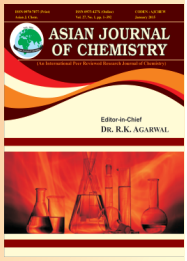
Asian Journal of Chemistry; Vol. 27, No. 2 (2015), 393-395

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.16901



Characterization of Hypoglycemic Constituents in *Momordica charantia* L. and their Hypoglycemic Effect

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Received: 20 December 2013; *Accepted*: 15 April 2014; *Published online*: 10 January 2015; AJC-16581

This study represents the active constituents in *Momordica charantia* L. and their hypoglycemic effect. Active constituents in *Momordica charantia* L. were extracted by ethanol percolation extraction and purified by macroporous resin method and preparative thin layer chromatography, post-administration blood glucose in mice was measured using mice model of STZ diabetes. Four compounds were isolated from *Momordica charantia* L., the determination of blood glucose, hepatic glycogen and fructosamine in mice indicated that blood glucose in mice was lowered after administration of *Momordica charantia* L. It is suggested that *Momordica charantia* L. has a hypoglycemic effect.

Keywords: *Momordica charantia* L., Aglycone, Momordicoside, Diabetic mice model.

INTRODUCTION

Ku Gua is the unripe fruit of *Momordica charantia* L. in the genus Momordica of the family Cucurbitaceae. It is widely distributed in tropical, subtropical and temperate regions, which is a commonly used Chinese medicine in clinical practice. *Momordica charantia* L. is bitter in taste and cold in nature, which has the actions of clearing heat, removing toxicity, nourishing, strengthening and lowering blood sugar, as early as in the "Compendium of Materia Medica", *Momordica charantia* L. is recorded to be able to "remove pathogenic heat, recover fatigue, clear away heart-fire and improve eyesight". Modern scholars have conducted extensive research on *Momordica charantia* L. and isolated a variety of compounds from it[1], which have been proven to have hypoglycemic, antitumor, antifertility and immuno suppressive physiological activities[2-4].

As both medicinal and edible, *Momordica charantia* L. has good medicinal and health care values, in addition, China has relatively abundant resources of *Momordica charantia* L. The research and development of drugs and health food containing *Momordica charantia* L. can not only enrich the public demand for clinical medication and ease the clinical pressure, but can also be of great benefit to public health care. In this paper, the ethanolic extract of *Momordica charantia* L. was purified and the isolated compounds were structurally characterized, meanwhile, the hypoglycemic activity of *Momordica charantia* ethanol extract (MCEE) was studied.

EXPERIMENTAL

Agilent 1200 HPLC (Agilent, USA); Bruker Avance 600 NMR spectrometer, X-6 micro melting point apparatus, Sephadex LH-20 (Pharmacia Bioteck); blood glucose monitor (Johnson & Johnson, USA).

Drugs: Fresh Ku Gua was purchased from the market, which was identified as the unripe fruit of *Momordica charantia* L. in the genus Momordica of the family Cucurbitaceae.

Glibenclamide (SFDA approval No. H44020768, Guangdong Huanan Pharmaceutical Group Co., Ltd.)

Animals: Kunming mice, 8-10 weeks old, weighing 18-22 g, male, were provided by the Laboratory Animal Center of China Medical University.

Methods: Isolation and purification of active constituents in *Momordica charantia* L.

Isolation: Fresh unripe *Momordica charantia* L. was crushed and extracted by percolation with 95 % ethanol twice, the percolates were combined, ethanol was removed and the residue was concentrated to give a liquid extract.

Purification: Above liquid extract was suspended by addition of an appropriate amount of water and extracted with ethyl acetate, followed by removal of ethyl acetate. The ethyl acetate layer was subjected to silica gel column chromatography, gradient-eluted with chloroform-methanol eluent; the same fractions were combined and purified by Sephadex LH-20 column chromatography, eluted with methanol, then isolated by

preparative thin layer chromatography to give three compounds. Water layer was concentrated to an appropriate concentration and enriched by macroporous resin column chromatography (AB-8). The same fractions were combined, concentrated and then isolated by preparative HPLC to give compound **4**.

