Biosci. Biotechnol. Biochem., **74** (3), 673–676, 2010

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Note

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Received November 9, 2009; Accepted December 22, 2009; Online Publication, March 7, 2010
[doi:10.1271/bbb.90832]

When administered to rats, mogroside V (a pentaglucose-conjugated mogroside), the main sweetening component of *Siraitia grosvenori*, was mostly degraded by digestive enzymes and intestinal microflora, and was excreted in the feces as mogrol (aglycone) and its mono- and diglucosides. However, trace amounts of mogrol and its monoglucoside were found in the portal blood as sulfates and/or glucuronide conjugates.

Key words: *Siraitia grosvenori* Swingle; mogroside V; glycoside; aglycone; conjugate

Siraitia grosvenori Swingle (SG) (*Siraitia grosvenorii* C. Jeffrey ex A. M. Lu et Zhi Y. Zhang (*Momordica grosvenori* Swingle)) is a traditional Chinese fruit, and an herbaceous perennial of the Cucurbitaceae family. SG has been used as a folk medicine in China for cough, sputum, asthma, bronchitis, pharyngitis, acute gastritits, and constipation.[1)] The most remarkable characteristics of SG are its unique properties of sweetness. The chemical structure of the sweetening components in SG have been found to belong to the triterpenoides, named mogroside for various glycosylated compounds, and mogrol for aglycone.[2,3)] The sweetening components in this plant include penta-, tetra-, and tri-glucose conjugated mogrosides. Pentaglucose-conjugated mogrosides include mogroside V (M-V) and 11-oxo-mogroside V (11oxoM-V), tetraglucose-conjugated mogrosides include mogroside IV (M-IV) and siamenoside I (S-I), and triglucose-conjugated mogrosides include mogroside III (M-III). The sweetness of M-V, 11oxoM-V, M-IV, S-I, and M-III has been reported to be 378, 68, 300, 465, and 195 times as sweet as sucrose respectively.[4)] Based on these sweetness characteristics, SG is utilized as a plant-derived substitute for sucrose as well as a folk medicine.[5)] Previous studies on the phamacological activities of SG extracts (SG-ex) revealed anti-atherosclerotic effects,[6)] anti-cancer activity,[7,8)] anti-allergy activity,[9)] and anti-diabetic effects in animal models.[10,11)] Previous findings suggest that on oral administration of SG, either M-V or its metabolites are likely to be absorbed, with pharmacological effects, but no reports have been published concerning the metabolism of triterpenoides in SG. This study was undertaken to investigate the process of digestion of M-V in the digestive tract of rats and its consequences. To this end, rats were orally given M-V, and the triterpenoide contents in their small intestine, portal blood, and feces were analyzed by LC-MS.

Fresh fruits of SG were crushed and boiled in water. The extract was filtrated and then concentrated under reduced pressure until its dry weight was about 60% of the total weight, referred to as SG-ex in this report. SG-ex was diluted with water, and its triterpenoids were selectively adsorbed onto a reverse-phase column (a gravity open column). The triterpenoid fraction was eluted from the column with a 70% ethanol solution. This fraction was evaporated, resuspended in water, and purified by repeated column chromatography as described above. The residue was made into powder in a spray-dryer to yield SG glycoside powder (SG-gly). The M-V content of SG-gly was 72.0% w/w determined by HPLC analysis. To obtain the standard M-V, M-IV, and S-I, SG-gly was dissolved in water and put onto a gravity open column. The column was equilibrated with water to adsorb SG triterpenoids. The SG triterpenoid fraction was then eluted with a linear gradient of ethanol. Fractions containing each SG triterpenoids were collected, and concentrated by evaporation. The standard M-V, M-IV, and S-I were fractionated from SG triterpenoids by re-chromatography.[3,4)] Standard M-III and mogroside IIE (M-IIE) were produced by enzymatic reaction. A reaction mixture containing SG-gly, 100 mM acetate buffer (pH 4.0), and cellulase was incubated at 40 °C for 24 h. After the reaction was stopped by boiling for 15 min, the mixture was centrifuged to remove precipitates. The supernatant was applied to repeated column chromatography to obtain M-III and M-IIE. Standard mogroside IE (M-IE) and mogrol were prepared by thermolysis. SG-gly was dissolved in 0.1 N-HCl and reacted by boiling for 20 h. The mixture was centrifuged to remove the supernatant. The precipitant was dissolved in ethanol (45% v/v) and applied to repeated column chromatography to obtain standard M-IE and mogrol. The purities of all standards were at least 96%. The chemical structures of these standard materials were confirmed by NMR analysis, as described previously[12–14)] (Fig. 1).

