Structures of Steroidal Alkaloid Oligoglycosides, Robeneosides A and B, and Antidiabetogenic Constituents from the Brazilian Medicinal Plant *Solanum lycocarpum*

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In the course of our studies on antidiabetogenic compounds from natural medicines and medicinal foodstuffs, a methanolic extract of the fruits of *Solanum lycocarpum* showed an inhibitory effect on the increase of serum glucose levels in oral sucrose-loaded rats. Through bioassay-guided separation, three known steroidal alkaloid oligoglycosides, solamargine (**1**), solasonine (**3**), and 12-hydroxysolasonine (**5**), were isolated from the active fraction together with two new steroidal alkaloid oligoglycosides, robeneosides A (**2**) and B (**4**). The major constituents, solamargine (**1**) and solasonine (**3**), exhibited an inhibitory effect on the increase of serum glucose levels in oral sucrose-loaded rats. In addition, these compounds suppressed gastric emptying in mice. However, this methanolic extract from *S. lycocarpum* fruits did not have any effect on intestinal α-glucosidase (sucrase and maltase) in vitro. It was concluded that these steroidal alkaloid oligoglycosides, **1** and **3**, inhibited the increase of rat serum glucose levels by suppressing the transfer of sucrose from the stomach to the small intestine.

The fruits of *Solanum lycocarpum* St.-Hil. (Solanaceae) are widely distributed in various parts of Brazil. The fruits of this plant are popularly called "fruta-de-lobo" (wolf-fruit) and are used as a folk medicine for the treatment of diabetes and obesity and to decrease cholesterol levels.[1−4] This species has been reported also to possess sedative, diuretic, antiepileptic, and antispasmodic activities.[1−4] Perez et al. reported that *S. lycocarpum* extract at a high dose (1000 mg/kg/day) reduced glycemia in alloxan-induced diabetic rats.[3] On the other hand, Oliveira et al. demonstrated that the starch obtained from the fruits of *S. lycocarpum* did not show hypoglycemic effects in mice.[4] In the course of our studies on antidiabetogenic compounds from natural medicines[5,6] and medicinal foodstuffs,[7] it was found that the methanolic extract from the fruit of *S. lycocarpum* inhibited the increase of serum glucose levels in sucrose-loaded rats without affecting intestinal α-glucosidase (IC_{50} > 400 μg/mL), as shown in Table 1.[5,8] By bioassay-guided separation, three known steroidal alkaloid oligoglycosides, solamargine (**1**),[9−11] solasonine (**3**),[9,10] and 12-hydroxysolasonine (**5**),[10] and the new steroidal alkaloid oligoglycosides robeneosides A (**2**) and B (**4**) were isolated from this MeOH-eluted fraction. The present paper deals with the inhibitory effects of the two principal known oligoglycosides, **1** and **3**, on serum glucose levels in oral sucrose-loaded rats as well as the isolation and structural elucidation of two new oligoglycosides, **2** and **4**. In addition, the steroidal alkaloid oligoglycosides **1** and **3** were found to show inhibitory effects on gastric emptying time in mice.

Results and Discussion

The dried fruits of *S. lycocarpum* were extracted with MeOH three times under reflux for 3 h. The MeOH extract (13.8%) was subjected to Diaion HP-20 column chromatography (H_2O → MeOH → acetone) to give a H_2O-eluted fraction (10.01%), a MeOH-eluted fraction (3.08%), and an acetone-eluted fraction (0.44%). As shown in Table 2, the MeOH-eluted fraction showed a significant inhibitory effect on serum glucose levels in oral sucrose-loaded rats. This MeOH-eluted fraction was then subjected to normal-phase silica gel [$CHCl_3$−MeOH → $CHCl_3$−MeOH−H_2O → MeOH] and reversed-phase silica gel (ODS) open column chromatography [MeOH−H_2O → MeOH], and finally HPLC [MeOH−H_2O], to give **1**[9−11] (0.42%), **2** (0.008%), **3**[9,10] (0.67%), **4** (0.0005%), and **5**[10] (0.0009%). The known compounds (**1**, **3**, and **5**) were identified by comparison of their physical data (^1H NMR, ^{13}C NMR, MS) with reported values.[9−11]

