JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Triterpene Glycosides of *Siraitia grosvenori* Inhibit Rat Intestinal Maltase and Suppress the Rise in Blood Glucose Level after a Single Oral Administration of Maltose in Rats

YASUSHI A. SUZUKI,*,† YUJI MURATA,† HIROSHI INUI,‡ MASAKI SUGIURA,†,‡ AND YOSHIHISA NAKANO‡

Biochemical Laboratory, Saraya Company, Ltd., Kashiwara, Osaka 582-0028, Japan, and Department of Applied Biological Chemistry, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan

The effect of the crude extract from *Siraitia grosvenori* Swingle (SG-ex) on the postprandial rise in blood glucose level was investigated. The increase in plasma glucose level in response to the oral administration of maltose was significantly suppressed in rats when SG-ex was given orally 3 min before the maltose administration. There was, however, no effect when glucose was administered instead, suggesting that the antihyperglycemic effect of SG-ex is elicited by inhibition of maltase in the small intestinal epithelium. In vitro, SG-ex inhibited rat small intestinal maltase. Similar effects were also observed both in vivo and in vitro when the concentrate of the sweet elements (triterpene glycosides) prepared from SG-ex was used. Furthermore, the main sweet element of SG-ex, mogroside V, and some minor elements such as mogroside IV, siamenoside I, and mogroside III also exhibited maltase inhibitory effect with IC_{50} values of 14, 12, 10, and 1.6 mM, respectively. These results suggest that SG-ex exerts anti-hyperglycemic effects in rats by inhibiting maltase activity and that these effects are at least partially exerted by its sweet elements, triterpene glycosides.

KEYWORDS: ***Siraitia grosvenori*; maltase; glycoside; rat; hypoglycemic; postprandial blood glucose**

INTRODUCTION

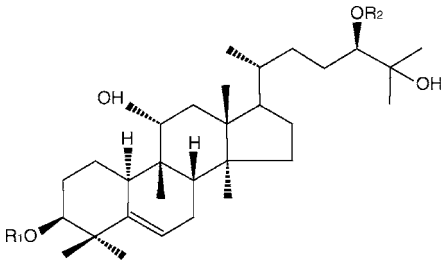
Siraitia grosvenori Swingle, a traditional Chinese fruit, belongs to Cucurbitaceous species and has been used as a folk medicine for sore throats, cough, and minor stomach and intestinal troubles. One of the most intriguing characteristics of this fruit is its unique sweetness. The extract from this fruit (SG-ex) has been reported to be ∼150 times sweeter than sucrose despite having minimal caloric content (*1*). Chemical compositions of the dry fruit have been intensively investigated, and three triterpene glycosides in which mogrol was involved as an aglycon were initially identified, namely, mogroside IV (M-IV), mogroside V (M-V), and mogroside VI (*2*). Two more sweet components, siamenoside I (S-I) and 11-oxomogroside V (11oxoV), were later identified (*3*). The relative sweetnesses of M-IV, M-V, S-I, and 11oxoV were 392, 425, 563, and 84 times as high as that of sucrose, respectively, but another triterpene glycoside, mogroside III (M-III), was tasteless (*4*). On the basis of these characteristics, the triterpene glycoside concentrate from SG-ex (SG-gly) is commercially utilized as a sweet component in sugar substitutes.

Physiological functions of SG-gly and its components have been paid more attention lately, and some interesting findings have been reported. For example, 11oxoV was found to have a strong inhibitory effect on low-density lipoprotein (LDL) oxidation and, thus, can reduce the atherogenic potential of LDL (*5*). In addition, SG-gly has been shown to have inhibitory effects on the initiation and promotion of cancer (*6*). We expected to find other physiological functions in *S. grosvenori* and thus looked for one related with its sweet characteristics. Among natural plants having a physiological function as food, there are many components revealed to exert suppression on the elevation of postprandial blood glucose levels by inhibiting small intestinal α-glucosidase, such as polyphenol in guava (*7*), catechin in tea (*8*), anthocyanin extracts from sweet potato, cabbage, and morning glory (*9*−*11*), and touchi in soybean (*12*−*15*). Furthermore, L-arabinose, an α-glucosidase (sucrase) inhibitor, has been proposed to be useful for preventing lipogenesis and obesity, in addition to the control of postprandial blood glucose levels (*16*, *17*). We thus hypothesized that *S. grosvenori* similarly has inhibitory effects on the elevation of postprandial blood glucose levels even though there is no direct implication from previous reports regarding *S. grosvenori*.

Diabetes mellitus is one of the most prevalent diseases in Japan. Poor control of this disease results in diabetogenic failures such as kidney dysfunction, retinopathy, and neuropathy (*18*). It is therefore important to prevent an excess postprandial rise of blood glucose level or to improve insulin resistance. Agents with α-glucosidase inhibitory activity have been useful as oral

* Author to whom correspondence should be addressed (telephone 81-729-77-8000; fax 81-729-77-2224; e-mail suzuki-y@saraya.com).
† Saraya Co. Ltd.
‡ Osaka Prefecture University.