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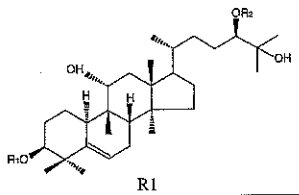
Abbreviations: SG, *Siraitia grosvenori* Swingle; SG-ex, *Siraitia grosvenori* Swingle extract; SG-gly, *Siraitia grosvenori* Swingle glycoside; M-V, mogroside V; M-IV, mogroside IV; S-I, siamenoside I; M-III, mogroside III; M-IIE, mogroside IIE; M-IIA, mogroside IIA; M-IA, mogroside IA; M-IE, mogroside IE



	R1	R2
Mogroside V	-Glc^{6}Glc	-Glc^{2}Glc (6 Glc)
Mogroside IV	-Glc^{6}Glc	-Glc^{2}Glc
Siamenoside I	-Glc	-Glc^{2}Glc (6 Glc)
Mogroside III	-Glc	-Glc^{2}Glc
Mogroside IIE	-Glc	-Glc
Mogroside IIA	-	-Glc^{2}Glc
Mogroside IE	-Glc	-
Mogroside IA	-	-Glc
Mogrol	-	-

Fig. 1. Structures of SG Triterpenoides.

Wistar rats (Clea Japan, Osaka, Japan) were purchased at 9 weeks of age and reared for 1 week to acclimatize them to the environment in the animal quarters, where the temperature was kept at 23 ± 2 °C, the relative humidity was controlled at 60 ± 10%, and the room was lighted 9:00 to 21:00 each day. The rats were allowed free access to a standard pellet diet (CE-2; Clea Japan, Osaka, Japan) and water. After 16 h of fasting, 1 ml of SG-gly solution (117 mg/ml) was orally administered. The small intestinal contents and portal blood were collected at 120 min after administration. The small intestinal contents were collected by passing 3 ml of PBS through the small intestinal lumen. The passed fluid was collected, and 95% v/v ethanol was added to fill a total volume to 10 ml, followed by centrifugation at 12,000 rpm for 10 min. The supernatant was filtrated with a 0.45-μm membrane filter and then diluted with ethanol. Portal blood was heparinated and centrifuged at 12,000 rpm for 10 min. One hundred μl of sodium acetate buffer (100 mM, pH 5.0), 50 μl (1,667 units) of β-glucuronidase solution (Type H-5 From Helix pomatia, 654,200 units/g, Sigma-Aldrich, St. Louis, MO, USA), and 50 μl (8.3 units) of sulfatase solution (Type VIII, From Abalone Entrails, 29,000 units/g, Sigma-Aldrich) were added to 400 μl of plasma, and the mixture was incubated at 37 °C for 17 h. After incubation, 600 μl of acetic acid in methanol (0.83 M acetic acid) was added to the mixture, followed by agitation with a voltex for 0.5 min. This was followed by sonication for 30 s and centrifugation at 12,000 rpm for 5 min. The supernatant was subjected to LC-MS analysis. Feces were collected at 24 h after administration. Ethanol (95% v/v) was added to the feces to make a total volume of about 5 ml, followed by crushing into slurry form, which was then combined with 5-fold ethanol and boiled on a hot plate with agitation for 10 min. The uppermost layer was harvested. This extraction process was repeated for five sessions, and the pooled fractions were filtered. The total volume of the extract was adjusted to 1.8 ml by evaporation and the addition of ethanol, followed by centrifugation at 12,000 rpm for 10 min. The supernatant, passed through a 0.45-μm membrane filter, was subjected to LC-MS analysis. All these animal studies were carried out in accordance with the standard procedures for experimental animals (Notification no. 6, Prime Minister's Office, March 27, 1980, partially amended May 28, 2002).

The separation column used for LC-MS analysis was a reverse-phase semi-micro column (Semi-Micro Asahipak ODP-50 2D, ϕ2.0 mm, 150 mm). The mobile phase was water/acetonitrile and the flow rate was 0.2 ml/min. The LC-MS device (LCMS-2010EV, Shimadzu, Kyoto, Japan) setting was as follows: ionization mode APCI negative, vaporized nitrogen gas flow rate 2.5 l/min, CDL temperature 200 °C, APCI probe temperature 400 °C, and analysis was performed at selected ion monitoring of m/z 1,285.6 (M-V), m/z 1,123.6 (M-IV and S-I), m/z 962.5 (M-III), m/z 799.4 (M-II), m/z 637.4 (M-I), and m/z 518.4 (mogrol). Complete separation of standard SG triterpenoids without peak-overlapping was achieved when the percentage of acetonitrile in the mobile phase was 10–85% with a gradient curve setting of 1 during 30 min elution time and all standard SG triterpenoids were eluted at from 12.5 to 24 min (Fig. 2A).