Robeneoside A (**2**) was isolated as a white powder, mp 205−208 °C (from $CHCl_3$−MeOH), with a negative optical rotation ($[\alpha]_D^{29}$ −112.2 in pyridine). In the positive- and negative-ion FABMS of **2**, quasimolecular ion peaks were observed at *m*/*z* 906 $[M + Na]^+$ and 882 $[M - H]^-$, respectively. HRFABMS analysis revealed the molecular formula of **2** to be $C_{45}H_{73}NO_{16}$. The IR spectrum of **2** showed strong absorption bands at 3450, 1071, and 1046 cm^{-1}, suggestive of a glycosidic moiety.[12,13] Acid hydrolysis of **2** with 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[14−19] The ^1H and ^{13}C NMR (Table 2) spectra of **2**, which were assigned by various NMR experiments,[20] showed signals assignable to four methyls [δ 0.82 (3H, d, $J = 5.2$ Hz, H_3-27), 0.99 (3H, s, H_3-18), 1.10 (3H, s, H_3-19), 1.18 (3H, d, $J = 7.2$ Hz, H_3-21)], three methines bearing an oxygen function [δ 3.85 (1H, m, H-3), 3.98 (1H, brs, H-12), 4.58 (1H, brd, $J \approx 8$ Hz, H-16)], an olefinic proton [δ 5.34 (1H, d, $J = 4.9$ Hz, H-6)] in the aglycon moiety, and three glycopyranosyl units {a β-D-glucopyranosyl [δ 4.93 (1H, d, $J = 7.2$ Hz, H-1′)], two α-L-rhamnopyranosyl [δ 1.77 (3H, d, $J = 6.3$ Hz, H-6″), 1.64 (3H, d, $J = 6.0$ Hz, H-6‴), 6.40 (1H, brs, H-1″), 5.86 (1H, brs, H-1‴)]}. The planar structure in **2** was determined by a detailed HMBC NMR experiment. Thus, long-range correlations were observed between the following protons and carbons: H-6 and C-4, 7, 8, 10; H-12 and C-9, 13, 14, 18; H-17 and C-13, 16, 18, 20, 21; H_3-18 and C-12, 13, 14, 17; H_3-19 and C-1, 5, 9, 10; H_3-21 and C-17, 20, 22; H_3-27 and C-24, 25, 26; H-1′ and C-3; H-1″ and C-2′; and H-1‴ and C-4′ (Figure 1). Next, the relative stereochemistry of **2** except for the 22-position was characterized by a ROESY experiment, which showed correlations between the following proton pairs: H-3 and H-1α, H-4α; H-19 and H-1β, H-4β; H_3-18 and H-8, H-12, H-20; and H-17 and H-14, H-16, H_3-21. The configuration at the C-22 position of **2** was characterized by comparison of the 23-methylene carbon signal of **2** in the ^{13}C NMR (pyridine-d_5) spectrum with those of known compounds having a spirosol-5-ene unit.[11] The ^{13}C NMR signal (δ 27.0) of xylosyl-β-solamargine,[21] having

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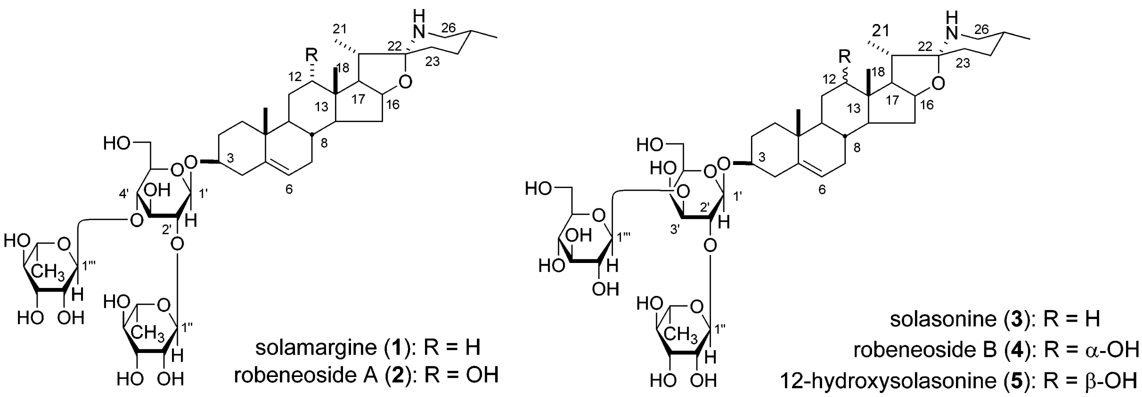
Table 1. Effects of the MeOH Extract of *Solanum lycocarpum* and Its MeOH-, H_2O-, and Acetone-Eluted Fractions on the Increase of Serum Glucose Levels in Sucrose-Loaded Rats

