Bioactive Saponins and Glycosides. VII.¹⁾ On the Hypoglycemic Principles from the Root Cortex of *Aralia elata* SEEM.: Structure Related Hypoglycemic Activity of Oleanolic Acid Oligoglycoside

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The hypoglycemic component, elatoside E, was isolated from the root cortex of *Aralia elata* SEEM. (Araliaceae) together with elatoside F and eight known oleanolic acid glycosides, elatosides A and C, oleanolic acid 3-*O*-[α -L-arabinofuranosyl (1 $\rightarrow$ 4)]- β -D-glucopyranosiduronic acid, oleanolic acid 3-*O*- β -D-glucopyranosiduronic acid, stipuleanosides R₁ and R₂, and chikusetsusaponins IV and IVa. The structures of elatosides E and F were determined on the basis of chemical and physicochemical evidence as oleanolic acid 3-*O*-[β -D-xylopyranosyl (1 $\rightarrow$ 2)][β -D-glucopyranosyl (1 $\rightarrow$ 3)]- α -L-arabinopyranoside and its 28-*O*- β -D-glucopyranosyl ester, respectively.

The hypoglycemic activity of oleanolic acid and nine oleanolic acid oligoglycosides from the root cortex of *Aralia elata* was determined by monitoring inhibition effect on the elevation of plasma glucose level by oral sucrose tolerance test in rats, and some structure–activity relationships of oleanolic acid glycoside were obtained.

Key words hypoglycemic principle; elatoside E; *Aralia elata*; oral sucrose tolerance test; oleanolic acid oligoglycoside; elatoside F

Many natural drugs and traditional preparations are used in Chinese traditional medicine as antidiabetics and several active components have been identified by *in vivo* bioassay using an experimental diabetic animal induced by alloxan or streptozotocin, and by *in vitro* bioassay testing aldose reductase inhibitory activity and glucose transport activity.²⁾ The bark and root cortex of *Aralia elata* SEEM. (Japanese name Taranoki, Araliaceae) are prescribed for tonic, antiarthritic, and antidiabetic purposes in Chinese traditional preparations, and in Japanese folk medicine, the root cortex of this plant is said to be useful for treating diabetes. In regard to the biologically active constituents of *Aralia elata*, many triterpene oligoglycosides having cytoprotective activity in hepatic injury have been isolated from the leaves of this plant,³⁾ while no report on its antidiabetic component has yet been published to our knowledge. We recently isolated potent inhibitors of ethanol absorption called elatosides A (3) and B from the bark of *Aralia elata* together with elatosides C (7) and D and, in the detailed pharmaceutical assessment, oleanolic acid 3-*O*-glucuronide structure was found to be essential to the inhibitory activity.^{1,4)} Furthermore, from the young shoots, which are known to be the edible part of *Aralia elata*, we isolated various olean-12-ene type triterpene glycosides called elatosides G, H, I, J, and K and reported their hypoglycemic activity.⁵⁾ In the course of our studies on the bioactive constituents of natural medicines,⁶⁾ we found that the glycoside fractions from the root cortex and bark of *Aralia elata* inhibit the rise of plasma glucose level in an oral sucrose tolerance test in rats, while that of the leaves showed no activity (Table 1). From the glycoside fraction of the root cortex with hypoglycemic activity, two oleanolic acid oligoglycosides, elatosides E (1) and F (2), were isolated together with eight known oleanolic acid glycosides.⁷⁾ This paper offers a full account of the structural elucidation of 1 and 2. In addition, we describe the

hypoglycemic activity of oleanolic acid glycosides from the root cortex of *Aralia elata* and the structural requirement of the glycosides for this activity.⁸⁾

The fresh root cortex of *Aralia elata* collected in Kyoto Prefecture was extracted with methanol under reflux. The methanolic extract was partitioned into an ethyl acetate–water mixture and the water phase was further extracted with 1-butanol. Since the 1-butanol-soluble portion (so-called glycoside fraction) showed hypoglycemic activity in the oral sucrose tolerance test, it was subjected to normal and reversed-phase silica-gel column chromatography and finally HPLC to afford elatosides A^{1,4)} (3, 0.004%), C^{1,4)} (7, 0.04%), E (1, 0.012%), and F (2, 0.015%), oleanolic acid 3-*O*- α -L-arabinofuranosyl (1 $\rightarrow$ 4)- β -D-glucopyranosiduronic acid⁹⁾ (5, 0.002%), ole-

Chart 1

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Table 1. Inhibitory Activity of the Glycoside Fractions from Root Cortex, Bark, and Leaves of *Aralia elata* on the Rise in Plasma Glucose Level by Oral Sucrose Tolerance Test

