹³C-NMR spectrum were assigned to a 12(13)-ene grouping by comparison with literature data [5].

The ¹H and ¹³C-NMR (Table 1) spectra of **C1** exhibited seven sugar anomeric protons assignable to a β -D-glucopyranosiduronic acid moiety [δ_H 4.76, 1H, d, 6.5 Hz], a β -D-glucopyranosyl moiety [δ_H 5.38, 1H, s], a β -D-fucopyranosyl moiety [δ_H 6.30, 1H, d, 5.5 Hz], two α -L-rhamnopyranosyl moieties [δ_H 5.74, 1H, s (RhmI-1-H); δ_H 5.67, 1H, s (RhmII-1-H)], two β -D-xylopyranosyl moieties [δ_H 5.08, 1H, d, 7.0 Hz (XylII-1-H); δ_H 5.21, 1H, d, 7.0 Hz (XylIII-1-H)], and a gynosogenin moiety [δ_H 3.04, 1H, dd-like, (18-H), δ_H 4.04, 1H, m (3-H), δ_H 5.44, 1H, s, (12-H); δ_H 9.89, 1H, s (23-H)]. The identities of the monosaccharides and the oligosaccharide sequence were determined by a combination of DEPT and two-dimensional NMR experiments (such as HMQC, HMBC, TOCSY NMR). The sequence of the glycan part was deduced from the following HMBC correlations: the anomeric proton signals at δ_H 4.76 and δ_C 83.5 (H-1 of the β -D-glucopyranosiduronic acid attached to the C-3 of the aglycone), δ_H 5.38 and δ_C 82.6 (H-1 of the β -D-glucopyranosyl moiety attached to the C-2 of the β -D-glucopyranosiduronic acid), and on the other hand, the pentasaccharide part at C-28 was established by the following HMBC information between the following protons and carbons: Fuc-1-H and 28-C, RhmI-1-H and Fuc-2-C, RhmII-1-H and Fuc-3-C, XylII-1-H and RhmI-4-C, XylIII-1-H and XylII-3-C (Figure 2). On the basis of the foregoing evidence, the structure of **C1** was determined as 28-O- β -D-xylopyranosyl(1→3)- β -D-xylopyranosyl(1→4)- α -L-rhamnopyranosyl(1→2)-[α -L-rhamnopyranosyl(1→3)]- β -D-fucopyranosylgynosogenin 3-O- β -D-glucopyranosyl (1→2)- β -D-glucopyranosiduronic acid.

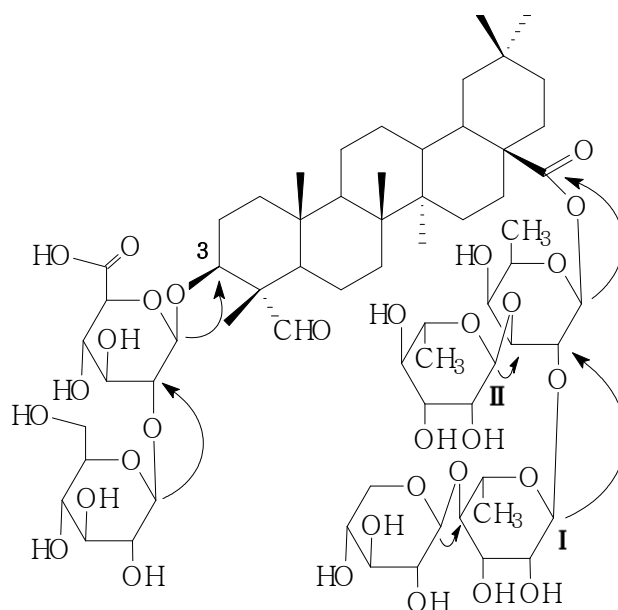
Table 1. ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) data for **C1** in pyridine- d_5 (δ , ppm).