			serum glucose levels (mg/dL)		
treatment[a]	dose (mg/kg, po)	*n*	0.5 h	1.0 h	2.0 h
normal		6	84.7 ± 4.6**	88.8 ± 3.2**	82.8 ± 2.6*
control		6	168.9 ± 7.0	141.4 ± 5.0	113.8 ± 3.2
MeOH ext.	250	6	131.0 ± 7.4**	123.4 ± 4.1	121.0 ± 2.4
control		6	173.8 ± 3.3	141.0 ± 3.7	110.6 ± 2.9
H_2O-eluted fraction	100	7	166.3 ± 1.9	136.4 ± 2.5	115.4 ± 2.6
MeOH-eluted fraction	50	5	157.6 ± 10.5	137.9 ± 5.8	116.9 ± 4.3
	100	7	135.6 ± 1.8**	121.5 ± 3.2**	126.1 ± 4.4*
acetone-eluted fractions	100	7	162.7 ± 6.1	142.5 ± 2.0	121.2 ± 3.1

[a] Test samples were given orally to fasted rats 30 min before administration of 20% sucrose. The acacia solution was given orally 30 min before administration of water in normal group or before administration of sucrose in control group. Blood samples were collected 0.5, 1, and 2 h after sucrose loading. Values represent the means ± SEM. Significantly different from the control group, $*p < 0.05$, $**p < 0.01$.

Chart 1



the 22βN configuration, was shifted upfield relative to that (δ 34.7) of xylosyl-σ-solamargine,[21] having the 22αN configuration, as a result of the strong γ interaction of the 21α-methyl group. The C-23 signal of **2** was observed at δ 34.4, so that the C-22 configuration of **2** was determined to be in the αN form. Furthermore, in the ROESY experiment on **2**, no ROE correlation was observed between H_3-21 and H_2-23. On the basis of these results, the configuration of the spiroketal position in **2** was elucidated to be in the 22α*N*-form, similar to those of **1**, **3**, and **5**. These findings and comparison of the ^1H NMR and ^{13}C NMR spectra of **2** with those of **1**,[9−11] **3**,[9,10] and **5**[10] led us to formulate the structure of robeneoside A (**2**) as (25*R*)-12α-hydroxy-3β-{*O*-α-L-rhamnopyranosyl(1→2)-[*O-a*-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyloxy}-22α*N*-spirosol-5-ene.[22,23]

Table 2. ^{13}C NMR (125 MHz, pyridine-d_5) Data of Robeneosides A (**2**) and B (**4**)

carbon	**2**	**4**	carbon	**2**	**4**
1	37.4	37.5		Glc	Gal
2	30.2	30.2	1′	100.2	100.5
3	78.1	77.7	2′	78.0	76.3
4	39.0	39.0	3′	77.8	85.0
5	140.9	141.2	4′	78.6	70.3
6	122.0	121.9	5′	76.9	75.0
7	32.4	32.5	6′	61.3	62.5
8	32.0	32.1			
9	44.5	44.5		Rha	Rha
10	37.0	37.0	1″	102.0	102.1
11	29.4	29.4	2″	72.6	72.5
12	71.4	71.7	3″	72.8	72.8
13	45.3	45.3	4″	74.2	74.2
14	48.2	48.2	5″	69.5	69.4
15	32.7	32.7	6″	18.7	18.6
16	79.2	78.8			
17	54.2	54.2		Rha	Glc
18	17.3	17.3	1‴	103.0	105.7
19	19.3	19.3	2‴	72.6	75.0
20	42.0	42.0	3‴	72.7	78.5
21	15.6	15.5	4‴	73.9	71.6
22	98.5	98.2	5‴	70.4	78.3
23	34.5	34.5	6‴	18.5	62.7
24	30.8	30.8			
25	31.3	31.3			
26	47.9	48.0			
27	19.7	19.6			