MCEE hypoglycemic experiment

Establishment of experimental STZ-diabetic mouse model: Forty mice, half male and half female, were fasted for 24 h. The mice which were fasted but watered for 24 h were intraperitoneally injected with STZ citrate buffer (pH 4.4) at a dose of 200 mg/kg, 72 h later, tails were severed and blood was collected to measure blood glucose and the mice with blood glucose level higher than 11.1 mmol/L were selected as successfully modeled hyperglycemia animals[5] and set aside.

Effect of MCEE on blood glucose in STZ-diabetic mice: The successfully modeled mice were randomly divided into five groups according to body weight and blood glucose level, which were namely hyperglycemia model control group, glibenclamide group (25 mg/kg) and MCEE experimental groups (200, 400 and 600 mg/kg). Among them, the mice in the hyperglycemia model control group and glibenclamide group were intragastrically administered distilled water and corresponding volume of glibenclamide and the mice in the MCEE groups were intragastrically administered according to the doses once a day for two weeks. After the last administration, the mice in each group were fasted for 3 h, then tails were severed and blood was collected for measurement of blood glucose, hepatic glycogen and fructosamine levels.

RESULTS AND DISCUSSION

Structural identification of compounds

Compound 1: Colourless needle crystals (acetone), positive in Liebermann-Burchard reaction. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.12 (H, dd, *J* = 9.5, 1.8 Hz, H-6), 5.69 (H, dd, *J* = 9.5, 3.5 Hz, H-7), 5.62 (2H, m, H-23, H-24); 3.54 (H, d, *J* = 8.5 Hz, H-19e), 3.72 (H, d, *J* = 8.5 Hz, H-19b); 3.48 (H. br.s, OH-25), 4.05 (H, br.d, OH-3). ^{13}C-NMR ($CDCl_3$, 500 MHz) δ: 21.4 (C-1), 28.6 (C-2), 76.4 (C-3), 41.6 (C-4), 145.6 (C-5), 124.2 (C-6), 66.4 (C-7), 49.7 (C-8), 49.8 (C-9), 36.4 (C-10), 23.2 (C-11), 28.5 (C-12), 45.6 (C-13), 47.9 (C-14), 34.4 (C-15), 27.6 (C-16), 49.7 (C-17), 49.2 (C-18), 79.4 (C-19), 36.4 (C-20), 17.7 (C-21), 37.3 (C-22),125.4 (C-23), 139.6 (C-24), 70.3 (C-25), 30.3 (C-26), 27.3 (C-27), 20.7 (C-28), 24.9 (C-29), 20.4 (C-30).

Structural data of compound **1** (Fig. 1) were basically consistent with the known compound[6,7], so its structure was aglycone of momordicoside L (3β,7β,25-trihydroxy-cucurbita-5,(23E)-diene-19-al).

