Food Chemistry 228 (2017) 567–573

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cucurbitane glycosides from the fruit of *Siraitia grosvenori* and their effects on glucose uptake in human HepG2 cells *in vitro*

Fu Li [a,b], Fumei Yang [c], Xin Liu [d], Lun Wang [a,b], Bin Chen [a,b], Lianhong Li [e], Mingkui Wang [a,b,*]

[a] School of Pharmacy and Bioengineering, Chengdu University, Chengdu 610106, China
[b] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
[c] Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, China
[d] Beijing Entry-Exit Inspection and Quarantine Bureau, Beijing 100026, China
[e] Chengdu MUST Bio-technology Company Limited, Chengdu 610000, China

ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form 20 January 2017
Accepted 6 February 2017
Available online 10 February 2017

Keywords:
Siraitia grosvenori
Cucurbitane glycosides
Glucose uptake
In vitro

ABSTRACT

The mogrosides in the fruit of *Siraitia grosvenori* can serve as a sugar substitute for diabetics due to their sweetness, low calorie and positive effects on blood glucose level control. The present study was to purify the mogrosides from the fruit of *S. grosvenori* and evaluate their enhancement of glucose uptake rate in HepG2 cells *in vitro*. As a result, eighteen mogrosides were isolated, including six new ones and a known but new naturally occurring compound. The chemical structures of the new compounds were identified by 1D, 2D-NMR and HR-ESI-MS techniques, together with chemical methods. Compared to the positive control (metformin), all the obtained mogrosides showed equivalent or more potent effects on the glucose uptake in HepG2 cells *in vitro*. These results suggested the mogrosides in the fruit of *S. grosvenori* were worthy of further research to confirm their potential benefits for obese and diabetic patients.

1. Introduction

Siraitia grosvenori (Swingle) C. Jeffery (Cucurbitaceae) is a unique plant mainly distributed in Guangxi province, China. The fruit of *S. grosvenori*, also known as Luo Han Guo, has been used for centuries in traditional Chinese medicine to treat dry coughs, sore throats, dire thirst, and constipation (Jia & Yang, 2009). Nowadays, Luo Han Guo is well known throughout the world due to its ability to produce abundant of mogrosides (Jin & Lee, 2012), which are natural low-calorie sweeteners and can serve as alternatives to sugar for obese and diabetic patients without side effects (Takasaki et al., 2003). Some individual mogrosides, such as mogroside IV, V and siamenoside I containing four or five glucose moieties, were judged to be more than 400 times sweeter than sucrose (Matsumoto, Kasai, Ohtani, & Tanaka, 1990). In 2010, *S. grosvenori* extracts were approved by the U.S. Food and Drug Administration (FDA) for use as food additives (Chiu, Wang, Lee, Lo, & Lu, 2013).

Modern pharmaceutical studies showed that mogrosides from the fruit of *S. grosvenori* possessed various biological activities, including anti-inflammatory (Di, Huang, & Ho, 2011), anticarcinogenic (Takasaki et al., 2003), anti-virus (Akihisa et al., 2007; Ukiya et al., 2002), antioxidative (Qi, Chen, Zhang, & Xie, 2008) properties. It was interesting that, as natural sweeteners, the *S. grosvenori* mogrosides not only were safe for diabetics but also exhibited anti-diabetic activity *in vitro* and *in vivo*. Suzuki, Murata, Inui, Sugiura, and Nakano (2005) firstly advocated that mogroside V and some minor elements from the fruit of *S. grosvenori* can suppress the rise in blood glucose level after a single oral administration of maltose in rats by inhibiting rat intestinal maltase. Afterwards, Zhou, Zheng, Ebersole, and Huang (2009) demonstrated the *S. grosvenori* extract and pure mogroside V exhibited a significant activity in stimulating insulin secretion in pancreatic beta cells. A recent study by Chen et al. (2011) revealed cucurbitane triterpenoids obtained through acid hydrolysis of *S. grosvenori* mogrosides might be potential AMPK activators.