Robeneoside B (**4**) was isolated as a white powder, mp 206−210 °C (from $CHCl_3$−MeOH) with a negative optical rotation ($[\alpha]_D^{29}$ −22.2 in pyridine). In the positive- and negative-ion FABMS of **4**, quasimolecular ion peaks were observed at *m/z* 922 $[M + Na]^+$ and 898 $[M - H]^-$, respectively. HRFABMS analysis revealed the molecular formula of **4** to be $C_{45}H_{73}NO_{17}$. The IR spectrum of **4** showed strong absorption bands at 3450, 1071, and 1046 cm^{-1}, suggestive of a glycosidic moiety.[12,13] Acid hydrolysis of **4** with 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v) liberated D-galactose, D-glucose, and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[14−19] The proton and carbon signals due to the steroidal aglycon part of **4** in the ^1H and ^{13}C NMR (Table 2) spectra of **4** were superimposable on those of **2**, whereas the signals due to the saccharide part were similar to those of **3**. The ^1H and ^{13}C NMR spectra of **4**, which were assigned by various NMR experiments,[20] showed signals assignable to four methyls [δ 0.81 (3H, brs, H_3-27), 0.97 (3H, s, H_3-18), 1.09 (3H, s, H_3-19), 1.20 (3H, brs, H_3-21)], three methines bearing an oxygen function [δ 3.96 (1H each, m, H-3, H-12), 4.60 (1H, m, H-16)], an olefinic

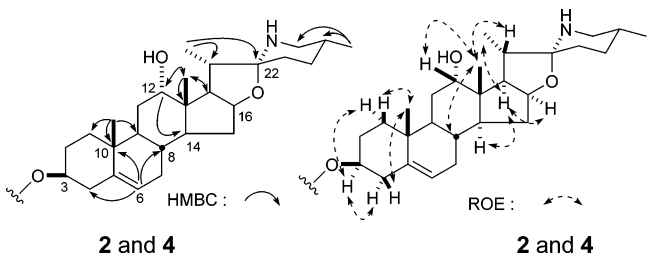


Figure 1. Selected HMBC and ROESY correlations.

Table 3. Effects of Solamargine (**1**) and Solasonine (**3**) on Increase in Serum Glucose Levels in Sucrose-Loaded Rats

treatment[a]	dose (mg/kg, po)	*n*	serum glucose levels (mg/dL) 0.5 h	1.0 h	2.0 h
normal		15	82.3 ± 2.3**	86.1 ± 3.0**	88.2 ± 2.7**
control		18	167.8 ± 3.1	148.1 ± 3.0	123.2 ± 2.9
solamargine (**1**)	25	5	141.7 ± 8.0**	139.9 ± 4.1	133.7 ± 1.6
	50	10	124.3 ± 5.7**	117.1 ± 3.9**	118.7 ± 3.4
	100	5	118.5 ± 5.6**	116.6 ± 4.7**	122.3 ± 7.0
solasonine (**3**)	25	5	158.8 ± 7.2	157.6 ± 7.8	129.5 ± 1.7
	50	10	132.7 ± 4.8**	136.7 ± 4.8	135.4 ± 2.5*
	100	5	124.7 ± 1.3**	122.4 ± 6.6**	121.9 ± 4.7
normal		6	81.4 ± 3.3**	80.8 ± 2.2**	84.7 ± 2.4**
control		7	169.6 ± 6.7	138.6 ± 3.7	118.5 ± 3.0
tolbutamide	12.5	6	152.6 ± 2.8*	130.5 ± 4.0	114.9 ± 3.8
	25	6	138.1 ± 3.5**	106.3 ± 3.5**	99.5 ± 2.1**
normal		5	85.6 ± 3.6**	88.9 ± 4.1**	87.1 ± 2.9*
control		6	159.6 ± 7.0	145.0 ± 6.9	110.9 ± 5.4
metformin	125	5	134.8 ± 4.3*	136.0 ± 5.1	115.5 ± 4.7
	250	5	124.1 ± 5.5**	116.3 ± 5.5**	126.4 ± 1.9
	500	5	113.1 ± 5.3**	105.6 ± 4.5**	119.8 ± 7.7

[a] Refer to footnote in Table 1.