10.1021/jf0478105 CCC: $30.25
Published on Web 03/25/2005



	R_1	R_2
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

Figure 1. Chemical structures of mogrosides isolated from the fruit of *S. grosvenori.*

hypoglycemic drugs for the control of hyperglycemia in patients with diabetes. These drugs inhibit the digestion of disaccharides, and thus the absorption of glucose, eliciting attenuated postprandial blood glucose levels. We here examined the inhibitory effect for the elevation of postprandial blood glucose levels and α-glucosidase inhibition profiles of *S. grosvenori* both in vivo and in vitro.

MATERIALS AND METHODS

Materials. SG-ex and SG-gly were prepared in Guilin S&T new tech company (Guilin, China). To obtain SG-ex, fresh fruits of *S. grosvenori* were crushed and boiled in water with a ratio of 2:3 (w/w) at 100 °C for 6 h. After centrifugation, the supernatant was concentrated at 70−80 °C under reduced pressure until soluble solids of a 64.0 °Brix paste, measured by refractometry at 20 °C. The supernatant after another centrifugation was utilized as SG-ex in our study. SG-ex was then diluted to 0.16 g/mL (soluble solids of ∼10 °Brix in water) and was applied to a reverse-phase column (gravity open column) equilibrated with 100% water to adsorb triterpene glycosides. The column was washed by water with a 10-fold excess of the bed volume. The glycosides fraction was then eluted with a 70% ethanol solution. Ethanol in the eluate was subsequently removed at 60−70 °C by vacuum concentration and dried with a spray dryer. The resultant was named SG-gly. Four glycosides, M-V, M-IV, S-I, and M-III, were then purified from SG-gly by repeated column chromatography as described previously (*3*, *4*). Rat intestinal acetone powder was purchased from Sigma (St. Louis, MO).

Analysis of Glycosides in *S. grosvenori*. Constituents of SG-ex and SG-gly were analyzed by HPLC. A Shimadzu Class-VP chromatograph coupled with a photodiode array UV detector was used for the analysis. The ODS column (Nucleosil 5-C18, ∅ 4.6 mm, length 150 mm) was equilibrated with acetonitrile/water (10:90, v/v), the flow rate was 1 mL/min, the column temperature was held at 40 °C, and the absorbance was monitored at 203 nm. The standards were M-V (98%), S-I (97%), M-IV (96%), and M-III (85%). The chemical structures of these glycosides are shown in **Figure 1** and were previously confirmed by ^{1}H NMR and IR (*19*, *20*). Twenty microliters of each standard (0.1−0.2 mM), SG-ex (1.0%) and SG-gly (0.12%), was subjected to HPLC analysis. After injection of the sample, the mobile phase was held at 10% acetonitrile for 10 min (to wash unbound materials in the crude sample), and then glycoside materials were eluted by linear gradient to 55% acetonitrile by 50 min. The amount of each glycoside in samples was calculated according to the area under the curve of the corresponding standard glycoside.

Animals. Five-week-old male Wistar strain rats (Clea Japan, Inc., Tokyo, Japan) were acclimatized for at least 1 week in the animal room prior to study. The room was maintained at a relative humidity of 55% and a temperature of 23 ± 2 °C under controlled lighting from 9:00 a.m. to 9:00 p.m. Rats were fed a laboratory diet, CE-2 (Clea Japan, Inc), and given water ad libitum. The animal treatments conformed to the Guidelines for Care and Use of Experiemtal Animals.

Oral Maltose and Glucose Tolerance Test. After overnight (16−20 h) food deprivation, SG-ex, SG-gly (0.1 g/kg of body weight in 500 μL of water), or water (500 μL for the control) was administered orally by direct stomach intubation (first administration). Either maltose or glucose solution in 1 mL of water (2 g/kg of body weight) was administered as the second administration at 3 min after the first administration. Blood samples were collected from the tail vein into heparinized tubes at 0 min (before the first administration), and 30, 60, 90, and 120 min after the second administration. Plasma was separated by centrifugation at 500*g* for 10 min at 4 °C. Plasma glucose levels were measured by a glucose oxidase method using a commercial kit (Glucose B-test; Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions.

Assay for Maltase and Sucrase Inhibitory Activities. The inhibitory activities for maltase and sucrase were assessed according to the procedure described elsewhere (*21*). The reaction was started by adding 20 μL of rat intestinal enzyme solution (2 mg/mL) to 80 μL of substrate solution (various concentrations of maltose or sucrose in 20 mM phosphate buffer at pH 7.0) with various amounts of inhibitors, followed by incubation at 37 °C for 10 min. The reaction was stopped by adding 150 μL of 0.1 N NaOH and then neutralized with 150 μL of 0.1 N acetic acid. The amount of glucose formed during the reaction was determined by a hexokinase/glucose 6-phosphate dehydrogenase method using a commercially available kit (D-glucose kit; R-Biopharm GmbH, Darmstadt, Germany) according to the manufacturer's instructions. Patterns of inhibition were assessed by drawing Lineweaver−Burk plots. Inhibitor concentrations for 50% inhibition (IC_{50}) were determined with 5 mM maltose for the maltase inhibition and with 20 mM sucrose for the sucrase inhibition by drawing Dixon plots. The competitive inhibition constant (K_{ic}) and the uncompetitive inhibition constant (K_{iu}) were determined by drawing Dixon plots and modified Dixon plots, respectively (*22*).