We analyzed the distributions of M-V and its metabolites in the small intestine, and portal and whole blood after a single ingestion of SG-gly. Partial M-V was degraded to tetra- (S-I and M-IV) and triglucose (M-III) conjugated mogrosides in the small intestine (Table 1). We have reported that SG-gly suppressed postprandial blood glucose level after maltose administration.[10] An *in vitro* assay to assess maltase inhibitory activity revealed that M-III was much more active than M-V.[10] Because M-III is produced in the small intestine, our previous finding might be physiologically significant, and smaller digests such as M-II, M-I, and mogrol might also have similar functions. This remains to be elucidated.

SG triterpenoids in the free form were not detected in the portal blood plasma after administration of SG-gly (Fig. 2B). When portal blood extracts were analyzed after β-glucuronidase and sulfatase treatment, M-IE and mogrol were detected (Fig. 2C). On the other hand, no SG-triterpenoids were detected in the whole blood, either the free or the conjugated form (data not shown). Stevia is another plant-derived sweetener. Its main sweetness components, composed of diterpene aglycon (steviol) conjugated with glucoses, include stevioside (triglucosylated steviol) and rebaudioside A (tetraglucosylated steviol), both of which are metabolized to a steviol glucuronide in rats.[15] In a study on the absorption and metabolism of soybean isoflavones (genistein and daidzein), Piskula reported that when these substances were administered to rats, they were absorbed after hydrolysis and were detected in the form of glucuronic or sulfuric acid conjugates *in vivo*.[16] Furthermore, quercetin glycosides, one of the most abundant flavonoides found in plant foods, are converted to glucuronic and/or sulfate conjugates in small intestine.[17] Therefore, it is conceivable that SG triterpenoids such as M-IE and mogrol are converted to conjugated form in small intestine, which are then detected in the portal blood of rats.

In the feces after an oral dose of SG-gly, the excreted metabolites were mostly mogrol, M-IIA, and M-IE,