proton [δ 5.35 (1H, d, J = 4.9 Hz, H-6)] in the aglycon moiety, and three glycopyranosyl units {a galactopyranosyl [δ 4.88 (1H, d, J = 8.0 Hz, H-1′)], an α-L-rhamnopyranosyl [δ 1.67 (3H, d, J = 6.0 Hz, H-6″), 6.24 (1H, br s, H-1″)], a β-D-glucopyranosyl [δ 5.15 (1H, d, J = 7.7 Hz, H-1′)]}. The oligoglycoside moiety in **4** was characterized by HMBC experiments. Thus, long-range correlations were observed between the following protons and carbons: H-1′ and C-3; H-1″ and C-2′; and H-1‴ and C-3′. Next, ROESY difference experiments on **4** showed correlations between the following proton pairs: H-3 and H-1α, H-4α; H-19 and H-1β, H-4β; H_3-18 and H-8, H-12, H-20; and H-17 and H-14, H-16, H_3-21. Thus, the relative stereochemistry of **4**, including the 12α-hydroxyl group, could be characterized. Consequently, the structure of robeneoside B (**4**) was determined as (25*R*)-12α-hydroxy-3β-{*O*-α-L-rhamnopyranosyl(1→2)-[*O*-β-D-glucopyranosyl(1→3)]-β-D-galactopyranosyloxy}-22α*N*-spirosol-5-ene.

We examined the effects of the major isolated constituents of *S. lycocarpum*, solamargine (**1**) and solasonine (**3**), on the increase in serum glucose levels in sucrose-loaded rats.[24] As shown Table 3, **1** and **3** showed significant inhibitory activities against the increase of serum glucose levels, and the inhibition effects of **1** tended to be more potent than those of **3**. In this experimental model, an insulin-secretion stimulant, tolbutamide, exhibited substantial inhibition, and metformin, which shows an inhibitory effect on intestinal glucose absorption as well as improving of peripheral insulin sensitivity, exhibited moderate inhibition.[25] Next, the mode of action for the antihyperglycemic activities of the steroidal alkaloids was studied. The regulation of serum glucose is controlled by many factors such as transport of sugar in the digestive tract, the secretion and release of hormones, and absorption of glucose thorough membranes of the small intestine.[6,7,25] We previously reported that several triterpene oligoglycosides exhibited antihyperglycemic activity by suppressing the transfer of glucose from the stomach to the small intestine.[24,26−29] Accordingly, the effects of the steroidal alkaloid oligoglycosides **1** and **3** on gastric emptying were examined. As shown in Table 4, **1** significantly suppressed the gastric emptying time in mice at a dose of 50 mg/kg, and **3** also tended to suppress gastric emptying similarly to the antihyperglycemic effects of **1** and **3**.[7,26,28] These findings led us to conclude that these steroidal alkaloid oligoglycosides inhibit the increase of serum glucose in oral sucrose-loaded rats by suppressing the transfer of sucrose from the stomach to the small intestine, at least in part. However, the detailed mechanism of action of these compounds should be studied further.

In conclusion, two new steroidal alkaloid oligoglycosides, robeneoside A (**2**) and robeneoside B (**4**), were isolated from the fruits of *S. lycocarpum*, and their structures were determined on the basis of chemical and physicochemical evidence. In addition, this study has demonstrated that the principal constituents of these fruits, solamargine (**1**) and solasonine (**3**), had an inhibitory effect on serum glucose levels in oral sucrose-loaded rats, and this effect may be relevant for the prevention and treatment of diabetes and morbid obesity with accelerated gastric emptying time. Potential antidiabetogenic effects by steroidal alkaloids are reported for the first time in this investigation.

Table 4. Effects of Solamargine (**1**) and Solasonine (**3**) on Gastric Emptying Time in 1.5% CMC-Na-Loaded Mice

treatment[a]	dose (mg/kg, po)	*n*	GE (%) at 30 min
control		5	85.0 ± 3.6
solamargine (**1**)	25	5	73.7 ± 3.8
	50	5	58.4 ± 4.6**
control		6	82.0 ± 3.6
solasonine (**3**)	25	6	75.4 ± 2.7
	50	6	74.8 ± 4.9

[a] Values represent the means ± SEM. Significantly different from the control group, $^{**}p < 0.01$.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: melting points, Yanaco micromelting point apparatus (MP-500D); specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; HPLC, Shimadzu RID-6A refractive index detector; and HPLC, Cosmosil $5C_{18}$-MS-II (Nacalai Tesque Inc., 250 × 4.6 mm i.d. and 250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); Diaion HP-20 column chromatography (Nippon Rensui); TLC, precoated TLC plates with silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. The fruits of *S. lycocarpum* were collected in Minas Gerais state, Brazil, in January 2005. The plant was identified by one of the authors (M.Y.). A voucher of the plant is on file in our laboratory (2005.01. Brazil-01).