Statistical Analysis. Data on oral maltose and glucose tolerance tests were analyzed by two-way ANOVA for repeated measures, and multiple comparisons were done by Newman−Keuls method. These statistical analyses were performed with GB-Stat 5.4 (Dynamic Microsystems, Silver Spring, MD). Each result is expressed as the mean ± SD.

RESULTS

Effects of SG-ex and SG-gly on Postprandial Blood Glucose Levels in Rats. To test our hypothesis that *S. grosvenori* lowers postprandial blood glucose, the glycemic response after a single oral maltose ingestion was examined in Wistar rats. At 30, 60, and 90 min after the maltose administration, the rise of plasma glucose level was significantly suppressed in rats when SG-ex was given orally 3 min before the administration of the sugar (**Figure 2A**). It should be noted that the most effective inhibition was achieved at 30 min, when the rise in plasma glucose level was only ∼70% compared to control. This inhibitory effect was diminished gradually as time went on and reverted to nonsignificance at 120 min. When glucose was administrated orally, however, SG-ex did not have any inhibitory effects (**Figure 2B**), suggesting that the effect of SG-ex is elicited by inhibition of maltase in the small intestinal epithelium but not by inhibition of the glucose passage through the glucose transporters.

We made an assumption that SG-gly, the sweet element (triterpene glycosides) in SG-ex, is the major contributor for this effect. We thus analyzed concentrations of four sweet elements, M-V, S-I, M-IV, and M-III in SG-ex, and those in SG-gly (**Figure 3**). The retention times of M-V, S-I, M-IV, and M-III were 30.9, 31.9, 32.6, and 33.7 min, respectively (**Figure**

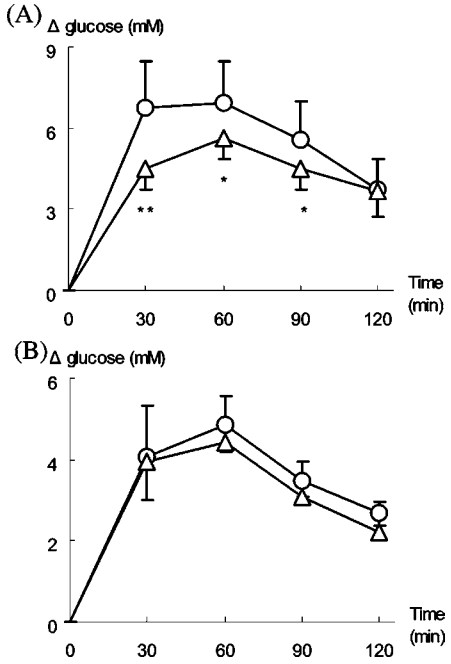


Figure 2. Effect of SG-ex (△) on plasma glucose levels after a single oral administration of maltose (**A**) or glucose (**B**) in male Wistar rats. Substrate solution (maltose or glucose, 2 g/kg of body weight, 1 mL) was administered at 3 min after intubation of SG-ex (0.1 g/kg of body weight, 0.5 mL) to the 16 h food-deprived rat. For the control (○), 0.5 mL of water without SG-ex was administered as a substitute for the inhibitor. Each plot represents the mean ± SD ($n = 7$; *, $P < 0.05$; **, $P < 0.01$).

3A,**D**). On the basis of the area under the curve (AUC) of each standard peak, concentrations of M-V, S-I, M-IV, and M-III in SG-ex are roughly estimated to be 2.1, 0.3, 0.8, and 0.7%, respectively (**Figure 3B**,**D**), whereas concentrations of these sweet elements in SG-gly are 30.9, 1.4, 1.6, and 0.8%, respectively (**Figure 3C**,**D**).

To test our second hypothesis that SG-gly is the major contributor to the hypoglycemic effect of SG-ex, we examined inhibitory effects of SG-gly on the rise of plasma glucose levels in the same manner as for SG-ex. The rise of plasma glucose level was significantly inhibited by SG-gly at 30, 60, and 90 min (**Figure 4A**), which is similar to the result we obtained with SG-ex. A glucose tolerance test was also performed to examine if this inhibitory effect by SG-gly is elicited by inhibition on the glucose transporters or not. When glucose was administered, postprandial blood glucose levels were identical between control and SG-gly-treated rats (**Figure 4B**). Thus, neither SG-ex nor SG-gly inhibited glucose absorption (**Figures 2B** and **4B**), suggesting that glucose transporter was not affected and that intestinal maltase was the target for both SG-ex and SG-gly to exert their inhibitory activities.