	Dose (mg/kg, <i>p.o.</i>)	<i>n</i>	Plasma glucose concentration (mg/dl)		
			0.5 h	1 h	2 h
Control (normal)	—	5	79.2 ± 5.4**	105.7 ± 7.1**	102.7 ± 6.8
Control (sucrose tolerance)	—	5	165.2 ± 12.3 (86.0 ± 12.3)	155.0 ± 8.6 (49.3 ± 8.6)	101.1 ± 6.0 (-1.6 ± 6.0)
Glycoside fraction of the root cortex	200	5	108.9 ± 15.3* (29.8 ± 15.3*)	139.2 ± 8.7 (33.5 ± 8.7)	121.6 ± 11.0 (18.9 ± 11.0)
Glycoside fraction of the bark	200	5	116.2 ± 7.5** (37.0 ± 7.5**)	146.0 ± 12.4 (40.3 ± 12.4)	136.5 ± 11.4 (33.8 ± 11.4)
Glycoside fraction of the leaves	200	5	187.2 ± 12.7 (108.0 ± 12.7)	171.6 ± 11.2 (65.9 ± 11.2)	109.6 ± 8.4 (6.9 ± 8.4)

* $p < 0.05$, ** $p < 0.01$.

anolic acid 3-*O*- β -D-glucopyranosiduronic acid¹⁰⁾ (**6**, 0.001%), stipuleanosides R₁¹¹⁾ (**4**, 0.005%) and R₂¹¹⁾ (**8**, 0.007%), and chikusetsusaponins IV¹²⁾ (**9**, 0.017%) and IVa⁹⁾ (**10**, 0.002%).

Chemical Structures of Elatosides E (**1**) and F (**2**)

Elatoside E (**1**) was isolated as colorless fine crystals of mp 192.5–194.0 °C from chloroform–methanol–aqueous acetic acid. The IR spectrum of **1** showed absorption bands at 1698 cm⁻¹ ascribable to carboxyl group and strong absorption bands at 3432 and 1076 cm⁻¹ suggestive of its oligoglycosidic structure. In the positive-mode FAB-MS of **1**, quasimolecular ion peaks were observed at m/z 905 (M+Na)⁺ and 889 (M+Li)⁺ and high-resolution MS analysis revealed the molecular formula of **1** to be C₄₆H₇₄O₁₆. Methanolysis of **1** with 9% hydrogen chloride–dry methanol liberated oleanolic acid (**11**) and methyl glycosides of arabinose, glucose, and xylose in a 1 : 1 : 1 ratio.¹³⁾ The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra of **1**, which were assigned by various NMR analytical methods,¹⁴⁾ showed signals due to an oleanolic acid part, α -L-arabinopyranosyl [δ 4.77 (d, $J = 7.3$ Hz, 1'-H)], β -D-xylopyranosyl [δ 5.40 (d, $J = 7.3$ Hz, 1''-H)], and β -D-glucopyranosyl [δ 5.31 (d, $J = 7.9$ Hz, 1'''-H)] moiety. The oligosaccharide structure bonding to the 3-position of **11** was characterized by an heteronuclear multiple bond correlation (HMBC) experiment. Namely, long-range correlations were observed between the anomeric proton (1'-H) of the arabinopyranosyl moiety and the 3-carbon of the oleanolic acid part, between the anomeric proton (1''-H) of the xylopyranosyl moiety and the 2'-carbon of the arabinopyranosyl moiety, and between the anomeric proton (1'''-H) of the glucopyranosyl moiety and the 3'-carbon of the arabinopyranosyl moiety. On the basis of this evidence and comparison of the ¹³C-NMR data for **1** with those for various known oleanolic acid oligoglycosides,^{3–5,9–11)} the structure of elatoside E was determined to be oleanolic acid 3-*O*-[β -D-xylopyranosyl (1→2)][β -D-glucopyranosyl (1→3)]- α -L-arabinopyranoside (**1**).

Elatoside F (**2**) was also obtained as colorless fine crystals of mp 212.5–214.0 °C and its IR spectrum showed absorption bands due to hydroxyl, ester, and carboxyl groups. Here again, the molecular formula C₅₂H₈₄O₂₁ was determined from the positive-mode FAB-MS [m/z 1067 (M+Na)⁺] and by high-resolution MS measure-

Table 2. ¹³C-NMR Data of Elatosides E (**1**) and F (**2**)