Extraction and Isolation. The dried fruits of *S. lycocarpum* (8.7 kg) were extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (1203 g, 13.8%). The MeOH extract (380 g) was subjected to Diaion HP-20 column chromatography [3.0 kg, H_2O → MeOH → acetone] to give H_2O-, MeOH-, and acetone-eluted fractions (275, 85, and 12 g, respectively). The MeOH-eluted fraction (70 g) was subjected to normal-phase silica gel column chromatography [2.1 kg, $CHCl_3$−MeOH−H_2O (40:10:1 → 30:10:1→ 6:4:1, v/v/v) → MeOH] to give 10 fractions [1 (3.8 g), 2 (2.2 g), 3 (1.0 g), 4 (0.2 g), 5 (1.6 g), 6 (1.6 g), 7 (13.0 g), 8 (18.0 g), 9 (28.0 g), and 10 (2.0 g)].

Fraction 1 (3.8 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH−H_2O (50:50 → 70:30 → 90:10, v/v) → MeOH] to afford seven fractions [1-1 (300 mg), 1-2 (500 mg), 1-3 (720 mg), 1-4 (γ-linolenic acid, 500 mg, 0.02%), 1-5 (1040 mg), 1-6 (linoleic acid, 500 mg, 0.02%), and 1-7 (220 mg)]. Fraction 7 (2 g) was subjected to reversed-phase silica gel column chromatography [60 g, MeOH−H_2O (50:50, v/v) → MeOH] to afford two fractions [7-1 (980 mg) and 7-2 (solamargine, **1**, 1.02 g, 0.29%)]. Fraction 8 (2.0 g) was subjected to reversed-phase silica gel column chromatography [60 g, MeOH−H_2O (30:70 → 50:50 → 70:30, v/v) → MeOH] to afford seven fractions [8-1 (980 mg), 8-2 (60 mg), 8-3 (10 mg), 8-4 (solasonine, **3**, 550 mg, 0.22%), 8-5 (30 mg), 8-6 (solamargine, **1**, 330 mg, 0.13%), and 8-7 (20 mg)]. Fraction 8-5 (30 mg) was purified by HPLC [MeOH−H_2O (60:40, v/v)] to furnish solasonine (**3**, 5 mg, 0.002%) and robeneoside A (**2**, 5 mg, 0.002%). Fraction 9 (3.0 g) was subjected to reversed-phase silica gel column chromatography [60 g, MeOH−H_2O (30:70 → 60:40, v/v) → MeOH] to afford seven fractions [9-1 (1520 mg), 9-2 (220 mg), 9-3 (200 mg), and 9-4 (solasonine, **3**, 980 mg, 0.40%)]. Fraction 9-3 (200 mg) was purified by HPLC [MeOH−H_2O (60:40, v/v)] to furnish solasonine (**3**, 91 mg, 0.04%) and robeneoside A (**2**, 15 mg, 0.006%). Fraction 10 (2.0 g) was subjected to reversed-phase silica gel column chromatography [60 g, MeOH−H_2O (10:90 → 30:70 → 50:50, v/v) → MeOH] to afford six fractions [10-1 (1100 mg), 10-2 (220 mg), 10-3 (100 mg), 10-4 (130 mg), 10-5 (solasonine, **3**, 300 mg, 0.01%), and 10-6 (160 mg)]. Fraction 10-4 (130 mg) was purified by HPLC [MeOH−H_2O (30:70, v/v)] to furnish 12-hydroxysolasonine (**5**, 20 mg, 0.0009%) and robeneoside B (**4**, 12 mg, 0.0005%). The known compounds (**1**, **3**, and **5**), γ-linolenic acid, and linoleic acid were identified by comparison of their ^{1}H NMR, ^{13}C NMR, and MS with reported values or commercial samples.

