Antidiabetic Principles of Natural Medicines. III.[1] Structure-Related Inhibitory Activity and Action Mode of Oleanolic Acid Glycosides on Hypoglycemic Activity

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We examined the structure-related activity of oleanolic acid glycosides with respect to their inhibitory effect on the increase in serum glucose in oral glucose-loaded rats and their mechanism of action using oleanolic acid 3-*O*-glucuronide and momordin Ic. Both the 3-*O*-monodesmoside structure and 28-carboxyl group were confirmed to be essential for such activity, and the 3-*O*-glucuronide was more potent than 3-*O*-glucoside. On the other hand, the 28-ester glucoside moiety and 6′-methyl ester of the glucuronide moiety reduced such activity. Oleanolic acid 3-*O*-glucuronide and momordin Ic, both of which inhibited the increase in serum glucose in oral glucose-loaded rats, did not lower serum glucose in normal or intraperitoneal glucose-loaded rats, or alloxan-induced diabetic mice. These glycosides were found to suppress gastric emptying in rats, and also inhibit glucose uptake in the rat small intestine *in vitro*. These results indicate that oleanolic acid 3-*O*-glucuronide and momordin Ic, given orally, have neither insulin-like activity nor insulin releasing-activity. They exhibit their hypoglycemic activity by suppressing the transfer of glucose from the stomach to the small intestine and by inhibiting glucose transport at the brush border of the small intestine.

Key words momordin Ic; oleanolic acid glycoside; gastric emptying; glucose absorption; glucose uptake

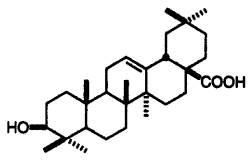
Many Chinese and Japanese traditional medicines are known to have preventive and therapeutic effects in diabetes and obesity, but their active components have not yet been characterized except in a few cases. In the course of our studies on bioactive saponin constituents in natural medicines,[2] we have recently found that extracts of several natural medicines inhibit the increased serum glucose in oral glucose-loaded rats. Through bioassay-guided separation, we have characterized the active saponins from *Aralia elata* (roots, bark, and young shoots),[3] *Aesculus hippocastanum* (seeds),[4] *Beta vulgaris* (roots and leaves),[5] *Polygala senega* var. *latifolia* (roots),[6] *Gymnema sylvestre* (leaves),[7] and *Kochia scoparia* (fruit).[8] In addition, by examination of the structure requirements for inhibition of the increased serum glucose, the active saponins could be classified into the following three types: 1) olean-12-en-28-oic acid 3-*O*-monodesmoside; 2) acylated polyhydroxyolean-12-ene 3-*O*-monodesmoside; and 3) olean-12-ene 3,28-*O*-acylated bisdesmoside. As a continuing study, we examined the inhibition by several oleanolic acid glycosides on the increased serum glucose in oral glucose-loaded rats. Furthermore, the mode of action for the hypoglycemic activity of saponins was studied using oleanolic acid 3-*O*-monodesmosides, oleanolic acid 3-*O*-glucuronide (**2**), and momordin Ic (**5**). In this paper, we describe the detailed structure requirement and plausible mechanism for inhibition.

Experimental

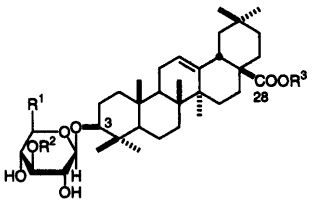
Chemicals Oleanolic acid 3-*O*-glucuronide (**2**) and momordins Ic (**5**) and IIc (**8**) were isolated from the fruit of *Kochia scoparia*, as described.[8]

Methyl(oleanolic acid 3-*O*-β-D-glucopyranosid)uronate (**3**)[9] and Momordin Ic 6′-methyl ester (**6**)[10]: A solution of **2** or **5** (both 50 mg) in 3% hydrogen chloride in dry methanol was heated under reflux for 2.5 h. After cooling, the reaction solution was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by ordinary-phase silica gel column chromatography ($CHCl_3$–MeOH–H_2O) to give **3** (32 mg) or **6** (26 mg), which was identified by comparison of its TLC behavior, ^{1}H-NMR, and ^{13}C-NMR spectra with authentic samples.[9,10]

