Charantoside L, A New Cucurbitane-Type Glycoside from *Momordica charantia* L. with α-Glucosidase Inhibitory Activities

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**Abstract**

A new cucurbitane-type glycoside (1) and two known compounds (2-3) were isolated from the ethanol extract of the fruits of *Momordica charantia* L. Their chemical structures were determined as (19S,23E)-5β,19-epoxy-19-methoxy cucurbita-6,23-diene-3β,25-diol 3-O-β-D-allopyranoside (1), goyaglycoside d (2), and (19S,23E)-5β,19-epoxy-19-methoxy cucurbita-6,23-diene-5β,25-diol (3) on the basis of the extensive spectroscopic methods, including 1D, 2D NMR, HRESIMS, and in comparison with the reported data. Compounds 1 to 3 were evaluated for α-glucosidase inhibitory effects. Compounds 1 and 2 showed anti-α-glucosidase activity with IC₅₀ values of 134.12 ± 11.20 and 163.17 ± 13.71 µM, respectively, compared with the positive control, acarbose, IC₅₀ 160.99 ± 14.30 µM. Compounds 2 and 3 were first isolated from plant *M. charantia* growing in Vietnam.

**Keywords**

cucurbitaceae, *momordica charantia*, cucurbitane-type glycoside, α-glucosidase, charantoside L

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**Introduction**

The plant *Momordica charantia* L. (Cucurbitaceae) is widely cultivated in many tropical regions and its fruit has been used as a vegetable, which has effect of reducing blood sugar and therapeutic effect on diabetes. Cucurbitane-triterpenoids are major components of this plant and some of which have been reported to unti-diabetes a bitter stomachic, a laxative, an antidiabetic, and an anthelmintic agents.¹⁻²³ In the previous papers, we reported twenty-one cucurbitane-type glycosides from the fruits of this plant and their α-glucosidase inhibitory effects.²⁰⁻²² Continue researching on bioactive compounds from *M. charantia*, we further reported herein the isolation and structural elucidation of one new and two known cucurbitane-type compounds and their α-glucosidase inhibition activities.

**Results and Discussion**

Phytochemical study on the water layer of the ethanol extract of *M. charantia* fruits led to the isolation of three compounds (1-3) as white amorphous powders. Compounds 2 and 3 were identified to be (19R,23E)-5β,19-epoxy-19,25-dimethoxy cucurbita-6,23-diene-3β,25-diol 3-O-β-D-allopyranoside (goyaglycoside d)⁶ and (19S,23E)-5β,19-epoxy-19-methoxy cucurbita-6,23-diene-3β,25-diol,²⁴⁻²⁶ respectively (Figure 1 and Table 1).

The molecular formula of 1 was determined to be C₃₇H₆₀O₉Na base on the quasi-molecular ion peaks at m/z 649.4130 [M + H]⁺ (calcd for [C₃₇H₅₁O₉Na]⁺, 649.4130, ∆ = 0 ppm) and m/z 671.4130 [M + Na]⁺ (calcd for [C₃₇H₆₀O₉Na]⁺, 671.4130, ∆ = 0 ppm) in the high-resolution electron spray ionization mass spectrometry (HRESIMS). The ¹³C nuclear magnetic resonance (¹³C NMR) spectrum of 1 showed signals of 37 carbons including 6 non-protonated carbons, 15 CH, 8 CH₂, and 8 CH₃.