Up to now, nearly thirty cucurbitane glycosides have been reported from *S. grosvenori* (Jin & Lee, 2012). However, only several high content compounds, such as mogroside V, IV, III and siamenoside I, had been evaluated for their anti-diabetic activity. Hence, the present study focused on the chemical composition of *S. grosvenori*, including not only major mogrosides but also minor

* Corresponding author at: Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China.
E-mail address: wangmk@cib.ac.cn (M. Wang).

http://dx.doi.org/10.1016/j.foodchem.2017.02.018
0308-8146/

ones. In addition, all the isolated mogrosides were preliminarily evaluated for their anti-diabetic activity. As a result, six new cucurbitane glycosides, a new naturally occurring compound and eleven known ones were obtained. Herein, the isolation and structure elucidation of these compounds and their ability to increase glucose uptake in human HepG2 cells *in vitro* are reported.

2. Materials and methods

2.1. General procedures

Optical rotations were determined in methanol on a Perkin-Elmer 341 polarimeter (Perkin-Elmer Corporation, Wellesley, MA, USA). Low resolution MS were recorded on a Finnigan LCQ^{DECA} ion-trap mass spectrometer (Finnigan Co. Ltd., San Jose, CA, USA) while high resolution MS were measured on a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) with both electrospray ionization sources operated in positive ion mode. NMR spectra, including ^{1}H NMR, ^{13}C NMR, H–H COSY, HSQC, HMBC, NOESY and TOCSY experiments, were recorded on an Advance III spectrometer (Bruker Group, Fallanden, Switzerland) operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C with TMS as an internal standard. HPLC analysis of the sugar derivatives was performed on a Shimadzu LC-20AT series apparatus with an SPD-M20A UV–vis spectrophotometric detector, equipped with a 250 × 4.6 mm i.d. Grace Alltima^{TM} C_{18} (5 μm) column (Alltech Associates Inc., Deerfield, IL, USA) with isocratic elution mode using a mobile phase of 23% CH_3CN in 50 nM H_3PO_4 at a flow rate of 0.8 mL/min. HPLC purifications were performed on a CXTH system, equipped with a UV3000 detector at 210 nm (Beijing Chuangxintongheng Instruments Co. Ltd., Beijing, People's Republic of China). The preparative HPLC column used was a 50 × 250 mm i.d., 10 μm, YMC-pack ODS-AM (YMC Co. Ltd., Kyoto, Japan). The flow rate was 90 mL/min. Macroporous resin (HPD-100A, 26–60 mesh) was used to enrich total saponins (Cangzhou Bon Adsorber Technology Co. Ltd., Cangzhou, People's Republic of China). MCI-gel was used for column chromatography to remove colored impurities (Mitsubishi Chemical Corp., Tokyo, Japan). Silica gel (100–200 mesh) for column chromatography and silica gel GF254 (10–40 μm) for TLC were purchased from Qingdao Haiyang Chemical Group Co. Ltd. (Qingdao, People's Republic of China). Chemical reagents for isolation were of analytical grade and purchased from Chengdu Kelong Chemical Reagent Co. Ltd. (Chengdu, People's Republic of China).

2.2. Plant material

The fruit of *S. grosvenori* was purchased in March 2012 from Lotus Pond Chinese Herbal Medicine Market, Sichuan province, People's Republic of China. The plant material was identified by Professor Weikai Bao, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (LHG-100) was deposited at the Laboratory of Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences.