Oleanolic acid 3-*O*-β-D-glucopyranoside (**4**)[10] and Oleanolic acid 3-*O*-β-

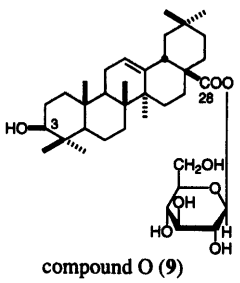


oleanolic acid (**1**)



	R^1	R^2	R^3
oleanolic acid 3-*O*-glucuronide (**2**) :	COOH	H	H
methyl(oleanolic acid 3-*O*-β-D-glucopyranosid)uronate (**3**) :	COOMe	H	H
oleanolic acid 3-*O*-β-D-glucopyranoside (**4**) :	CH_2OH	H	H
momordin Ic (**5**) :	COOH	Xyl	H
momordin Ic 6′-methyl ester (**6**) :	COOMe	Xyl	H
oleanolic acid 3-*O*-β-D-xylopyranosyl (1→3)-β-D-glucopyranoside (**7**) :	CH_2OH	Xyl	H
momordin IIc (**8**) :	COOH	Xyl	Glc

Xyl : β-D-xylopyranosyl
Glc : β-D-glucopyranosyl



compound O (**9**)

Chart 1

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Table 1. ^{13}C-NMR Data of Oleanolic Acid 3-*O*-*β*-D-Xylopyranosyl (1→3)-*β*-D-Glucopyranoside (**7**)

	δc		δc
C- 1	38.8	C-22	33.3
C- 2	26.5	C-23	17.0
C- 3	89.1	C-24	28.2
C- 4	39.5	C-25	15.5
C- 5	55.9	C-26	17.4
C- 6	18.5	C-27	26.2
C- 7	33.3	C-28	180.1
C- 8	39.8	C-29	33.3
C- 9	48.1	C-30	23.8
C-10	37.1	Glc-1′	106.4
C-11	23.8	2′	74.6
C-12	122.5	3′	87.9
C-13	144.9	4′	69.7
C-14	42.2	5′	77.9
C-15	28.4	6′	62.7
C-16	23.8	Xyl-1″	106.2
C-17	46.7	2″	75.2
C-18	42.0	3″	78.1
C-19	46.6	4″	70.9
C-20	31.0	5″	67.4
C-21	34.3		

68 MHz, pyridine-d_5.

D-xylopyranosyl (1→3)-*β*-D-glucopyranoside (**7**): A solution of **3** or **6** (both 50 mg) in methanol (2 ml) was treated with sodium borohydride ($NaBH_4$, 10 mg) and the mixture was stirred at room temperature for 1 h. Excess $NaBH_4$ was quenched with acetone, then the solution was neutralized with Dowex HCR×W2 (H^+ form) and the resin was filtered off. Removal of the solvent from the filtrate yielded **4** (43 mg) or **7** (45 mg). **4** was identified by comparison of its physical data with reported values.[10]

7: Colorless fine crystals from $CHCl_3$–MeOH, mp 208—209 °C, $[\alpha]_D^{22}$ +32.3° (c=0.15, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{41}H_{66}O_{12}Na$ (M+Na): 773.4452. Found: 773.4470. IR (KBr) cm^{-1}: 3425, 1691, 1080. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 0.83, 0.96, 1.02, 1.30, 1.31 (3H each, all s, 25, 29, 24, 27-H_3), 0.99 (6H, s, 26, 30-H_3), 3.28 (1H, dd-like, 18-H), 3.37 (1H, dd-like, 3-H), 4.88 (1H, d, J=7.6 Hz, Glc-1′-H), 5.20 (1H, d, J=7.3 Hz, Xyl-1″-H), 5.47 (1H, br s, 12-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C : given in Table 1. Positive-ion FAB-MS (m/z): 751 $(M+H)^+$, 773 $(M^+Na)^+$.

Compound O (**9**)[11]: A solution of chikusetsusaponin V[12] (100 mg) in 0.1 M acetate buffer (pH 4.4, 5.0 ml) was treated with glycyrrhizinic acid hydrolase (50 mg) and the solution was stirred at 44 °C for 3 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was purified by ordinary-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give **9** (62 mg), which was identified by comparison of its TLC behavior, ^{1}H-NMR and ^{13}C-NMR spectra with an authentic sample.[5]

Pharmacological Methods

Reagents D-[U-^{14}C]glucose (11.3 GBq/mmol, Amersham), phlorizin (Sigma); other reagents were purchased from Wako Pure Chemical Industries.