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Of these, one methoxy group was confirmed at δC 57.1, two double bonds at δC 134.0/129.8 and 125.3/139.5, and one allose moiety at δC 74.0, 72.1, 70.1, 68.2 (5xCH), and 63.0 (CH2) (Supplemental Figures S1-S8). The above observation in comparison with those of 2 suggesting that 1 was a cucurbitane-type glycoside, a typical compound from M. Charantia. The NMR signals at δC 83.9 (C), δC 115.0/δH 4.42 (s), and at δC 57.1/δH 3.69 (s) suggested for the 5β,19-epoxy-19-methoxy structure. The NMR data of 1 were further assigned by 2D NMR spectra (Table 1, [Supplemental Figures S9-S20]). In the heteronuclear multiple bond correlation [HMBC] spectra, the correlations of H3-28 [δH 1.21]/H3-29 [δH 0.88] to C-3 [δC 83.7]/C-4 [δC 38.5]/C-5 [δC 83.9], H-6 [δH 6.11]/H-7 [δH 5.49] to C-5/C-8 [δC 49.7], H-19 [δH 4.42] to C-5/C-8, and methoxy protons [δH 3.69] to C-19 [δC 115.0] were observed. These evidence further confirmed the 5β,19-epoxy-19-methoxy structure. The HMBC correlations from H-26/H-27 to C-24 [δC 139.5]/C-25 [δC 70.7], and from H-1′ [δH 4.80] to C-3 [δC 83.7] indicated that one hydroxy group was at C-25, one double bond at C-23/C-24, and the allose moiety attached to C-3 [Figure 2]. The large J value [15.5 Hz] of H-23/H-24 suggested the trans configuration of this double bond, which was further confirmed by the nuclear overhauser effect spectroscopy [NOESY] cross peak of H2-22 [δH 1.75 and 2.17] and H-24 [δH 5.58] [Figure 3]. The carbon chemical shifts of C-5, C-8, C-10, and C-19 of 1 differed with that of 2 [19R] and similar to that of 3 [19S] [Table 1] suggested 19S configuration, which was further indicated by the cross peaks between H-19 and H-8/H-18 in the NOESY spectrum of 1. The observation of correlation spectroscopy [COZY] cross peaks of H-1′ [δH 4.80]/H-2′ [δH 3.41]/H-3′ [δH 4.26]/H-4′ [δH 3.61]/H-5′ [δH 3.67]/H-6′ [δH 3.90 and 3.77] and H-3′ appeared as a broad singlet at δH 4.26 confirmed the allose moiety of 1. Which was further indicated by the NOESY cross peaks of H-2′/H-3′ and H-3′/H-4′. The large coupling constant of the anomeric proton at δH 4.80 [J = 7.8 Hz] and the NOESY cross peaks of H-1′/H-3′ indicated β-form of the glycosyl linkage. Acid hydrolysis of 1 obtained D-allose, which were identified by the positive sign of their optical rotations and TLC analysis in comparison with authentic monosaccharide. Based on the above evidence, the structure of 1 was elucidated to be [19S,23E]-5β,19-epoxy-19-methoxy cucurbita-6,23-diene-3β,25-diol 3-O-β-D-allopyranoside, a new compound named charantoside L [Figure 1].

The cucurbitane-type glycosides have been reported for their significant anti α-glucosidase activity. Therefore, compounds 1-3 were evaluated for their anti α-glucosidase activity. Acarbose, an antidiabetic drug was used as a positive control in the test. All the experiments were performed in triplicate and the biological results were described in the half maximal inhibitory concentration (IC50). Compounds 1 and 2 showed anti α-glucosidase activities with IC50 values of 134.12 ± 11.20 and 163.17 ± 13.71 μM, respectively, comparing with the positive control (acarbose, IC50 160.99 ± 14.30 μM), whereas compound 3 is inactive (Table 2).
Material and Methods

General Experimental Procedures

Optical rotation was measured on a Jasco P-2000 polarimeter. IR spectrum was recorded on a Spectrum Two FT-IR spectrometer, NMR spectra on a Bruker AvanceNEO 600 MHz spectrometer, HRESIMS on a SCIEX X500 QTOF LC/MS. Fractionation was monitored by thin layer chromatography (TLC) to combine test tubes showing similar TLC pattern. For TLC, a precoated silica gel 60 F254 (0.25 mm, Merck) and RP-18 F 254S plates (0.25 mm, Merck) were used. Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or YMC RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd). Compounds were visualized by UV irradiation (254 and 365 nm) and by spraying with H2SO4 solution (5%) followed by heating with a heat gun. HPLC was carried out using an AGILENT 1100 HPLC system.

Plant Material

The fruits of *Momordica charantia* L. were collected in Thai Binh province in May 2021, and identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources. A voucher specimen was deposited at the Institute of Marine Biochemistry, VAST.

Extraction and Isolation

The dried powders of *M. charantia* fruits (2.0 kg) were sonicated with hot ethanol (96°) (3 times × 5 L, each 3 h) to give EtOH extract (200 g) after evaporation of the solvent. The EtOH extract was suspended in water and successively partitioned with EtOAc to obtain the EtOAc extract (MCE, 60 g) and H2O layer (MCW). The MCE extract (58 g) was chromatographed on a silica gel column eluting with gradient solvent of hexane: acetone (40:1, 20:1, 10:1, 5:1, 1:1, and 0:1, v/v) to give six fractions, MCE1-MCE6. Fraction MCE3 (2.2 g) was chromatographed on an RP-18 column eluting with acetone:H2O (3:1, v/v) to give three smaller fractions, MCE3A-MCE3C. MCE3C was chromatographed on a J′sphere H-80 column (150 × 20 mm), solvent condition of 100% CH3CN to give to yield compound 3 (10.2 mg). Fraction MCE5 (6.5 g) was chromatographed on an RP-18 column eluting with acetone:H2O (3:1, v/v) to give four smaller fractions, MCE5A-MCE5D. MCE5B was chromatographed on a J′sphere H-80 column (150 × 20 mm), solvent condition of 70% CH3CN in H2O to give compounds 1 (15.5 mg) and 2 (16.2 mg).