Maltase Inhibition by SG-gly and Its Components. On the basis of the in vivo study, we examined maltase inhibitory assay for SG-ex, SG-gly, and purified triterpene glycosides in vitro. All tested materials were found to inhibit rat intestinal maltase activity. The IC_{50} of SG-ex for 5 mM maltose was 14 ± 2 mg/mL, and that of SG-gly for 5 mM maltose was 5.0 ± 0.4 mg/mL (**Table 1**). IC_{50} values of the main constituents in SG-gly, M-V, M-IV, S-I, and M-III, for 5 mM maltose were also determined to be 18, 14, 11, and 1.6 mg/mL, respectively. The numbers of glucoses glycosylated to its frame structure (an aglycon named mogrol) for M-V, M-IV, S-I, and M-III are 5, 4, 4, and 3, respectively (**Figure 1**). It should be noted that the IC_{50} became gradually lower as the number of glucoses glycosylated became fewer.

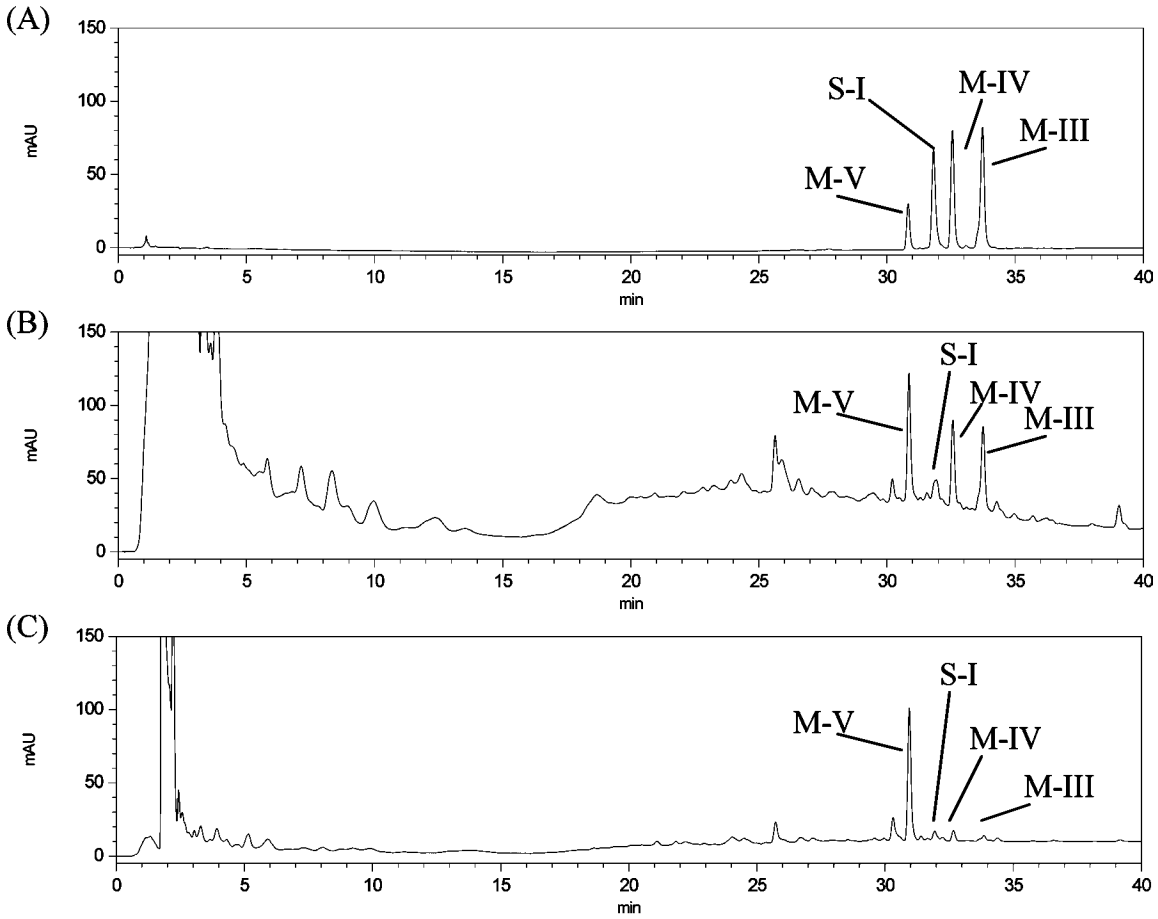
Constants (K_i) and patterns of maltase inhibition were determined for each inhibitor (**Table 2**). First, patterns of maltase inhibition were the mixed type of competitive and uncompetitive for all of the materials tested here. A representative Lineweaver−Burk plot to determine the type of maltase inhibition for M-III is shown in **Figure 5**. An intersection point of four linear lines was located in the second quadrant, indicating that the type of maltase inhibition by M-III is a mixed inhibition. We determined the K_i of competitive inhibition (K_{ic}) and the K_i of uncompetitive inhibition (K_{iu}) for each material according to the method described by Cortes et al. (*22*). All six materials exhibited the mixed inhibition with a predominant competitive component, which was indicated by the fact that K_{ic} is lower than K_{iu} (**Table 2**). These glycosides thus interact with maltase predominantly to inhibit its activities, rather than with the maltase−maltose complex, which, however, also occurs to some extent.