Animals Male Wistar rats and male ddY mice were purchased from Kiwa Laboratory Animal Co., Ltd. The animals were maintained at a room temperature of 23±2 °C and were fed standard laboratory chow (MF, Oriental Yeast Co., Ltd.). They were fasted for 20—24 h prior to experiments, but were supplied with water *ad libitum*. Test samples were suspended in 5% acacia solution and given orally at 5 ml/kg to rats and 10 ml/kg to mice in each experiment. The experiments were performed in conscious animals unless otherwise noted.

Serum Glucose in Glucose-Loaded or Normal Rats Rats weighing 130—170 g were fasted for 20—24 h and the test compounds were given orally. Thirty minutes later, a) *oral glucose-loaded rat*: 10% D-glucose solution was administered orally (*p.o.*) at 5 ml/kg, b) *intraperitoneal glucose-loaded rat*: 10% glucose solution in saline was administered intraperitoneally (i.p.) at 5 ml/kg, or c) *normal rat* (*non-glucose-loaded rat*): water was given orally at 5 ml/kg instead of glucose solution. Blood samples (*ca.* 0.4 ml) were collected from the jugular vein at 0.5, 1, and 2 h after glucose loading in these experiments. 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).

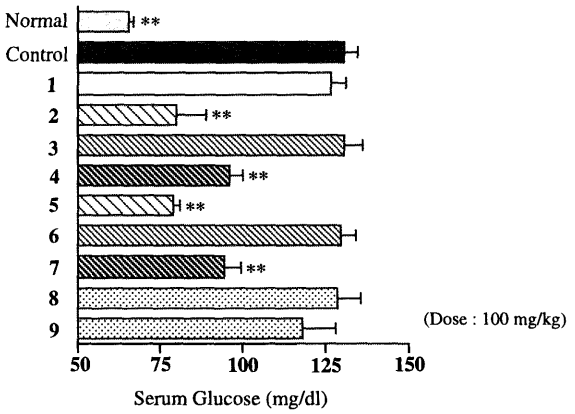

Fig. 1. Effects of Oleanolic Acid (**1**) and Its Glycosides (**2**—**9**) on Serum Glucose in Oral Glucose-Loaded Rats

Each column represents the mean with S.E. of serum glucose at 30 min after administration of D-glucose (0.5 g/kg, *p.o.*). Significantly different from the control group, $^{**}p<0.01$ (n=5, 6).

Serum Glucose in Alloxan-Induced Diabetic Mice Mice weighing 29—32 g were fasted for 20—24 h and alloxan (50 mg/kg) was administered intravenously. Two days later, the mice were again fasted for 20—24 h, and test compounds were given orally. Blood samples (*ca.* 0.1 ml) were collected from the infraorbital venous plexus before (0 h), and at 1 and 3 h after the administration of test compound.

Gastric Emptying in Rats Rats weighing 130—170 g were used. Test food consisting of 10% glucose, 1% carboxymethyl cellulose sodium salt (CMC-Na), and 0.05% phenol red was given orally (0.75 ml/rat) to rats, and the stomach was removed and homogenized with 50 ml 0.1 N NaOH. Then, 0.5 ml 20% trichloroacetic acid (TCA) was added to 5 ml homogenate, and the solution was centrifuged. NaOH (0.5 N) was added to the supernatant and the amount of phenol red was determined from the absorbance at 560 nm. Each test compound was given orally 30 min before administration of test food. Gastric emptying (%) was calculated as shown below.

$$\text{gastric emptying (\%)} = (PR_o - PR_s)/PR_o \times 100$$

PR_o: amount of phenol red given orally

PR_s: amount of phenol red remaining in the stomach

Glucose Uptake in Rat Small Intestine *in Vitro* Rats weighing 130—170 g were used. The method described by Meir *et al.*[13] was modified for this experiment. Small fragments (0.1—0.15 g) of everted rat jejunum were placed in 950 μl modified Krebs–Henseleit solution, pH 7.4, with D-[U-^{14}C]glucose and unlabeled D-glucose (final concentration: 2 mM, 1.0—1.5×10^5 cpm/ml). Then, 50 μl of test sample solution in dimethyl sulfoxide (DMSO) was added to the medium. Incubation was carried out at 30 °C for 6 min, followed by washing twice for 3—5 s with medium containing 1 mM phlorizin without D-[U-^{14}C]glucose. Samples were placed on filter paper to absorb water from the tissue, then placed in scintillation vials and digested with 0.5 ml of tissue solubilizer (Soluene 350, Packard). The solubilized samples were mixed with scintillator and the radioactivity was determined using a liquid scintillation counter (LS6500, Beckmann) to estimate glucose uptake (μmol glucose/100 mg tissue). Phlorizin was used as a reference.