Position	C1-Aglycone		Position	C1-Sugar Chain	
	^{13}C	^1H		^{13}C	^1H
1	39.8		GlcA-1	102.7	4.76, 1H, d, 6.5 Hz
2	25.8		2	82.6	4.32, 1H, m
3	83.5	4.04, 1H, m	3	77.8	4.65, 1H, m
4	54.7	-----	4	72.4	4.20, 1H, m
5	48.1		5	76.9	4.50, 1H, m
6	18.7		6	175.9	
7	32.9		Glc-1	105.9	5.38, 1H, s
8	39.8		2	75.1	4.15, 1H, m
9	47.5		3	77.8	4.25, 1H, m
10	35.9		4	71.4	4.02, 1H, m
11	23.5		5	77.9	4.20, 1H, m
12	122.4	5.44, 1H, s	6	61.8	3.65, 2H, m
13	143.8	-----	Fuc-1	93.43	6.30, 1H, d, 5.5 Hz
14	41.7	-----	2	76.7	4.25, 1H, m
15	27.9		3	83.7	3.95, 1H, m
16	23.5		4	72.4	3.86, 1H, m
17	46.7	-----	5	74.0	3.80, 1H, m
18	41.8	3.04, 1H, dd-like	6	18.3	1.54, 3H, d, 5.5 Hz
19	46.7		RhmI-1	100.9	5.74, 1H, s
20	30.6	-----	2	71.4	4.33, 1H, m
21	32.1		3	72.3	4.28, 1H, m
22	32.9		4	82.9	4.50, 1H, m
23	209.7	9.89, 1H, s	5	68.7	4.01, H, m
24	10.9	1.36, 3H, s	6	18.3	1.67, 3H, d, 5.5 Hz
25	15.4	0.76, 3H, s	RhmII-1	101.2	5.67, 1H, s
26	17.3	0.92, 3H, s	2	71.4	3.26, 1H, m
27	26.1	1.18, 3H, s	3	72.1	4.32, 1H, m
28	175.9	-----	4	70.6	4.71, 1H, m
29	32.1	0.89, 3H, s	5	68.7	4.51, 1H, m
30	23.5	0.96, 3H, s	6	18.3	1.61, 3H, d, 5.5 Hz
			XylI-1	106.2	5.08, 1H, d, 7.0 Hz
			2	74.8	4.01, 1H, m
			3	86.7	4.18, 1H, m
			4	70.2	4.13, 1H, m
			5	66.6	4.28/3.45, 2H, m
			XylII-1	105.6	5.21, 1H, d, 7.0 Hz
			2	76.3	4.04, 1H, m
			3	77.9	4.16, 1H, m
			4	70.6	4.13, 1H, m
			5	67.0	4.28/3.61, 2H, m

pyranosiduronic acid), and on the other hand, the oligosaccharide part at C-28 was established by the HMBC correlations between the following protons and carbons: Fuc-1-H and 28-C, RhmI-1-H and Fuc-2-C, RhmII-1-H and Fuc-3-C, Xyl-1-H and RhmI-4-C (Figure 3). On the basis of the foregoing evidence, the structure of **C2** was established as 28-*O*- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamno-pyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)- β -D-glucopyranosiduronic acid.

Table 2. ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) data for **C2** in pyridine- d_5 (δ , ppm).

Position	C2-Aglycone		Position	C2-Sugar Chain	
	^{13}C	^1H		^{13}C	^1H
1	38.0		GlcA-1	102.9	4.77, 1H, brs
2	25.9		2	82.2	4.36, 1H, m
3	83.9	4.04, 1H, m	3	77.8	4.65, 1H, m
4	54.9	-----	4	72.4	4.20, 1H, m
5	48.2		5	76.9	4.54, 1H, m
6	18.6		6	175.6	
7	32.4		Glc-1	106.2	5.37, 1H, s
8	40.0		2	75.4	4.51, 1H, m
9	47.6		3	78.4	4.52, 1H, m
10	35.6		4	71.6	4.06, 1H, m
11	23.6		5	77.9	4.36, 1H, m
12	122.5	5.37, 1H, s	6	62.0	4.57, 2H, m
13	143.9	-----	Fuc-1	93.6	6.29, 1H, d, 5.5 Hz
14	41.8	-----	2	76.5	3.99, 1H, m
15	28.0		3	83.3	4.31, 1H, m
16	23.7		4	72.3	4.05, 1H, m
17	46.9	-----	5	74.2	4.07, 1H, m
18	42.0	3.10, 1H, dd-like	6	18.4	1.53, 3H, d, 6.0 Hz
19	46.1		RhmI-1	100.8	5.77, 1H, s
20	30.7	-----	2	70.8	4.33, 1H, m
21	32.2		3	72.4	4.28, 1H, m
22	33.0		4	83.0	4.52, 1H, m
23	209.8	9.90, 1H, s	5	68.7	3.13, 1H, m
24	10.9	1.37, 3H, s	6	18.8	1.72, 3H, d, 6.0 Hz
25	15.6	0.76, 3H, s	RhmII-1	101.5	5.68, 1H, s
26	17.4	0.93, 3H, s	2	71.6	3.85, 1H, m
27	25.9	1.18, 3H, s	3	72.4	4.32, 1H, m
28	176.2	-----	4	70.4	4.75, 1H, m
29	32.0	0.88, 3H, s	5	68.7	4.53, 1H, m
30	23.1	0.95, 3H, s	6	18.6	1.62, 3H, d, 6.5 Hz
			Xyl-1	106.9	5.15, 1H, d, 7.0 Hz
			2	74.8	4.04, 1H, m
			3	86.9	4.21, 1H, m
			4	70.0	4.13, 1H, m
			5	67.3	4.27/3.48, 2H, m

Figure 3. Key HMBC correlations of compound **C2**.

3. Experimental Section

3.1. General

Melting points were determined of an XT₄-100_x micromelting apparatus (Beijing Keyi Electric Light Instrument Factory, Beijing, China) and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter (PerkinElmer Inc., Waltham, MA, USA). IR spectra were obtained of Nicolet 5700 IR spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). NMR spectra were recorded on an Inova 500 (¹H, 500 MHz; ¹³C, 125 MHz) spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). ESI-MS was performed with Agilent 1100 LC/MSD (Agilent Technologies, Inc., Santa Clara, CA, USA). For column chromatography, silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), ODS (40–60 μm, Alltech), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used. The analytical HPLC was performed on an Agilent 1200 LC equipped with a DAD and the preparative HPLC was performed on a Shimadzu LC-20A (Shimadzu LC-20A, Kyoto, Japan) equipped with a YMC-Pack ODS column (20 × 250 mm, 10 μm, YMC Co. Ltd. Kyoto, Japan).