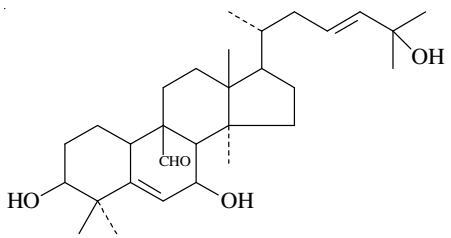

Fig. 1. 3β,7β,25-Trihydroxy-cucurbita-5,(23E)-diene-19-al

Compound 2: Colourless needle crystals, m.p. 145-146.5 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ:4.45 (H, dd, H-3), 4.87 (H, s, H-19), 0.86 (6H, s, H-23, H-24), 0.92 (3H, s, H-25), 1.06 (3H, s, H-26), 0.95 (3H, s, H-27), 0.96 (3H, s, H-28), 0.75 (3H, S, H-29), 1.09 (3H, s, H-30), 2.05 (3H, s, H-2'),; ^{13}C NMR ($CDCl_3$, 500 MHz) δ:38.5 (C-1), 23.8 (C-2), 81.2 (C-3), 37.5 (C-4), 55.7 (C-5), 18.4 (C-6), 33.7 (C-7), 40.3 (C-8), 51.7 (C-9), 37.4 (C-10), 21.5 (C-11), 26.5 (C-12), 38.6 (C-13), 43.7 (C-14), 27.9 (C-15), 37.5 (C-16), 34.7 (C-17), 142.1 (C-18), 129.6 (C-19), 32.1 (C-20), 34.7 (C-21), 37.7 (C-22), 28.2 (C-23), 16.9 (C-24), 16.4 (C-25), 16.7 (C-26), 14.8 (C-27), 25.5 (C-28), 31.6 (C-29), 29.2 (C-30).

^{1}H NMR and ^{13}C NMR data of compound **2** were basically consistent with the structure of known compound[8], so compound **2** (Fig. 2) was aglycone of momordicoside I and its structure was 5,19-epoxy-5β-cucurbita-6,23E-diene-3β,25-diol.

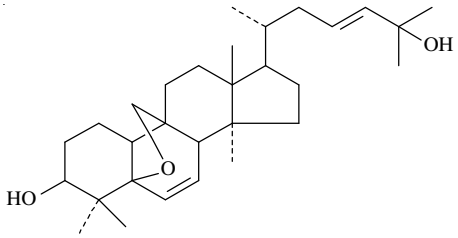

Fig. 2. 5,19-Epoxy-5β-cucurbita-6,23E-diene-3β,25-diol

Compound 3: White needle crystals (acetone), blue-purple in Liebermann-Burchard reaction, three different development systems were selected for TLC identification on the same TLC plate with β-sitosterol standard substance, the results all presented the same spot. Compound **3** (Fig. 3) was therefore identified as β-sitosterol.

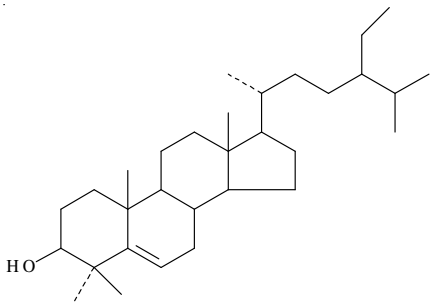

Fig. 3. β-Sitosterol

Compound 4: White solid, ^{1}H NMR (D_2O, 500 MHz) δ: 0.85 (s, H-12), 1.11 (s, H-13), 1.82-1.94 (m, H-2ax, H-4), 1.92 (s, H-8), 2.69 (m, H-11), 4.11 (m, H-3), 4.44 (d, *J* = 8.5 Hz, H-1'), 5.76 (s, H-14), 6.28 (d, *J* = 15.0 Hz, H-7), 7.46 (d, H-8). ^{13}C-NMR (pyridine-d5, 500 MHz) δ:50.5 (C-1), 43.6 (C-2), 76.3 (C-3), 43.2 (C-4), 89.5 (C-5), 85.3 (C-6), 133.7 (C-7), 133.7 (C-8), 147.6 (C-9), 22.2 (C-10), 78.5 (C-11), 17.1 (C-12), 21.4 (C-13), 125.8 (C-14), 176.2 (C-15), 103.5 (C-1'), 75.2 (C-2'), 78.9 (C-3'), 72.8 (C-4'), 78.7 (C-5'), 63.5 (C-6').

Structural data of compound **4** (Fig. 4) were basically consistent with the known compound[9], so its structure was dihydrophaseic acid-3-O-β-D-glucopyranoside.