We also assessed sucrase inhibitory activities of SG-ex and SG-gly (**Table 1**). SG-gly showed sucrase inhibitory activity ($IC_{50} = 110 \pm 20$ mg/mL), but its potency is much lower than that of maltase inhibition ($IC_{50} = 5.0 \pm 0.4$ mg/mL). In addition, SG-ex did not inhibit sucrose hydrolysis but SG-gly did. A reason for this may be that the concentration of mogrosides in SG-ex is below the threshold to detect an inhibitory effect on sucrase in vitro. These results suggest that *S. grosvenori* inhibits primarily maltase but not sucrase, which is favorable because SG-gly (or SG-ex) can be used as a replacement for sucrose.

DISCUSSION

We here for the first time demonstrated that *S. grosvenori* has the activity of inhibiting hyperglycemia after maltose ingestion in rats, suggesting that SG-ex and SG-gly may be effective agents to manage diabetes mellitus or obesity. The most notable point of our finding is that SG-gly possesses an anti-hyperglycemic effect while displaying a unique characteristic as a natural sweetener. In fact, SG-ex and SG-gly are both commercially utilized as noncalorie substitutes for sugar (sucrose). It is now likely that they both have the added benefit of serving as a hypoglycemic agent. Among the many low-calorie natural sweeteners that are commercially available, stevioside (*23*) and glycyrrhizin (*24*) are examples of what has been reported to have antidiabetic effects. Stevioside appears to have a distinct mechanism of α-glucosidase inhibition: first, it must be absorbed into the circulation and then acts directly on β cells in the pancreas to stimulate insulin secretion (*23*, *25*, *26*). Antidiabetic effects of glycyrrhizin are also elicited by a distinct mechanism: it has been reported to inhibit glucose transporter and to have an antiglycemic effect when glucose is given (*24*). Although the mechanisms appear to be different, each sweetener would be beneficial for diabetic patients in two ways: its sweetness with less calories and its antidiabetic effect. SG-gly is known to have several hundred times the sweetness of table sugar (*4*), and therefore a minimum amount can provide preferable sweetness without offering significant caloric content. Thus, SG-gly may be a good candidate as a sweet ingredient, providing beneficial effects to diabetes and obesity. Because SG-gly has a different mechanism from the other two natural sweeteners for controlling the blood glucose level properly, additive effects can be expected when the two of them are utilized simultaneously.

There are many natural resources with the α-glucosidase inhibitory activity. Some of them are more specific for sucrase



(D) Concentration of glycosides in SG-gly and in SG-ex

retention time (min)	ID	SG-ex (%)	SG-gly (%)
30.9	M-V	2.1	30.9
31.9	S-I	0.3	1.4
32.6	M-IV	0.8	1.6
33.7	M-III	0.7	0.8

Figure 3. HPLC analysis of SG-ex and SG-gly: chromatogram chart of (**A**) standard mixture of M-V (1.3 μg), S-I (2.2 μg), M-IV (2.2 μg), and M-III (1.9 μg); (**B**) SG-ex (180 μg); (**C**) SG-gly (12 μg). (**D**) Contents of each glycoside (w/w %) were determined by the AUC (area under curve) ratio of corresponding peaks (AUC of sample/AUC of the standard).

inhibition rather than maltase inhibition, such as catechin and the touchi extract. The touchi extract, a traditional Chinese food derived from fermented soybean, is an example having sucrase inhibitory effects (*12*). Its antiglycemic effect after a single oral treatment of sucrose is effective both in rats and in humans (*12*). Rats and humans do not always respond similarly, however. It is thus noteworthy that in the case of the touchi extract, the sucrase inhibitory activity observed in rats was applicable to humans. Although *S. grosvenori* acts mainly on the maltase−maltose system, which is different from the touchi extract, it could be conceivable for *S. grosvenori* to exert a similar antiglycemic effect in humans.

Attenuating the postprandial rise of blood glucose level is believed to be an effective means to manage diabetes mellitus, in particular non-insulin-dependent type 2 diabetes mellitus. The touchi extract has proven to be effective for borderline and mild type 2 diabetes in humans (*14*). Long-term administration of the touchi extract on a type 2 diabetic mice model has also indicated its beneficial antidiabetic effect (*15*). These studies suggest that preventing an excessive postprandial rise of blood glucose level by an α-glucosidase inhibitor from natural resources is effective in real life as well. It is therefore possible that long-term administration of SG-ex and/or SG-gly may have similar effects on borderline or mild type 2 diabetes by preventing an excessive postprandial rise of blood glucose level.

The competitive inhibition is the most common type of α-glucosidase inhibitions, and sucrase inhibition is a more common type than maltase inhibition. Both SG-ex and SG-gly effectively inhibit maltase and the rise of blood glucose level in rats after maltose administration, but do not inhibit sucrase. In practice, inhibiting maltase would be more preferable than inhibiting sucrase because carbohydrates derived from general food are mostly made from maltose and some isomaltose and because sucrose would be the first material restricted for type 2 diabetic patients, who would be thus less likely to take too much sucrose by themselves. In particular, because of its sweetness with high intensity, SG-gly or SG-ex can be used as a replacement for sucrose. Considering these unique properties,