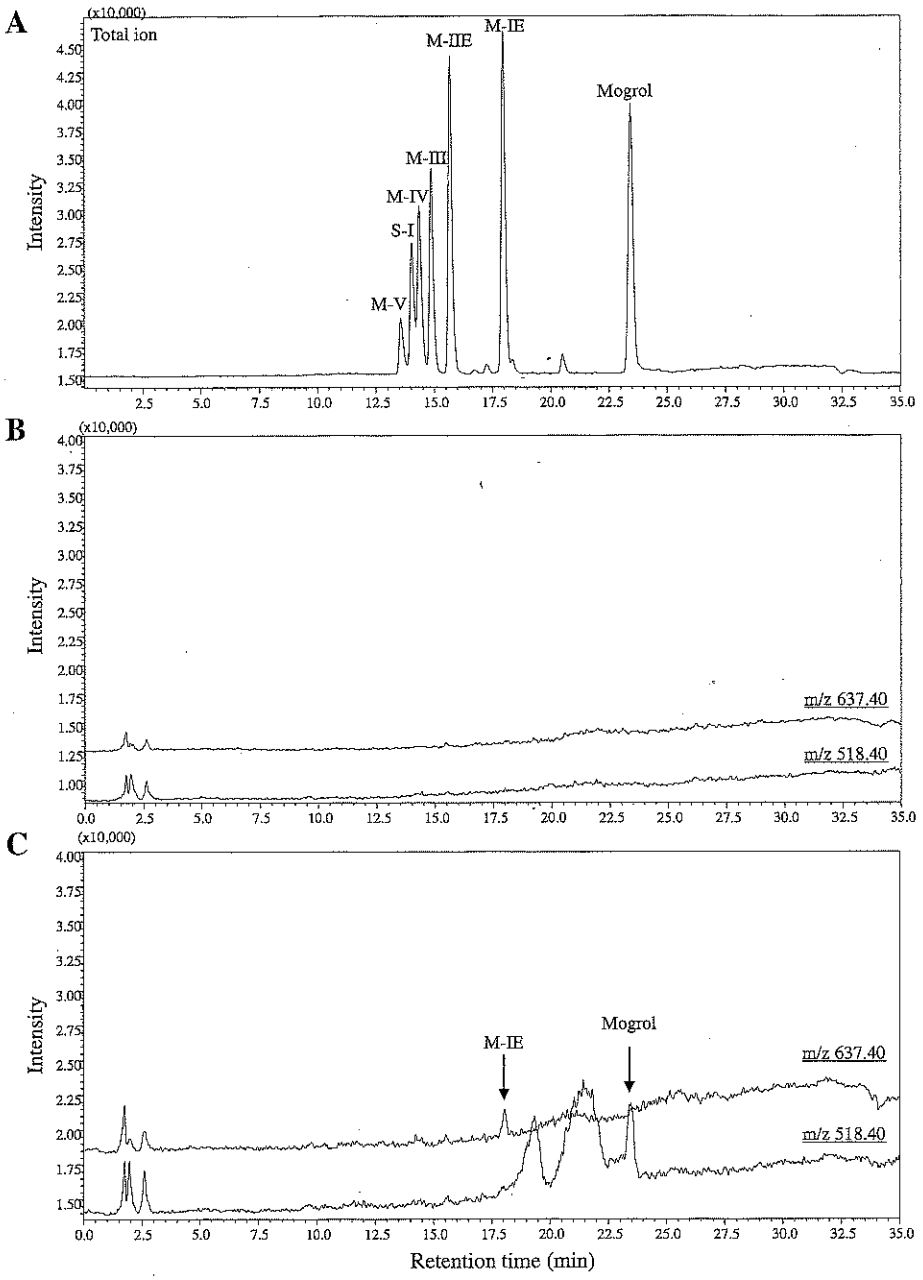


Fig. 2. Mass Chromatograms of SG Triterpenoid Analysis.
(A) standard SG triterpenoid mixture; (B), (C) portal blood plasma at 120 min after oral SG-gly ingestion; without enzyme treatment (B), with β-glucuronidase and sulfatase treatment (C).

Table 1. Quantification of M-V and Its Metabolites in the Small Intestine, Portal Blood Plasma, and Feces after a Single Oral Administration

	Small intestine[1] (μmol/ml)	Portal blood[1] (nmol/ml)	Feces[2] (μmol/d)
Mogroside V	2.70 ± 1.03	ND	0.13 ± 0.24
Siamenoside I	0.41 ± 0.22	ND	0.17 ± 0.19
Mogroside IV	0.41 ± 0.22	ND	0.18 ± 0.26
Mogroside III	0.19 ± 0.08	ND	0.63 ± 0.83
Mogroside IIE	0.03 ± 0.01	ND	0.13 ± 0.14
Mogroside IIA	ND	ND	11.46 ± 7.92
Mogroside IE	0.0003 ± 0.0003	0.07 ± 0.02	10.16 ± 0.17
Mogroside IA	ND	ND	0.01 ± 0.03
Mogrol	0.0003 ± 0.0001	0.36 ± 0.22	21.34 ± 12.25

[1]At 2 h and [2]24 h after a single oral administration.
ND, not detected.
Values are presented as the mean of 4–6 rats in each group with the standard deviation.

although all the identifiable SG triterpenoids were detected (Table 1). The total amount of mogrosides in the feces was about 40 μmol, which corresponds to 61% of administered M-V (65.5 μmol). On the other hand, no SG triterpenoids were detected in the urine, either in free or conjugated form (data not shown). Considering that no SG triterpenoids were detected in the whole blood or urine, the absorbed amount of SG-gly and its metabolites was extremely low, although mogrol and M-IE were absorbed in the portal vein. Therefore, most of the orally ingested M-V is excreted without absorption, and the reminder is likely to be degraded to unknown metabolites. A large amount of M-IIA was excreted in the feces but was not detected in small intestine. On the other hand, the M-IIE found in small intestine was almost equivalent to that in the feces (Table 1). These results suggest that M-IIA can be produced only by

intestinal microflora, while M-IIE can form only by enzymatic digestion in the small intestine.

In summary, SG-gly was mostly degraded by digestive enzymes and intestinal microflora, and was excreted in the feces as mogrol and its mono- and diglucosides after a single ingestion by rats, whereas a trace amount of mogrol and its monoglucoside were found in the portal blood as sulfates and/or glucuronides conjugates. Numerous pharmacological effects of SG-gly have been reported in animal models.[6–11] While further investigation is required to establish a precise knowledge of how SG-gly is metabolized *in vivo*, our results here suggest mogrol as a candidate that is responsible for various physiological functions of SG-gly. Thus, *S. grosvenori* appears to be a useful, noncaloric sugar substitute that has the added benefit of various pharmacologic actions. To clarify in more detail the pharmacologic actions of SG-gly, we are progressing toward an analysis of the metabolic pathway of mogrol.

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