	1	2		1	2
C-1	38.8	38.8	C-27	26.2	26.1
C-2	26.7	26.7	C-28	180.2	176.5
C-3	89.2	89.2	C-29	33.3	33.2
C-4	39.7	39.8	C-30	23.8	23.7
C-5	56.0	56.0	3- <i>O</i> -Ara-1'	105.7	105.7
C-6	18.5	18.6	2'	77.4	77.4
C-7	33.3	33.2	3'	83.7	83.7
C-8	39.8	39.9	4'	69.0	69.0
C-9	48.1	48.1	5'	66.2	66.2
C-10	37.1	37.1	2'- <i>O</i> -Xyl-1''	105.1	105.2
C-11	23.8	23.8	2''	76.0	76.0
C-12	122.6	122.9	3''	79.1	79.1
C-13	144.8	144.1	4''	71.5	71.6
C-14	42.2	42.2	5''	67.1	67.1
C-15	28.3	28.3	3'- <i>O</i> -Glc-1'''	105.1	105.2
C-16	23.7	23.4	2'''	75.3	75.3
C-17	46.7	47.0	3'''	78.4	78.4
C-18	42.0	41.8	4'''	71.4	71.4
C-19	46.5	46.2	5'''	78.5	78.5
C-20	31.0	30.8	6'''	62.6	62.6
C-21	34.2	34.0	28- <i>O</i> -Glc-1''''		95.8
C-22	33.2	32.5	2''''		74.2
C-23	27.8	27.9	3''''		78.9
C-24	16.5	16.5	4''''		71.1
C-25	15.5	15.6	5''''		79.4
C-26	17.4	17.5	6''''		62.2

Pyridine-*d*₅, 68 MHz.

ment. Upon methanolysis with 9% hydrogen chloride–dry methanol, **2** gave **11** and methyl glycosides of arabinose, glucose, and xylose in a 1 : 2 : 1 ratio,¹³⁾ while alkaline hydrolysis of **2** with 5% aqueous sodium hydroxide furnished elatoside E (**1**). The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra¹⁴⁾ of **2** showed signals assignable to the elatoside E part [δ 4.75 (d, $J = 7.9$ Hz, 1'-H), 5.40 (d, $J = 7.6$ Hz, 1''-H), and 5.29 (d, $J = 7.6$ Hz, 1'''-H)] and the β -D-glucopyranosyl ester moiety [δ 6.32 (d, $J = 7.6$ Hz, 1''''-H)]. The HMBC experiment of **2** showed long-range correlations between the following protons and carbons: 1'-H and 3-C; 1''-H and 2'-C; 1'''-H and 3'-C; 1''''-H and 28-C. Consequently, the structure of elatoside F was elucidated as 28-*O*- β -D-glucopyranosyl oleanolic acid 3-*O*-[β -D-xylopyranosyl (1→2)][β -D-glucopyranosyl (1→3)]- α -L-arabinopyranoside (**2**).

Inhibitory Effect of Oleanolic Acid (**11**) and Elatosides

A (3), C (7), E (1), and F (2) and Other Oleanolic Acid Oligoglycosides (4, 5, 6, 8, 9) from the Root Cortex of *Aralia elata* on the Elevation of Plasma Glucose Level in Rats by Oral Sucrose Tolerance Test Since the root of *Aralia elata* has been said to be useful for treating diabetes, we examined the hypoglycemic activity of elatosides (1, 2, 3, 7) in the oral sucrose tolerance test in rats. To identify the structure-activity relationships of oleanolic acid glycoside, the hypoglycemic activity of other oleanolic acid glycosides (4, 5, 6, 8, 9) from the root cortex of *Aralia elata* and their common sapogenol, oleanolic acid (11) was examined. As shown in Tables 3 and 4, the oleanolic

acid 3-*O*-monodesmosides elatosides A (3) and E (1), stipuleanoside R₁ (4), and oleanolic acid 3-*O*-glycosides (5, 6) showed potent inhibitory activity against the rise of plasma glucose level in rats by oral sucrose tolerance test after a single oral dose of 100 mg/kg. Since oleanolic acid (11) lacked the inhibitory activity, the 3-*O*-glycoside moiety of these oligoglycosides was found to be essential for exerting the hypoglycemic activity. Among oleanolic acid 3,28-*O*-bisdesmosides tested, elatosides C (7) and F (2) lacked the activity, while stipuleanoside R₂ (4) and chikusetsusaponin IV (9), both of which possessed an α -L-arabinofuranosyl moiety at the 4'-position of the

	R ¹	R ²	R ³		R ¹	R ²	R ³
elatoside A (3)	β -D-Xyl	β -D-Gal	H	elatoside C (7)	β -D-Xyl	β -D-Gal	H
stipuleanoside R ₁ (4)	H	β -D-Gal	α -L-Ara (f)	stipuleanoside R ₂ (8)	H	β -D-Gal	α -L-Ara (f)
5	H	H	α -L-Ara (f)	chikusetsusaponin IV (9)	H	H	α -L-Ara (f)
6	H	H	H	chikusetsusaponin IVa (10)	H	H	H

