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Mode of Action of Escins Ia and IIa and *E*,*Z*-Senegin II on Glucose Absorption in Gastrointestinal Tract

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Abstract—We examined the mode of action of escins Ia (**1**) and IIa (**2**) and *E*,*Z*-senegin II (**3**) for the inhibitory effect on the increase in serum glucose levels in oral glucose-loaded rats. Although **1**–**3** inhibited the increase in serum glucose levels in oral glucose-loaded rats, these compounds did not lower serum glucose levels in normal or intraperitoneal glucose-loaded rats, or alloxan-induced diabetic mice. Furthermore, **1**–**3** suppressed gastric emptying in rats, and also inhibited glucose uptake in the rat small intestine in vitro. These results indicated that **1**–**3** given orally have neither insulin-like activity nor insulin-releasing activity. Compounds **1**–**3** inhibited glucose absorption by suppressing the transfer of glucose from the stomach to the small intestine and by inhibiting the glucose transport system at the small intestinal brush border.

Introduction

Diabetes mellitus is one of the most common diseases associated with carbohydrate metabolism. Many Chinese and Japanese traditional medicines are known to have preventive and therapeutic effects in diabetes and obesity, but for the most part their active components have not yet been characterized. In the course of our studies on bioactive principles in natural medicines, we have recently found that the extracts of several natural medicines show inhibitory activity on the increase in serum glucose level in oral glucose-loaded rats. Through bioassay-guided separation, we have characterized the active saponin constituents from *Aralia elata* (roots, bark, and young shoots),[1] *Aesculus hippocastanum* (seeds),[2] *Beta vulgaris* (roots and leaves),[3] *Polygala senega* var. *latifolia* (roots),[4] *Gymnema sylvestre* (leaves),[5] and *Kochia scoparia* (fruit).[6] In addition, by examination of the structural requirements for inhibition of the increases in serum glucose level, 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*-glucuronide; and (3) olean-12-ene 3,28-*O*-acylated bisdesmoside. We reported previously that oleanolic acid 3-*O*-monodesmosides, momordin Ic and oleanolic acid 3-*O*-glucuronide, inhibited glucose absorption by both suppressing the transfer of glucose from the stomach to the small intestine and inhibiting the glucose uptake in small intestine.[7] In this paper, we describe a plausible mechanism for the inhibitory activity using two acylated polyhydroxyolean-12-ene 3-*O*-glucuronides, escins Ia (**1**) and IIa (**2**), and a olean-12-ene 3,28-*O*-acylated bisdesmoside, *E*,*Z*-senegin II (**3**).

Results and Discussion

The regulation of serum glucose level is controlled by many factors such as the secretion and release of hormones (e.g. insulin and glucagon etc.), transportation of sugar in the digestive tract, and the absorption of glucose from the membrane of the small intestine. As shown in Table 1, escins Ia (**1**, 100 mg/kg) and IIa (**2**, 100 mg/kg) and *E*,*Z*-senegin II (**3**, 100 mg/kg) significantly inhibited the increase in serum glucose level 30 min after oral loading with glucose. However, **1**–**3** did not lower serum glucose levels in intraperitoneal glucose-loaded rats and in normal rats (Tables 2 and 3). Tolbutamide (50 mg/kg), as a reference drug, strongly

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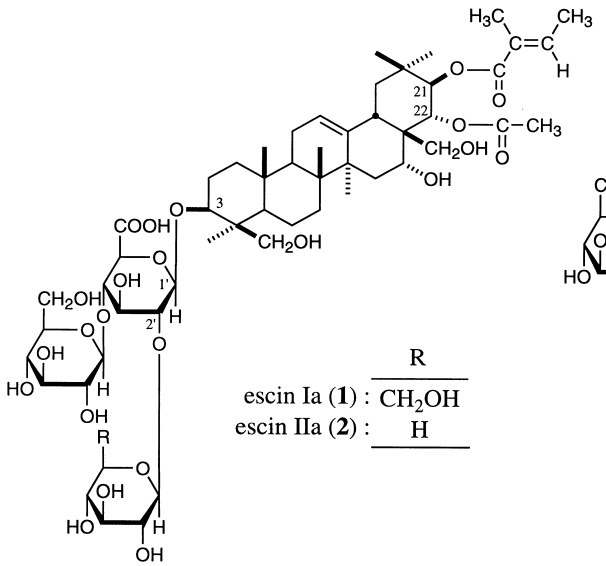