Statistics Values were expressed as means±S.E. Statistical significance was assessed by one-way analysis of variance following Dunnett's test.

Results

Strucure-Related Inhibitory Activity with Respect to Increased Glucose Levels in Oral Glucose-Loaded Rats

Figure 1 shows the inhibition by oleanolic acid glycosides (dose: 100 mg/kg) of increased glucose levels in oral glucose-loaded rats. As previously reported,[3e] although oleanolic acid (**1**) did not exhibit activity, oleanolic acid 3-*O*-glucuronide (**2**) and momordin Ic (**5**) strongly inhibited the in-

Table 2. Effects of Oleanolic Acid 3-*O*-Glucuronide (**2**) and Momordin Ic (**5**) on Serum Glucose in Oral Glucose-Loaded Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1 h	2 h
Normal	—	4	67.5±4.8**	95.5±6.6**	88.0±6.9
Control	—	5	145.3±5.3	141.2±6.6	106.1±4.2
			(77.8)	(45.7)	(18.1)
Oleanolic acid 3-*O*-glucuronide (**2**)	12.5	5	125.0±6.9	145.1±5.1	119.5±9.6
			(57.5)	(49.6)	(31.5)
	25	5	94.5±3.9**	124.9±4.9	113.0±2.7
			(27.0)	(29.4)	(25.0)
	50	5	79.7±10.2**	118.1±5.7*	106.2±5.5
			(12.2)	(22.6)	(18.2)
Normal	—	5	66.8±5.5**	92.0±12.6**	86.1±6.8
Control	—	6	156.1±7.3	138.5±9.1	106.9±4.6
			(89.3)	(46.5)	(20.8)
Momordin Ic (**5**)	12.5	6	125.8±13.6	139.1±9.5	118.8±6.6
			(59.0)	(47.1)	(32.7)
	25	6	102.7±7.6**	119.6±3.6	121.2±5.2
			(35.9)	(27.6)	(35.1)
	50	6	91.6±5.3**	125.4±7.2	115.0±4.7
			(24.8)	(33.4)	(28.9)
Normal	—	5	74.0±2.4**	94.3±3.3**	86.1±6.5*
Control	—	7	129.7±6.1	118.2±3.1	93.5±4.0
			(55.7)	(23.9)	(7.4)
Tolbutamide	25	5	86.0±5.6**	78.9±3.4**	59.5±2.2**
			(12.0)	(−15.4)	(−26.6)
	50	5	61.4±8.9**	67.9±7.2**	47.7±6.2**
			(−12.6)	(−26.4)	(−38.4)

Values in parentheses are expressed as the serum glucose concentration in glucose-loaded rat minus the mean concentration in the normal group. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

Table 3. Effects of Oleanolic Acid 3-*O*-Glucuronide (**2**) and Momordin Ic (**5**) on Serum Glucose in Intraperitoneal Glucose-Loaded Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1 h	2 h
Normal	—	5	70.9±9.1**	109.5±11.8	105.8±7.9
Control	—	5	123.8±12.0	135.4±10.7	110.3±11.4
			(52.9)	(25.9)	(4.5)
Oleanolic acid 3-*O*-glucuronide (**2**)	50	5	120.0±7.5	136.7±9.5	120.4±7.2
			(49.1)	(27.2)	(14.6)
Momordin Ic (**5**)	50	5	122.3±7.0	138.9±6.2	114.2±1.6
			(51.4)	(29.4)	(8.4)
Normal	—	5	59.4±2.9**	76.4±4.2**	74.4±4.2**
Control	—	7	118.6±5.5	119.0±5.2	102.4±2.9
			(59.2)	(42.6)	(28.0)
Tolbutamide	50	5	81.8±5.2**	78.2±4.0**	67.2±3.9**
			(22.4)	(1.8)	(−7.2)

Values in parentheses are expressed as the serum glucose concentration in glucose-loaded rat minus the mean concentration in the normal group. Significantly different from the control group, ** $p<0.01$.

crease in serum glucose levels. **4** and **7**, having the 3-*O*-β-D-glucopyranosyl moiety, also produced significant inhibition but were weaker than the corresponding 3-*O*-glucuronides (**2**, **5**), respectively. But **3** and **6**, having a 6′-methyl ester of glucuronic acid moiety, lacked activity. Momordin IIc (**8**) and compound O (**9**), having a 28-ester glucoside moiety, also lacked activity.