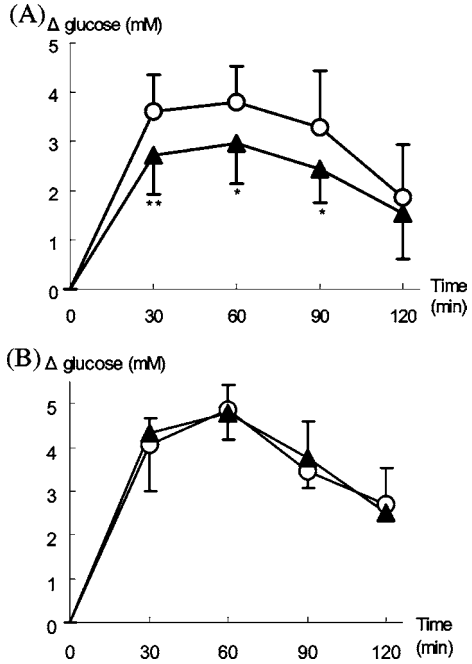


Figure 4. Effect of SG-gly (▲) on blood glucose levels after a single oral administration of maltose, $n = 10$ (**A**), or glucose, $n = 7$ (**B**), in male Wister rats. Substrate solution (maltose or glucose, 2 g/kg of body weight, 1 mL) was administered at 3 min after intubation of SG-gly (0.1 g/kg of body weight, 0.5 mL) to the 16 h food-deprived rat. For the control (○), 0.5 mL of water without SG-gly was administered as a substitute for the inhibitor. Each plot represents the mean ± SD (*, $P < 0.05$; **, $P < 0.01$.)

Table 1. Inhibitor Concentrations for 50% Inhibition (IC_{50})

	substrate		
	maltose (5 mM)		sucrose (20 mM)
inhibitor	mg/mL	mM	mg/mL
SG-ex	14 ± 2		NI[a]
SG-gly	5.0 ± 0.4		110 ± 20
M-V	18 ± 4	14 ± 3	ND[b]
M-IV	14 ± 7	12 ± 6	ND
S-I	11 ± 2	10 ± 2	ND
M-III	1.6 ± 0.2	1.6 ± 0.2	ND

[a] No inhibition. [b] Not determined.

Table 2. Inhibition Constant for Maltase

	K_{ic}		K_{iu}	
inhibitor	mg/mL	mM	mg/mL	mM
SG-ex	9.3 ± 1.5		61 ± 20	
SG-gly	3.4 ± 1.1		16 ± 6	
M-V	16 ± 3	13 ± 2	22 ± 2	17 ± 1
M-IV	12 ± 6	11 ± 5	23 ± 8	20 ± 7
S-I	5.5 ± 1.2	4.9 ± 1.1	17 ± 3	15 ± 2
M-III	1.5 ± 0.5	1.5 ± 0.5	1.6 ± 0.2	1.7 ± 0.2

sucrase inhibitions by SG-ex or SG-gly are not needed necessarily. The maltase inhibition pattern by SG-gly and by its constituents that we observed in this study consists of both competitive and uncompetitive inhibitions. This could be beneficial because the range of actions will be wider than just one type of inhibition; for example, SG-gly may be used simultaneously with most other α-glucosidase inhibitors.

We performed an oral maltose tolerance test with both SG-ex (0.1 g/kg of body weight) and SG-gly (0.1 g/kg of body weight) as inhibitors, and the inhibitory effects were similar

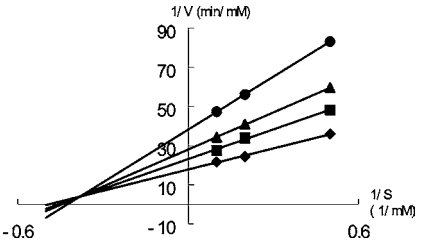


Figure 5. Double-reciprocal plots of the maltase activity against the maltose concentration in the presence of varied concentrations of M-III: (●) 4 mM; (▲) 3 mM; (■) 2 mM; (◆) 1 mM.

(**Figures 2A** and **4A**). If SG-gly is the only or major material having a hypoglycemic effect in SG-ex, SG-gly would have a more potent effect on inhibiting postprandial blood glucose than SG-ex. Therefore, SG-gly is less likely to be fully responsible for inhibiting the elevation of postprandial blood glucose that we observed as an effect of SG-ex administration, but does at least partly explain the effect by SG-ex.

The contribution ratio (CR) of the purified glycoside for the IC_{50} of *S. grosvenori*, both SG-ex and SG-gly, was defined for convenience by the following equation.

$$\mathrm{CR} = \frac{(IC_{50}\ \text{of SG}) \times (\text{concentration of purified glycoside in SG})}{IC_{50}\ \text{of purified glycoside}}$$

In SG-ex, the CR of M-III was 6.1%, which is the highest compared to the other three glycosides, but in SG-gly, the CR of M-V was 8.6%, and it was the most potent contributor. The total CR, which is determined by the sum of the CRs, was only around 9% in SG-ex and 12% in SG-gly. This indicates the existence of some unknown components that potently inhibit maltase activity. These components should exist in both SG-gly and SG-ex, which remains to be elucidated.