Robeneoside A (2): white powder ($CHCl_3$−MeOH); mp 205−208 °C; $[\alpha]_D^{29}$ −112.2 (*c* 1.0, pyridine); IR (KBr) ν_{max} 3450, 2941, 1626, 1280, 1071, 1046, 980 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.82 (3H, d, J = 5.2 Hz, H_3-27), 0.99 (3H, s, H_3-18), 1.01 (1H, ddd, J = 3.8, 13.8, 13.8 Hz, H_a-1), 1.10 (3H, s, H_3-19), 1.18 (3H, d, J = 7.2 Hz, H_3-21), 1.61, (1H, m, H_a-15), 1.64 (2H, m, H_2-24), 1.64 (3H, d, J = 6.0 Hz, H_3-6‴), 1.67 (1H, m, H_a-7), 1.70 (1H, m, H-9), 1.70 (1H, m, H-25), 1.74 (1H, m, H_b-1), 1.77 (1H, m, H_a-23), 1.77 (3H, d, J = 6.3 Hz, H_3-6″), 1.85 (1H, m, H_a-2), 1.85 (2H, m, H_2-11), 1.85 (1H, m, H_b-23), 1.94 (1H, m, H_b-7), 1.94 (1H, m. H-8), 2.07 (1H, qd, J = 7.2, 7.7 Hz, H-20), 2.08 (1H, m, H_b-2), 2.17 (1H, m, H-14), 2.17 (1H, m, H_b-15), 2.74 (1H, dd, J = 11.2, 13.8 Hz, H_a-4), 2.81 (1H, dd, J = 4.3, 13.8 Hz, H_b-4), 2.83 (2H, m, H_2-26), 3.08 (1H, dd, J = 7.7, 7.8 Hz, H-17), 3.62 (1H, m, H-5′), 3.85 (1H, m, H-3), 3.98 (1H, brs, H-12), 4.08 (1H, dd, J = 3.4, 12.3 Hz, H_a-6′), 4.20 (1H, brd, $J \approx$ 12.0 Hz, H_b-6′), 4.22 (1H, m, H-2′), 4.22 (1H, m, H-3′), 4.34 (1H, dd, J = 9.4, 9.5 Hz, H-4‴), 4.37 (1H, dd, J = 9.2, 9.5 Hz, H-4″), 4.39 (1H, dd, J = 8.9, 9.2 Hz, H-4′), 4.54 (1H, dd, J = 3.4, 9.4 Hz, H-3‴), 4.58 (1H, brd, $J \approx$ 8.0 Hz, H-16), 4.63 (1H, dd, J = 3.5, 9.2 Hz, H-3″), 4.68 (1H, dd, J = 1.5, 3.4 Hz, H-2‴), 4.83 (1H, dd, J = 1.2, 3.5 Hz, H-2″), 4.93 (1H, m, H-5‴), 4.93 (1H, d, J = 7.2 Hz, H-1′), 4.96 (1H, m, H-5″), 5.34 (1H, d, J = 4.9 Hz, H-6), 5.86 (1H, brs, H-1‴), 6.40 (1H, brs, H-1″); ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 2; positive-ion FABMS *m/z* 906 [M + Na]$^+$; negative-ion FABMS *m/z* 882 [M − H]$^-$; HRFABMS *m/z* 906.4824 (calcd for $C_{45}H_{73}NO_{16}Na$ [M + Na]$^+$, 906.4828).

Robeneoside B (4): white powder ($CHCl_3$−MeOH); mp 206−210 °C; $[\alpha]_D^{29}$ −22.2 (*c* 1.0, pyridine); IR (KBr) ν_{max} 3450, 2938, 1622, 1300, 1071, 1046, 980 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.81 (3H, brs, H_3-27), 0.97 (1H, m, H_a-1), 0.97 (3H, s, H_3-18), 1.09 (3H, s, H_3-19), 1.20 (3H, brs, H_3-21), 1.60 (1H, m, H_a-15), 1.62 (1H, m, H_a-7), 1.62 (2H, m, H_2-24), 1.64 (1H, m, H-9), 1.67 (3H, d, J = 6.0 Hz, H_3-6″), 1.72 (1H, m, H_b-1), 1.76 (1H, m, H_a-23), 1.76 (1H, m, H-25), 1.80 (2H, m, H_2-11), 1.85 (1H, m, H_a-2), 1.92 (1H, m, H_b-7), 1.92 (1H, m, H_b-23), 1.98 (1H, m, H-8), 2.06 (1H, m, H_b-2), 2.08 (1H, m, H-20), 2.13 (1H, m, H-14), 2.13 (1H, m, H_b-15), 2.72 (1H, dd, J = 11.2, 13.8 Hz, H_a-4), 2.79 (1H, dd, J = 4.1, 13.8 Hz, H_b-4), 2.88 (2H, m, H_2-26), 3.07 (1H, m, H-17), 3.90 (1H, m, H-5‴), 3.92 (1H, m, H-5′), 3.96 (1H, m, H-3), 3.96 (1H, m, H-12), 3.96 (1H, m, H-2‴), 4.12 (1H, dd, J = 8.9, 9.2 Hz, H-3‴), 4.16 (1H, dd, J = 8.6, 9.2 Hz, H-4‴), 4.25 (1H, m, H_a-6′), 4.25 (1H, m, H-4″), 4.25 (1H, m, H_a-6‴), 4.27 (1H, m, H-3′), 4.33 (1H, dd, J = 6.6, 11.2, H_b-6‴), 4.43 (1H, dd, J = 2.3, 11.8 Hz, H_b-6′), 4.56 (1H, dd, J = 3.5, 9.5 Hz, H-3″), 4.60 (1H, m, H-16), 4.64 (1H, dd, J = 8.0, 9.5 Hz, H-2′), 4.78 (1H, brd, $J \approx$ 2.0, H-4′), 4.86 (1H, brs, H-2″), 4.88 (1H, d, J = 8.0 Hz, H-1′), 4.88 (1H, m, H-5″), 5.15 (1H, d, J = 7.7 Hz, H-1‴), 5.35 (1H, d, J = 4.9 Hz, H-6), 6.24 (1H, brs, H-1″); ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 2; positive-ion FABMS *m/z* 922 [M + Na]$^+$; negative-ion FABMS *m/z* 898 [M − H]$^-$, 736 [M − $C_6H_{11}O_5$]$^-$; HRFABMS *m/z* 922.4792 (calcd for $C_{45}H_{73}NO_{17}Na$ [M + Na]$^+$, 922.4776).