3.2. Plant Material

The seeds of *Momordica charantia* L. were purchased from Anguo of Hebei Province in 2011, and identified by Lin Ma at Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen has been deposited at our lab at the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

3.3. Extraction and Isolation

The seeds of *M. charantia* L. (15.0 kg) were defatted three times by petroleum ether (90 L each time), and then extracted three times by heating to reflux with 95% ethanol (90 L each time), and the

combined solution was concentrated under reduced pressure to yield an extract (1.6 kg). The alcohol extract was partitioned successively with CHCl_3 , EtOAc and *n*-BuOH. The *n*-BuOH-soluble portions (175 g) was subjected to normal phase silica gel column chromatography with gradient elution [CHCl_3 -MeOH 20:1 (2 L), CHCl_3 -MeOH 9:1 (2 L), CHCl_3 -MeOH 4:1 (2 L), CHCl_3 -MeOH-H₂O 7:3:0.5 (2 L), CHCl_3 -MeOH-H₂O 6:4:0.5 (2 L), MeOH (2 L)] to give eleven fractions (Fr1-11). Compound C1 (8.0 mg) was purified from Fr9 (7.6 g) by normal-phase silica gel and Sephadex LH-20 chromatography. Compound C2 (5.0 mg) was purified from Fr10 (20.6 g) by repeated column chromatography as well as compound C1. Compound C3 (210.0 mg) was purified from Fr10 (20.6 g) by repeated column chromatography over silica gel with eluent as CHCl_3 -MeOH-H₂O 4:1:0.1. Compound C4 (102.0 mg) was purified from Fr11 (12.5 g) by repeated column chromatography over silica gel with eluent as CHCl_3 -MeOH-H₂O 6:4:0.5, and further purified by Sephadex LH-20 with eluent as MeOH and preparative HPLC (MeOH-H₂O, 60:40).

3.4. Acid Hydrolysis of Compounds C1 and C2

D-Glucose, D-galactose, L-rhamnose, D-fucose, D-xylose and L-arabinose aqueous solution (each 2 mg/mL, 80 μL) PMP CH_3OH solution (0.5 mol/L, 80 μL) and aqueous NaOH solution (0.3 mol/L, 80 μL) were heated at 70 °C for 30 min, cooled to room temperature for 10 min, HCl aqueous solution added (0.3 mol/L, 80 μL) and extracted with CHCl_3 (0.5 mL, three times). The aqueous fractions were identified by HPLC analysis (Phenomenex C18, 250 mm \times 4.6 mm, 5 μm column); flow phase A: CH_3CN -20 mmol/L NH_4OAc aqueous solution (15:85), B: CH_3CN -20 mmol/L NH_4OAc aqueous solution (40:60), flow rate: 1.2 mL/min; gradient elution, 0 \rightarrow 20 min, volume fraction of B from 0 to 60%; detection wavelengths: 245 nm; sample volume: 20 μL).

Compounds C1 (2 mg) and C2 (2 mg) were heated in an ampule with aqueous 2 mol/L HCl-1,4-dioxane (1:1, 2 mL) at 80 °C for 6 h. The aglycon was extracted with chloroform, and the aqueous layer was evaporated under reduced pressure and subjected to the normal preparation of sugar derivatives. Thus, compound C1 furnished D-xylose, L-rhamnose, D-glucose, and D-fucose in a ratio of 2:2:1:1, and compound C2 furnished D-xylose, L-rhamnose, D-glucose and D-fucose in the ratio of 1:2:1:1, which were identified by HPLC analysis of the thiazolidine derivatives following conversion to the 1-[(*S*)-*N*-acetyl-(*R*)-methylbenzylamino]-1-deoxyalditol acetate derivatives [7].

4. Conclusions

Two new bidesmoside triterpenoid glycosides identified as 28-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamnopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl (1 \rightarrow 2)- β -D-glucopyranosiduronic acid (**C1**) and 28-*O*- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-rhamnopyranosyl(1 \rightarrow 2)-[α -L-rhamnopyranosyl(1 \rightarrow 3)]- β -D-fucopyranosyl gypsogenin 3-*O*- β -D-glucopyranosyl(1 \rightarrow 2)- β -D-glucopyranosiduronic acid (**C2**) were isolated from the seeds of *M. charantia* L. together with two known ones identified as 3-*O*- β -D-glucopyranosyl-24 β -ethyl-5 α -cholesta-7-*trans*-22*E*,5(7)trien-3 β -ol (**C3**) and momordicoside S (**C4**). This finding represents an addition to the ongoing research on the pharmacological activity of these compounds, which may be helpful to understand the use of this plant in traditional medicine and should continue to clarify its actual health benefits.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/19/2/2238/s1>.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds **1–4** are available from the authors.