Recently, a diacylated anthocyanin from *Ipomoea batatas* has been shown to inhibit exclusively maltase activity in a noncompetitive manner (*27*), that is, the typical mixed type ($K_{ic} = K_{iu}$). The target (maltase) and type of inhibition for this anthocyanin are similar to those for SG-ex or SG-gly, making it suitable to compare them. The IC_{50} of isolated anthocyanins from *I. batatas* for maltase inhibition was 0.25 mg/mL (*7*), whereas that of SG-gly was 5.0 mg/mL in this study, and the *Ipomoea* extract (100 mg/kg of body weight) showed an antiglycemic effect in rats similar to that found in this study (*8*). In our study, 100 mg/kg of body weight of SG-gly was effective. The anti-hyperglycemic effect of SG-gly therefore seems to be much more effective in vivo than what we would expect from the in vitro data of IC_{50}. Thus, the maltase inhibitory activity of SG-gly does not fully explain the entire antiglycemic effect by its original constituents. We demonstrated that K_{ic}, K_{iu}, and IC_{50} became gradually lower as the number of glucoses glycosylated became fewer. This seems to imply that when M-V is hydrolyzed in the gut, M-IV, S-I, and M-III will be generated, and these digests will effectively inhibit α-glucosidase activities. Although there is no appreciable peak for mogroside I (M-I), mogroside II (M-II), or aglycon (mogrol) in the HPLC chart of either SG-ex or SG-gly, which should appear at a later retention time than that for M-III (**Figure 3**), those smaller mogrosides or mogrol may form by hydrolysis in the gut and exert a more potent inhibitory effect on maltase. It stands to reason that both SG-ex and SG-gly are more effective in vivo than in vitro. Acid hydrolysis of SG-gly was found to produce the aglycon of mogroside V (confirmed by LC-MS) and, thus, we attempted

to test maltase/sucrase inhibitory activities with partially purified aglycon (80% purity) in vitro. We did not find any inhibitory activities, suggesting that at least one glucose glycosylation to mogrol is required to exert α-glucosidase inhibition. Although mogrol was eliminated as an active ingredient for inhibiting α-glucosidase activity by SG-gly, the activities of M-I and M-II remain to be explored.

In summary, SG-ex and SG-gly exhibited strong inhibitory effects on the elevation of postprandial blood glucose levels after a single oral administration of maltose in rats. SG-ex, SG-gly, and isolated glycosides have maltase inhibitory effects in vitro, which are likely to be responsible for the antiglycemic activity observed in vivo. Therefore, *S. grosvenori* appears to be a useful, noncaloric sugar substitute that has the added benefit of attenuating postprandial glycemia through an inhibitory mechanism on maltase activity.

ABBREVIATIONS USED

SG-ex, extract of *Siraitia grosvenori* fruit; SG-gly, the triterpene glycoside concentrate from SG-ex; M-V, mogroside V; M-IV, mogroside IV; M-III, mogroside III; S-I, siamenoside I.

ACKNOWLEDGMENT

We gratefully acknowledge the technical advice of Dr. Tetsuya Adachi. We appreciate the help of Mayuko Tomoda with animal experiments.

LITERATURE CITED

(1) Lee, C. Intense sweetener from lo han kuo (*Momordica grosvenori*). *Experientia* **1975**, *31*, 533−534.

(2) Takemoto, T.; Arihara, S.; Nakajima, T.; Okuhira, M. Studies on the constituents of *Fructus momordicae*. I. On the sweet principle. *Yakugaku Zasshi* **1983**, *11*, 1151−1154.

(3) Kasai, R.; Nie, R. L.; Nashi, K.; Ohtani, K.; Zhou, J.; Tao, G. D.; Tanaka, O. Sweet cucurbitane glycosides from fruits of *Siraitia siamensis* (chi-zi luo-han-guo), a Chinese folk medicine. *Agric. Biol. Chem.* **1989**, *53*, 3347−3349.

(4) Matsumoto, K.; Kasai, R.; Ohtani, K.; Tanaka, O. Minor cucurbitane-glycosides from fruits of *Siraitia grosvenori* (Cucurbitaceae). *Chem. Pharm. Bull.* **1990**, *38*, 2030−2032.

(5) Takeo, E.; Yoshida, H.; Tada, N.; Shingu, T.; Matsuura, H.; Murata, Y.; Yoshikawa, S.; Ishikawa, T.; Nakamura, H.; Ohsuzu, F.; Kohda, H. Sweet elements of *Siraitia grosvenori* inhibit oxidative modification of low-density lipoprotein. *J. Atheroscler. Thromb.* **2002**, *9*, 114−120.

(6) Takasaki, M.; Konoshima, T.; Murata, Y.; Sugiura, M.; Nishino, H.; Tokuda, H.; Matsumoto, K.; Kasai, R.; Yamasaki, K. Anticarcinogenic activity of natural sweeteners, cucurbitane glycosides, from *Momordica grosvenori. Cancer Lett.* **2003**, *198*, 37−42.

(7) Yang, R. S.; Cheng, J. T. Hypoglycemic effect of guava juice in mice and human subjects. *Am. J. Chin. Med.* **1983**, *11*, 74−76.