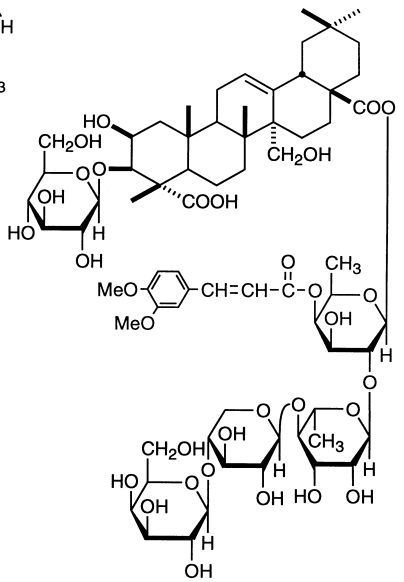

E,Z-senegin II (**3**)

decreased the serum glucose levels at 30, 60, and 120 min in these experiments. Tolbutamide can increase the secretion of insulin to decrease the serum glucose levels in normal and glucose-loaded rats. Insulin (1 U/kg, ip), as a reference drug, strongly decreased the serum glucose levels 60 and 180 min after intraperitoneal injection in alloxan-induced diabetic mice. But all of the test saponins (100 mg/kg, po) lacked hypoglycemic effects (Table 4). In normal rats, these saponins slightly increased the serum glucose levels, especially escins Ia (**1**) and Ib (**2**). It has been reported that the saponin fractions from the seeds of *Aesculus hippocastanum*, the roots of *Polygala senega* var. *latifolia* etc., when applied intraperitoneally, showed hyperglycemic activity due to their corticosterone secretion-inducing activity,[8] so this activity of saponins seem to be related to the hyperglycemic effects. These results indicated that **1**–**3** had neither insulin-like activity nor insulin-releasing activity like tolbutamide, and therefore it seemed that **1**–**3** affected glucose absorption in the gastrointestinal tract. Next, we examined the effects of **1**–**3** on mobility of glucose from the stomach to the small intestine (gastric emptying), and on glucose uptake at jejunum in vitro.

Table 1. Effects of escins Ia (**1**) and IIa (**2**) and *E,Z*-senegin II (**3**) on serum glucose levels in oral glucose-loaded rats

Treatment	Dose (mg/kg, po)		Increase of serum glucose levels (mg/dL)		
		N	0.5 h	1 h	2 h
Control	—	10	67.2 ± 3.4	36.8 ± 3.5	15.7 ± 4.0
Escin Ia (**1**)	50	5	55.6 ± 8.8	41.8 ± 5.7	27.7 ± 3.5
	100	5	40.4 ± 3.6**	32.2 ± 5.7	9.6 ± 6.1
Escin IIa (**2**)	50	5	53.4 ± 14.5	46.3 ± 5.9	32.1 ± 4.1*
	100	5	21.5 ± 7.2**	7.2 ± 7.8**	2.0 ± 7.4
Control	—	16	57.0 ± 2.3	35.9 ± 2.3	12.3 ± 2.5
E,Z-Senegin II (**3**)	25	7	59.9 ± 3.3	37.4 ± 3.2	12.4 ± 3.4
	50	7	51.5 ± 5.4	33.8 ± 4.0	19.3 ± 6.1
	100	12	32.3 ± 4.8**	31.7 ± 3.4	27.2 ± 4.0**
Control	—	7	55.7 ± 6.1	23.9 ± 3.1	7.4 ± 4.0
Tolbutamide	25	5	12.0 ± 5.6**	−15.4 ± 3.4**	−26.6 ± 2.2**
	50	5	−12.6 ± 8.9**	−26.4 ± 7.2**	−38.4 ± 6.2**

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

Table 2. Effects of escins Ia (**1**) and IIa (**2**) and *E*,*Z*-senegin II (**3**) on serum glucose levels in intraperitoneal glucose-loaded rats