Acid Hydrolysis of 2 and 4. Solutions of **2** and **4** (each 5.0 mg) in 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v) (1.0 mL) were each heated under reflux for 3 h. After cooling, each reaction mixture was poured into ice−water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN−H_2O (85:15, v/v); flow rate, 0.8 mL/min; column temperature, room temperature. Identifications of (i) l-rhamnose from **2** and **4**, (ii) D-glucose from **2** and **4**, and (iii) D-galactose from **4**, present in the aqueous layer, were carried out by comparison of their retention times and optical rotations with those of authentic samples. t_R: (i) 9.6 min (negative optical rotation); (ii) 13.5 min (positive optical rotation); (iii) 14.0 min (positive optical rotation).

Bioassay. Animals. Male Wistar rats weighing about 130−150 g and male ddY mice weighing about 30−32 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23 ± 2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 20−24 h prior to the beginning of the experiment, but were allowed free access to tap water. All experiments were performed with conscious rats or mice unless otherwise noted. Each test sample was suspended in 5% acacia solution, and the solution was orally administered at 10 mL/kg in each experiment, while the vehicle was administered orally at 10 mL/kg in the corresponding control groups. The experimental protocols were approved by Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Serum Glucose in Sucrose-Loaded Rats. Rats weighing about 130−150 g were fasted for 20−24 h. The test compounds suspended in 5% acacia solution were given orally, and the vehicle was given orally in the corresponding control groups. Thirty minutes later, 5 mL/kg of 20% (w/v) sucrose was administered orally (po). Water was given orally instead of sucrose in a normal group. Blood samples were collected at 0.5, 1, and 2 h after sucrose loading. The blood was centrifuged to obtain serum, and serum glucose levels were determined enzymatically by the glucose-oxidase method (glucose CII-test Wako, Wako Pure Chemical Industries).

Gastric Emptying in Mice. A solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na) containing 0.05% phenol red as a marker was given intragastrically (0.5 mL/mouse) to conscious mice. Thirty minutes later, mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the gastroesophageal junction and the pylorus were clamped, then the stomach was removed, weighed, placed in 14 mL of 0.1 M NaOH, and homogenized. The suspension was allowed to settle for 1 h at room temperature, and 5 mL of the supernatant was added to 0.5 mL of 20% trichloroacetic acid (w/v) and then centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 mL of 0.5 M NaOH, and the amount of phenol red was determined from the absorbance at 560 nm. Phenol red recovered from animals sacrificed immediately after the administration of the CMC-Na solution was used as standards (0% emptying). Gastric emptying (%) in the 30 min period was calculated according to the following equation:

$$\text{gastric emptying (\%)} = (1 - \text{amount of test sample/amount of standard}) \times 100$$

The test sample was given orally by means of a metal orogastric tube 30 min prior to the administration of the test meals.

Statistics. Values were expressed as means ± SEM. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (p) values less than 0.05 were considered significant.

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