Charantoside L (1). White amorphous powder; [α]D25 \( 37.0 \) (c 0.1, EtOH); IR (KBr) \( \nu_{max} \) 3397, 1455, 1376, 1053 cm\(^{-1}\), HRESIMS \( m/z \) 649.4130 [M + H]\(^+\) (calcd for \([C_{37}H_{61}O_{9}]^+\), 649.4130, \( \Delta = 0 \) ppm); 666.4576 [M + NH\(_4\)]\(^+\) (calcd for

| Table 1. NMR Spectroscopic Data for Compounds 1-3 in Deuterated Chloroform. |
|-----------------|-----------------|-----------------|
| Pos. | \( \delta_C \) | \( \delta_H \) (mult., \( J \) in Hz) | \( \delta_C \) | \( \delta_H \) |
| 1   | 17.6  | 1.28 (m), 2.03 (m) | 18.3  | 16.6  |
| 2   | 27.4  | 1.74 (m), 1.83 (m) | 27.3  | 27.1  |
| 3   | 83.7  | 3.48 (m)         | 82.6  | 76.2  |
| 4   | 38.5  | –                 | 38.8  | 37.1  |
| 5   | 83.9  | –                 | 85.6  | 85.1  |
| 6   | 134.0 | 6.11 (dd, 9.6, 1.2) | 132.1 | 133.1 |
| 7   | 129.8 | 5.49 (dd, 9.6, 3.6) | 132.0 | 130.5 |
| 8   | 49.7  | 2.26 (d, 4.8)     | 41.8  | 49.8  |
| 9   | 48.8  | –                 | 47.8  | 49.0  |
| 10  | 38.5  | –                 | 41.0  | 37.9  |
| 11  | 21.5  | 1.59 (m), 1.70 (m) | 22.9  | 21.4  |
| 12  | 30.4  | 1.60 (2H, m)      | 30.6  | 30.5  |
| 13  | 45.2  | –                 | 45.0  | 45.2  |
| 14  | 48.1  | –                 | 48.0  | 48.1  |
| 15  | 33.5  | 1.30 (m), 1.33 (m) | 33.5  | 33.5  |
| 16  | 27.8  | 1.37 (m), 1.95 (m) | 27.9  | 27.8  |
| 17  | 50.2  | 1.44 (m)         | 50.1  | 50.2  |
| 18  | 15.0  | 0.87 (s)         | 14.6  | 15.0  |
| 19  | 115.0 | 4.42 (s)         | 112.5 | 114.8 |
| 20  | 36.2  | 1.51 (m)         | 36.1  | 36.2  |
| 21  | 18.6  | 0.88 (d, 7.0)    | 18.7  | 18.6  |
| 22  | 39.1  | 1.75 (m), 2.17 (m) | 39.4  | 39.1  |
| 23  | 125.3 | 5.59 (dt, 15.5, 7.0) | 128.5 | 125.3 |
| 24  | 139.5 | 5.58 (d, 15.5)   | 136.7 | 139.6 |
| 25  | 70.7  | –                 | 74.9  | 70.8  |
| 26  | 30.0  | 1.31 (s)         | 25.8  | 30.0  |
| 27  | 29.9  | 1.31 (s)         | 25.8  | 30.0  |
| 28  | 25.2  | 0.88 (s)         | 24.8  | 24.4  |
| 29  | 20.9  | 1.21 (s)         | 21.2  | 20.6  |
| 30  | 19.9  | 0.85 (s)         | 19.7  | 19.9  |
| 19-OH  | 57.1  | 3.69 (s)      | 58.4  | 57.3  |
| 19-OMe | 57.1  | 3.69 (s)         | 58.4  | 57.3  |

| Figure 2. Important H-H COSY and HMBC correlations of compound 1. |
Conclusions

Three cucurbitane-type glycosides (1-3) were isolated from the ethanol extract of the fruits of *Momordica charantia* L. Their structures were established by extensive spectroscopic analysis. Of which, compound 1 was previously undescribed and compounds 2 and 3 were first reported from Vietnamese *M. charantia* plant. Compounds 1 and 2 showed anti-α-glucosidase activities with IC₅₀ values of 134.12 ± 11.20 and 163.17 ± 13.71 μM, respectively, compared with the positive control (acarbose, IC₅₀ 160.99 ± 14.30 μM), whereas compound 3 is inactive.

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Authors Contribution

Research idea, NX Nhiem, TTT Ha, PH Yen, PV Kiem; Isolation, DTH Yen, NQ Hop, NH Anh; Structure elucidation and writing, BH Tai, PH Yen, PV Kiem.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

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