Treatment	Dose (mg/kg, po)		Increase of serum glucose levels (mg/dL)		
		N	0.5 h	1 h	2 h
Control	—	7	59.2 ± 5.5	42.6 ± 5.2	28.0 ± 2.9
Escin Ia (**1**)	100	5	65.6 ± 3.9	47.6 ± 4.3	28.4 ± 3.4
Escin IIa (**2**)	100	5	48.2 ± 9.3	35.2 ± 7.8	26.8 ± 4.8
E,*Z*-Senegin II (**3**)	100	5	57.6 ± 7.6	40.0 ± 3.7	33.8 ± 2.2
Tolbutamide	50	5	22.4 ± 5.2**	1.8 ± 4.0**	−7.2 ± 3.9**

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

Table 3. Effects of escins Ia (**1**) and IIa (**2**) and *E*,*Z*-senegin II (**3**) on serum glucose levels in normal rats

Treatment	Dose (mg/kg, po)		Serum glucose levels (mg/dL)		
		N	0.5 h	1 h	2 h
Control	—	8	67.1 ± 2.9	79.3 ± 2.9	74.9 ± 3.7
Escin Ia (**1**)	100	6	83.3 ± 5.3*	84.8 ± 5.9	77.7 ± 5.3
Escin IIa (**2**)	100	6	88.2 ± 7.1*	87.3 ± 5.9	82.8 ± 7.4
E,*Z*-Senegin II (**3**)	100	6	78.5 ± 2.9	82.3 ± 3.1	73.5 ± 5.2
Tolbutamide	50	6	48.8 ± 1.2**	54.2 ± 2.2**	50.5 ± 2.3**

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

Table 4. Effects of escins Ia (**1**) and IIa (**2**) and *E*,*Z*-senegin II (**3**) on serum glucose levels in alloxan-induced diabetic mice

Treatment	Dose (mg/kg, po)		Serum glucose levels (mg/dL)		
		N	0 h	1 h	3 h
Control	—	12	676.3 ± 20.4	676.3 ± 10.5	579.3 ± 10.3
Escin Ia (**1**)	100	8	680.1 ± 26.2	614.3 ± 25.3	640.5 ± 41.5
Escin IIa (**2**)	100	8	676.8 ± 14.8	627.9 ± 14.9	662.4 ± 42.0
Control	—	8	625.3 ± 19.3	527.4 ± 31.4	533.6 ± 18.0
E,*Z*-Senegin II (**3**)	100	8	613.1 ± 31.2	581.1 ± 23.4	612.6 ± 48.9
Insulin	1 (U/kg, *i.p.*)	8	675.4 ± 10.1	107.1 ± 19.0**	107.4 ± 8.9**

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

1–**3** strongly suppressed gastric emptying in rats. In particular, escin IIa (**2**) strongly suppressed gastric emptying at the dose of 25 mg/kg (Table 5). These potent suppressing activities of **1**–**3** on gastric emptying seemed to be important for inhibition of the increase in serum glucose level after oral administration of glucose. The value of glucose uptake in the rat small intestine without test compound was about 0.2 μmol glucose/100 mg tissue for 6 min. As shown in Figure 1, phlorizin (0.1 mM), as a reference compound, strongly inhibited the glucose uptake. Phlorizin is well known as an inhibitor of the Na^+/glucose co-transport system at the intestinal brush border membrane.[9] **1**–**3** (0.5 mM) also significantly inhibited glucose uptake in rat small intestine fragments in vitro similarly to phlorizin.

On the basis of the above evidence, it was assumed that saponins such as **1**–**3** inhibited glucose absorption by suppressing the transfer of glucose from the stomach to the small intestine which are the sites of absorption of glucose, and by inhibiting the glucose transport system at the intestinal brush border membrane similar to oleanolic acid monodesmosides, previously reported.[7] Drugs which decrease postprandial hyperglycemia by suppressing the absorption of carbohydrates have been suggested to be effective for 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 the diabetes.

Experimental

Materials

Escins Ia (**1**) and IIa (**2**) and *E*,*Z*-senegin II (**3**) were isolated from the seeds of *Aesculus hippocastanum* and the roots of *Polygala senega* var. *latifolia*, as described.[2,4]

Table 5. Effects of escins Ia (**1**) and IIa (**2**) and *E*,*Z*-senegin II (**3**) on gastric emptying in rats