2.3. Extraction and isolation

The fruit of *S. grosvenori* (20.0 kg) were powdered and extracted with distilled water (3 × 60 L, each 4 h) at 80 °C. The extracted water solution was filtered and then poured into a HPD-100A macroporous resin column (HPD-100A macroporous resin, 10 kg; column, 30 × 90 cm) eluted with H_2O, 20% EtOH, 70% EtOH and 95% EtOH (50 L for each gradient elution), respectively. The 70% EtOH eluant solution was concentrated under reduced pressure to give a crude saponin (280 g), which was further separated by silica gel column chromatography (silica gel, 2.5 kg; column, 8 × 120 cm) with a gradient solvent system of MeOH/$CHCl_3$/H_2O (1:5:0.1, 1:4:0.15, 1:3:0.2, 1:2:0.3, and 1:1:0.4;), affording eight fractions (A–H) based on TLC analyses. Fraction C was decolorized with a MCI gel column (65% MeOH in H_2O) and separated by preparative HPLC (25% CH_3CN in H_2O) to give compound **17** (t_R = 38.1 min, 220.3 mg). Fraction D was decolorized by MCI (65% MeOH in H_2O) and further purified by preparative HPLC (25% CH_3CN in H_2O) to afford compounds **1** (t_R = 11.5 min, 480.2 mg), **2** (t_R = 10.6 min, 548.1 mg), and **18** (t_R = 25.7 min, 832.7 mg). Compounds **3** (t_R = 17.2 min, 1.11 g), **4** (t_R = 19.2 min, 730.0 mg), **5** (t_R = 23.1 min, 894.6 mg), **6** (t_R = 28.1 min, 47.4 mg), **7** (t_R = 25.4 min, 63.8 mg) and **13** (t_R = 14.9 min, 113.0 mg) were obtained from fraction E by repeated preparative HPLC using 23% CH_3CN in H_2O as elutant. Fraction F was separated by preparative HPLC (23% CH_3CN in H_2O) to give compounds **8** (t_R = 23.1 min, 39.2 g), **9** (t_R = 28.3 min, 751.3 mg), **14** (t_R = 17.1 min, 6.61 g), and **16** (t_R = 49.6 min, 218.8 mg). The purification of fraction G by preparative HPLC (23% CH_3CN in H_2O) yielded compound **10** (t_R = 13.2 min, 43.3 mg). Fraction H was decolorized by MCI gel (60% MeOH in H_2O) and further purified by preparative HPLC (50% MeOH in H_2O) to afford compounds **11** (t_R = 17.2 min, 52.0 mg), **12** (t_R = 20.9 min, 60.8 mg), and **15** (t_R = 13.4 min, 47.7 mg).

2.4. Determination the absolute configuration of the sugar residues

The absolute configurations of the sugar moieties were determined using the method previously described (Xu et al., 2010). Compounds **6**, **7**, **10**, **12**, **13** and **15** (each 3 mg) were mixed and heated with 5% H_2SO_4 (3 mL) under reflux for 8 h. The reaction mixture was extracted with $CHCl_3$. The H_2O layer was neutralized with $Ba(OH)_2$, filtered and subjected to TLC analysis with authentic glucose sample. The optical rotation of the acid hydrolysis solution was measured as $[\alpha]_D^{20}$ +50.2° (c 0.05, H_2O). Therefore, the configuration of the glucose in the new compounds should be in D-form.

2.5. Cell culture

HepG2 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (11.1 mM glucose) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 mg/ml), L-glutamine (0.03% (w/v)), and $NaHCO_3$ (2.2% (w/v)). Cell cultures were kept in a humidified incubator with 5% CO_2 at 37 °C. The culture solution was replaced every other day and passaged once for 2–3 d.

2.6. MTT assay

The cytotoxicity of all the isolated mogrosides against HepG2 cells was measured using the MTT assay (Zhang et al., 2016). The compounds showed no or very little cytotoxicity at concentrations used in the functional assay.

2.7. Glucose uptake assay

The glucose uptake assay was done according to the methods previously reported (Li et al., 2007; Lv et al., 2014). The cells were planted into 96-well plates with six wells left as blank wells. After reaching 80–90% confluence, the medium was replaced by RPMI-1640 (11.1 mM glucose) containing 0.2% bovine serum albumin (BSA). The medium was then added 1 μmol/L metformin or individual compounds at different concentrations, and dimethyl sulfoxide (DMSO) was used as the blank control. The glucose concentration in the medium was determined after 24 h treatment. Glucose uptake rate = [(glucose concentrations in blank wells – glucose concentrations of cell plated wells)/glucose