Effects on Serum Glucose in Glucose-Loaded and Normal Rats As shown in Table 2, oleanolic acid 3-*O*-glucuronide (**2**) and momordin Ic (**5**) inhibited the increase in serum glucose dose-dependently 0.5 h after oral loading with glucose. However, **2** and **5** did not lower serum glucose in intraperitoneal glucose-loaded rats (Table 3). In normal rats, **2** and **5** slightly increased the glucose level (Table 4). Tolbutamide (50 mg/kg), as a reference drug, strongly reduced the serum glucose in these experiments.

Effects on Serum Glucose in Alloxan-Induced Diabetic Mice As shown in Table 5, insulin (1 U/kg, i.p.), as a reference drug, strongly reduced the serum glucose 1 and 3 h after intraperitoneal injection in alloxan-induced diabetic mice. Oleanolic acid 3-*O*-glucuronide (**2**) and momordin Ic (**5**) (100 mg/kg, *p.o.*) lacked hypoglycemic effects.

Effects on Gastric Emptying in Rats As shown in Table 6, atropine sulfate (10 mg/kg), as a reference drug, significantly inhibited gastric emptying in rats 0.5, 1, and 2 h after oral application. Oleanolic acid 3-*O*-glucuronide (**2**)

Table 4. Effects of Oleanolic Acid 3-*O*-Glucuronide (**2**) and Momordin Ic (**5**) on Serum Glucose in Normal Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1 h	2 h
Control	—	5	72.3±5.2	88.7±7.9	79.9±5.5
Oleanolic acid 3-*O*-glucuronide (**2**)	50	5	79.4±6.4	98.0±8.9	76.6±7.0
Momordin Ic (**5**)	50	5	82.6±4.6	114.6±7.2	109.2±11.6
Tolbutamide	50	5	49.8±0.8**	46.8±2.8**	51.3±3.4

Significantly different from the control group, ** $p<0.01$.

Table 5. Effects of Oleanolic Acid 3-*O*-Glucuronide (**2**) and Momordin Ic (**5**) on Serum Glucose in Alloxan-Induced Diabetic Mice

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0 h	1 h	3 h
Control	—	8	689.3±29.2	604.3±15.4	580.4±10.0
Oleanolic acid 3-*O*-glucuronide (**2**)	100	8	685.8±23.8	546.0±13.0	600.5±17.2
Momordin Ic (**5**)	100	8	638.3±26.6	582.6±25.6	645.4±48.9
Insulin	1 (U/kg, i.p.)	8	688.1±12.9	68.3±9.6**	72.9±6.8**

Significantly different from the control group, ** $p<0.01$.

Table 6. Effects of Oleanolic Acid 3-*O*-Glucuronide (**2**) and Momordin Ic (**5**) on Gastric Emptying in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Serum glucose (mg/dl)		
			0.5 h	1 h	2 h
Control	—	6	74.6±3.7	88.2±2.3	97.8±0.5
Oleanolic acid 3-*O*-glucuronide (**2**)	12.5	5	70.0±3.5	80.7±2.2	95.7±0.7
	25	5	64.3±3.7	86.1±5.8	86.5±1.8**
	50	5	33.6±0.9**	69.0±3.6**	80.7±1.4**
Momordin Ic (**5**)	12.5	5	62.8±6.7	72.8±1.9	87.4±2.4*
	25	5	46.0±2.0**	57.3±4.0**	64.0±3.8**
	50	5	29.9±2.3**	36.1±3.1**	59.0±2.2**
Control	—	10	73.6±2.2	89.3±1.4	97.2±0.9
Atropine Sulfate	10	5	51.0±4.7**	68.0±3.1**	80.6±1.3**

Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

and momordin Ic (**5**) also strongly inhibited gastric emptying.

Effect on Glucose Uptake in Rat Small Intestine Fragments The glucose uptake without test sample was about 0.2 μmol glucose/100 mg tissue for 6 min. As shown in Fig. 2, phlorizin, as a reference substance, concentration-dependently inhibited glucose uptake in the rat small intestine. Oleanolic acid 3-*O*-glucuronide (**2**) and momordin Ic (**5**) also significantly inhibited the uptake.