Treatment	Dose (mg/kg, po)		Gastric emptying (%)		
		N	0.5 h	1 h	2 h
Control	—	6	78.7 ± 2.7	85.6 ± 2.7	94.0 ± 0.7
Escin Ia (**1**)	25	5	69.4 ± 4.7	88.9 ± 1.5	89.6 ± 1.1
	50	5	63.9 ± 6.8	74.5 ± 6.3	85.7 ± 2.5
	100	5	55.8 ± 3.1*	54.5 ± 4.5**	69.7 ± 3.9**
Escin IIa (**2**)	25	5	57.1 ± 7.2*	74.6 ± 3.5	88.4 ± 2.7
	50	5	42.3 ± 6.5**	71.3 ± 1.6*	75.4 ± 1.8**
	100	5	41.1 ± 3.0**	50.6 ± 5.6**	50.0 ± 4.8**
Atropine sulfate	10	5	51.0 ± 4.7**	66.0 ± 2.9**	65.5 ± 2.7**
Control	—	10	73.6 ± 2.2	89.3 ± 1.4	97.2 ± 0.9
E,*Z*-Senegin II (**3**)	25	5	73.4 ± 3.3	90.2 ± 1.7	96.6 ± 1.4
	50	5	73.5 ± 1.0	88.4 ± 1.2	96.4 ± 0.7
	100	5	50.0 ± 1.1**	74.1 ± 4.0**	77.0 ± 3.3**
Atropine sulfate	10	5	51.0 ± 4.7**	68.0 ± 3.1**	80.6 ± 1.3**

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

Reagents: D-[U-^{14}C]glucose (11.2 GBq/mmol, Amersham), phlorizin (Sigma), Soluene 350® (Packard); 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 constant temperature of 23 ± 2°C and were fed standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo). 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 were 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.

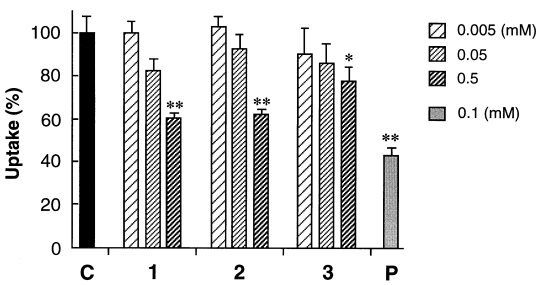

Figure 1. Effects of escins Ia (**1**) and IIa (**2**) and *E*, *Z*-senegin II (**3**) on glucose uptake in rat small intestine fragments (in vitro). C: control group ($N = 10$), **1**: escin Ia ($N = 8$), **2**: escin IIa ($N = 8$), **3**: *E*,*Z*-senegin II ($N = 5$), P: phlorizin ($N = 5$). The values obtained without test sample were taken to be 100%. Significantly different from the control group, $^{*}p < 0.05$, $^{**}p < 0.01$.

Blood glucose levels 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 at 5 mL/kg, (b) *intraperitoneal glucose-loaded rat*: 10% glucose solution in saline was administered intraperitoneally at 5 mL/kg, or (c) *normal rat (non-glucose-loaded rat)*: water was administered 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. Serum glucose levels were determined enzymatically using the glucose-oxidase method (kit reagent, Glucose CII-test Wako, Wako Pure Chemical Industries). In glucose-loading experiments, values are expressed as the serum glucose concentration in glucose-loaded rat minus the mean concentration in the normal group evaluated simultaneously.

Blood glucose levels 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 thereafter, 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 administration of test compound.

Gastric emptying in rats

Rats weighing 130–170 g were used. Test food consisting of 10% glucose, 1% 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 of 0.1 N NaOH. Then, 0.5 mL of 20% TCA was added to 5 mL of the 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 by the formula.

$$\text{Gastric emptying (\%)} = (\text{PRo-PRs})/\text{PRo} \times 100$$

PRo: amount of phenol red given orally.
PRs: 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 in Meir et al.[10] was modified and used for this experiment. Small fragments (0.1–0.15 g) of everted rat jejunum were placed in 950 μL of modified Krebs–Henseleit solution, pH 7.4, with D-[U-^{14}C]glucose and non-labeled D-glucose (final concentration: 2 mM, 1.0–1.5×10^5 cpm/mL). Then 50 μL of test sample solution in DMSO was added to the medium. Incubation was carried out at 30°C for 6 min, followed by washing twice for 3–5 s each time 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®). The solubilized samples were mixed with scintillator and the radioactivity was examined using a liquid scintillation counter (LS6500, Beckmann) to determine glucose uptake (μmol glucose/100 mg tissue). Phlorizin was used as a reference.

Statistics

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

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