(8) Matsumoto, N.; Ishigaki, F.; Ishigaki, A.; Iwashina, H.; Hara, Y. Reduction of blood glucose levels by tea catechin. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 525−527.

(9) Matsui, T.; Ueda, T.; Oki, T.; Sugita, K.; Terahara, N.; Matsumoto, K. α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. *J. Agric. Food Chem.* **2001**, *49*, 1948−1951.

(10) Matsui, T.; Ueda, T.; Oki, T.; Sugita, K.; Terahara, N.; Matsumoto, K. α-Glucosidase inhibitory action of natural acylated anthocyanins. 2. *J. Agric. Food Chem.* **2001**, *49*, 1952−1956.

(11) Matsui, T.; Ebuchi, S.; Kobayashi, M.; Fukui, K.; Sugita, K.; Terahara, N.; Matsumoto, K. Anti-hyperglycemic effect of diacylated anthocyanin derived from *Ipomoea batatas* cultivar Ayamurasaki can be achieved through the α-glucosidase inhibitory action. *J. Agric. Food Chem.* **2002**, *50*, 7244−7248.

(12) Fujita, H.; Yamagami, T.; Ohshima, K. Fermented soybean derived water-soluble Touchi extract inhibits α-glucosidase and is antiglycemic in rats and humans after single oral treatments. *J. Nutr.* **2001**, *131*, 1211−1213.

(13) Fujita, H.; Yamagami, T.; Ohshima, K. Efficacy and safety of Touchi extract, an α-glucosidase inhibitor derived from fermented soybeans, in non-insulin-dependent diabetic mellitus. *J. Nutr. Biochem.* **2001**, *12*, 351−356.

(14) Fujita, H.; Yamagami, T.; Ohshima, K. Long-term ingestion of a fermented soybean-derived Touchi-extract with α-glucosidase inhibitory activity is safe and effective in humans with borderline and mild type-2 diabetes. *J. Nutr.* **2001**, *131*, 2105−2108.

(15) Fujita, H.; Yamagami, T. Fermented soybean-derived Touchi-extract with anti-diabetic effect via α-glucosidase inhibitory action in a long-term administration study with KKA^y mice. *Life Sci.* **2001**, *70*, 219−227.

(16) Seri, K.; Sanai, K.; Matsuo, N.; Kawakubo, K.; Xue, C.; Inoue, S. L-Arabinose selectively inhibits intestinal sucrase in an uncompetitive manner and suppresses glycemic response after sucrose ingestion in animals. *Metabolism* **1996**, *45*, 1368−1374.

(17) Osaki, S.; Kimura, T.; Sugimoto, T.; Hizukuri, S.; Iritani, N. L-Arabinose feeding prevents increases due to dietary sucrose in lipogenic enzymes and triacylglycerol levels in rats. *J. Nutr.* **2001**, *131*, 796−799.

(18) Toeller, M. α-glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. *Eur. J. Clin. Invest.* **1994**, *24*, 31−35.

(19) Takemoto, T.; Arihara, S.; Nakajima, T.; Okuhira, M. Studies on the constituents of *Fructus momordicae*. II. Structure of sapogenin. *Yakugaku Zasshi* **1983**, *11*, 1155−1166.

(20) Takemoto, T.; Arihara, S.; Nakajima, T.; Okuhira, M. Studies on the constituents of *Fructus momordicae*. III. Structure of mogrosides. *Yakugaku Zasshi* **1983**, *11*, 1167−1173.

(21) Honda, M.; Hara, Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 123−124.

(22) Cortes, A.; Cascante, M.; Cardenas, M.; Cornish-Bowden, A. Relationships between inhibition constants, inhibitor concentrations for 50% inhibition and types of inhibition: new ways of analysing data. *Biochem. J.* **2001**, *357*, 263−268.

(23) Jeppensen, P. B.; Gregersen, S.; Poulsen, C. R.; Hermansen, K. Stevioside acts directly on pancreatic β cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K^+-channel activity. *Metabolism* **2000**, *49*, 208−214.

(24) Takii, H.; Matsumoto, K.; Kometani, T.; Okada, S.; Fushiki, T. Lowering effect of phenolic glycosides on the rise in postprandial glucose in mice. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1531−1535.

(25) Jeppensen, P. B.; Gregersen, S.; Alstrup, K. K.; Hermansen, K. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. *Phytomedicine* **2002**, *9*, 9−14.

(26) Jeppensen, P. B.; Gregersen, S.; Rolfsen, S. E. D.; Jepsen, M.; Colombo, M.; Agger, A.; Xiao, J.; Kruhoffer, M.; Orntoft, T.; Hermansen, K. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki Rat. *Metabolism* **2003**, *52*, 372−378.

(27) Matsui, T.; Ebuchi, S.; Fukui, K.; Matsugano, K.; Terahara, N.; Matsumoto, K. Caffeoylsophorose, a new natural α-glucosidase inhibitor, from red vinegar by fermented purple-fleshed sweet potato. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2239−2246.

Received for review December 27, 2004. Revised manuscript received February 17, 2005. Accepted February 22, 2005.

JF0478105