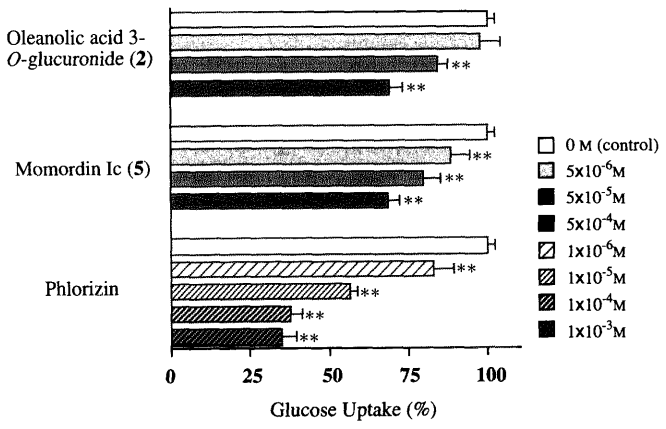

Fig. 2. Effects of Oleanolic Acid 3-*O*-Glucuronide (**2**) and Momordin Ic (**5**) on Glucose Uptake in Rat Small Intestine Fragments

Each column represents the mean with S.E. Significantly different from the control group, ** $p<0.01$ ($n=8$).

Discussion

We previously reported that saponins having the 3-*O*-glucuronic acid moiety of oleanolic acid, such as oleanolic acid 3-*O*-glucuronide (**2**) or momordin Ic (**5**), exhibited hypoglycemic activity while saponins having the 28-ester glucoside moiety, such as momordin IIc (**8**), did not.[8] In this study, the saponins (**4**, **7**) having the 3-*O*-glucopyranosyl moiety also exhibited significant activity, but this was apparently reduced, compared with **2** and **5**. **3** and **6** having the 6′-methyl ester of glucuronic acid moiety, lacked activity. The 28-monodesmoside, compound O (**9**), also lacked significant activity. These results strengthened the following structure requirements of oleanolic acid glycosides previously reported: 1) the 3-*O*-glycoside moiety was essential for activity; 2) the 28-ester glucoside moiety reduced the activity. Furthermore, the following structure requirements were found in this study: 3) the 3-*O*-glucuronic acid glycosides were more potent than the 3-*O*-glucopyranosyl analogs; 4) but the 6′-methyl ester of glucuronic acid moiety strongly re-

duced the activity.

The regulation of serum glucose is controlled by many factors such as the secretion and release of hormones (*e.g.* insulin and glucagon), transport of sugar in the digestive tract, and absorption of glucose *via* the membranes of the small intestine. Tolbutamide can increase the secretion of insulin to reduce serum glucose in normal and glucose-loaded rats. **2** and **5** dose-dependently inhibited the increased serum glucose in oral glucose-loaded rats. However, **2** and **5** lacked this effect on serum glucose in normal rats, intraperitoneal glucose-loaded rats and alloxan-induced diabetic mice. These results indicate that **2** and **5** have neither insulin-like activity nor insulin-releasing activity like tolbutamide and, therefore, it seems that **2** and **5** affect glucose absorption in the gastrointestinal tract. These saponins slightly increase serum glucose in normal rats. It has been reported that the saponin fractions from the rhizome of *Panax japonicus*, the seeds of *Aesculus hippocastanum*, and the roots of *Polygala senega* var. *latifolia*, *etc.*, when applied intraperitoneally, showed hyperglycemic activity due to their corticosterone secretion-inducing activity,[14] and this activity seems to be related to their hyperglycemic effects. **2** and **5** strongly suppressed gastric emptying in rats. These potent supression by **2** and **5** of gastric emptying seems to be important for inhibition of the increased serum glucose after oral administration of glucose. Phlorizin is well known as an inhibitor of the Na^+/glucose co-transport system at the intestinal brush border membrane.[15] **2** and **5** also inhibited glucose uptake in rat small intestine fragments *in vitro* like phlorizin. On the basis of the above evidence, it seems that saponins such as **2** and **5** inhibit the increase of serum glucose in oral glucose-loaded rats by suppressing the transfer of glucose from the stomach to the small intestine, the main site of glucose absorption, and by inhibiting glucose transport at the intestinal brush border membrane.

Drugs which reduce postprandial hyperglycemia by suppressing the absorption of carbohydrates have been shown to be effective for the prevention and treatment of non-insulin dependent diabetes mellitus. So, these saponins which suppress glucose absorption may also be effective for the prevention and treatment of diabetes.

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