TABLE-1
EFFECT OF MCEE ON BLOOD GLUCOSE IN STZ-DIABETIC MICE

Group	Blood glucose before administration (mmol/L)	Blood glucose after administration (mmol/L)	Difference value
Blank control group	17.62 ± 2.46	21.53 ± 3.64	-3.91 ± 3.24
Glibenclamide group	17.59 ± 3.12	14.25 ± 3.52*	3.34 ± 2.36##
MCEE group (200 mg/kg)	17.37 ± 2.51	16.46 ± 2.16	0.91 ± 2.35
MCEE group (400 mg/kg)	17.42 ± 3.25	14.37 ± 3.22*	3.05 ± 3.23##
MCEE group (600 mg/kg)	17.30 ± 3.33	11.85 ± 2.12**	5.45 ± 3.01##

Note: Comparison with the blank control group, * P < 0.05, ** P < 0.01; comparison within the same group before and after administration, # P < 0.05, ## P < 0.001

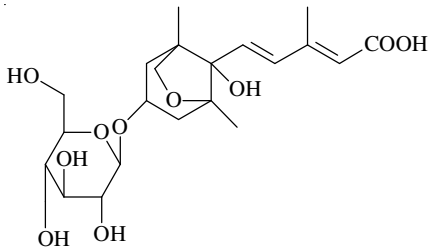

Fig. 4. Dihydrophaseic acid-3-O-β-D-glucopyranoside

Effect of MCEE on blood glucose in STZ-diabetic mice: After 2 weeks intragastric administration, blood glucose level markedly elevated in the blank control group and siginificantly elevated in the glibenclamide group, the effect in the MCEE medium- and high-dose groups were equivalent with that in the glibenclamide group (Table-1).

Effect of MCEE on hepatic glycogen and fructosamine in STZ-diabetic mice: Compared with the control group, MCEE could significantly increase the hepatic glycogen content in diabetic mice, while reducing the level of fructosamine, the differences were statistically significant (Fig. 5).

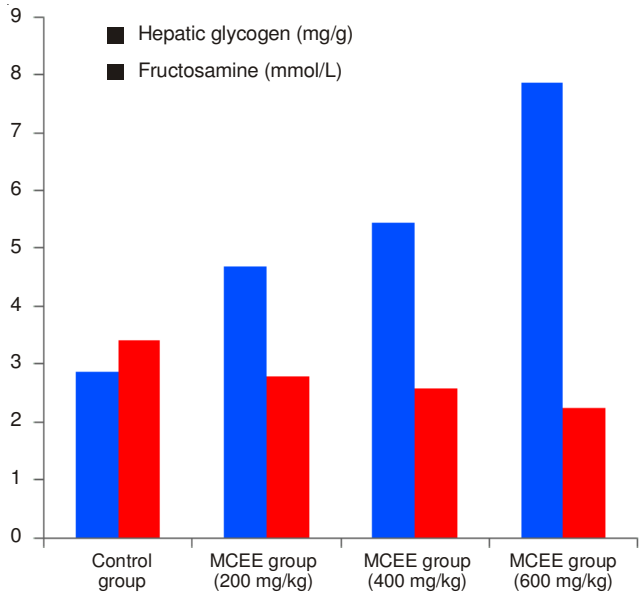

Fig. 5. Effect of MCEE on hepatic glycogen and fructosamine in STZ-diabetic mice

The results have demonstrated that blood pressure decreased significantly in alloxan diabetic mice after action of freeze-dried fresh *Momordica charantia* L. juice, dry *Momordica charantia* L. powder could relatively markedly reduce blood glucose in both normal and alloxan-induced diabetic rabbits, *Momordica charantia* L. extract, including momordicosides, had a hypoglycemic effect in normal and streptozotocin-diabetic animals. For normal glucose-fed rats, *Momordica charantia* L. showed somewhat less activity compared with sulfaphenazoles, but the results have shown that the hypoglycemic effect of *Momordica charantia* L. extract is obvious and generally accepted.

The results of this experiment showed that MCEE could significantly increase hepatic glycogen content in diabetic mice. It was thus speculated that the hypoglycemic effect of momordicosides is associated with increased hepatic glycogen synthesis.

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