β -D-Xyl : β -D-xylopyranosyl

β -D-Gal : β -D-galactopyranosyl

α -L-Ara (f) : α -L-arabinofuranosyl

oleanolic acid (11)

Table 3. Inhibitory Effect of Elatosides E (1), F (2), A (3), and C (7) on the Rise in Plasma Glucose Level by Oral Sucrose Tolerance Test

	Dose (mg/kg, <i>p.o.</i>)	<i>n</i>	Plasma glucose concentration (mg/dl)		
			0.5 h	1 h	2 h
Control (normal)		8	94.6±3.8**	108.6±3.5**	101.8±2.2
Control (sucrose tolerance)		6	170.0±5.2 (75.4±5.2)	153.0±1.9 (44.4±1.9)	124.7±3.6 (22.9±3.6)
Elatoside E (1)	100	6	112.8±3.8** (18.2±3.8**)	127.3±7.6* (18.7±7.6*)	117.3±7.5 (15.5±7.5)
Elatoside F (2)	100	6	171.2±2.2 (76.6±2.2)	153.5±4.5 (44.9±4.5)	120.7±2.8 (18.9±2.8)
Elatoside A (3)	100	6	122.3±7.6** (27.7±7.6**)	142.0±3.5* (33.4±3.5*)	138.2±5.4 (36.4±5.4)
Elatoside C (7)	100	6	166.0±4.7 (71.4±4.7)	154.0±6.4 (45.4±6.4)	134.2±3.6 (32.4±3.6)

* $p < 0.05$, ** $p < 0.01$.Table 4. Inhibitory Effects of Oleanolic Acid (11) and its Glycosides (4, 5, 6, 8, 9) from the Root Cortex of *Aralia elata* on the Rise in Plasma Glucose Level by Oral Sucrose Tolerance Test

	Dose (mg/kg, <i>p.o.</i>)	<i>n</i>	Plasma glucose concentration (mg/dl)		
			0.5 h	1 h	2 h
Control (normal)		5	78.8±4.2**	93.3± 5.0**	84.4±4.6**
Control (sucrose tolerance)		6	152.7±7.4 (73.9±7.4)	138.8± 4.7 (45.5± 4.7)	104.5±3.0 (20.1±3.0)
Stipuleanoside R ₁ (4)	100	6	126.5±7.3** (31.9±7.3**)	136.7± 5.7* (28.1± 5.7*)	131.3±5.2 (29.5±5.2)
5	100	6	96.8±2.9** (18.0±2.9**)	116.3± 4.3** (23.0± 4.3**)	101.0±2.2 (16.6±2.2)
6	100	6	105.7±6.4** (26.9±6.4**)	117.5± 7.2** (24.4± 7.2**)	113.0±4.4 (28.6±4.4)
Stipuleanoside R ₂ (8)	100	6	149.8±7.9 (55.2±7.9)	146.0±10.9 (37.4±10.9)	121.8±7.8 (20.0±7.8)
Chikusetsusaponin IV (9)	100	6	114.8±6.7** (36.0±6.7**)	111.0± 5.2** (17.7± 5.2**)	105.0±6.6 (20.6±6.6)
Oleanolic acid (11)	100	5	161.6±5.8 (82.8±5.8)	152.0± 6.1 (58.1± 6.1)	115.2±3.9 (30.8±3.9)

* $p < 0.05$, ** $p < 0.01$.

glucuronic acid part, exhibited hypoglycemic activity. This revealed that the 28-ester glucoside moiety significantly reduced the activity, while the 4'-*O*-arabinofuranosyl moiety tended to increase it. Detailed comparison of the hypoglycemic activities for **5** and **9** with those for **4** and **8** led us to suggest that the 3'-*O*-galactopyranosyl moiety tended to decrease the activity.

Hypoglycemic activity of oleanolic acid glycosides, which are the principal ingredients of the root cortex of *Aralia elata*, may be important evidence substantiating the traditional effect of this natural medicine such as its antidiabetic effect.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.¹⁾

Isolation of Elatosides E (1) and F (2) and Eight Known Oleanolic Acid Oligoglycosides (3, 4, 5, 6, 7, 8, 9, 10) from the Root Cortex of *Aralia elata* SEEM. The fresh root cortex of *Aralia elata* (1.7 kg, collected in Kyoto Prefecture) was cut finely and then extracted with MeOH three times under reflux. Evaporation of the solvent from the extract under reduced pressure gave the MeOH extract (98.9 g, 5.8% from natural medicine). The MeOH extract (93.9 g) was partitioned into AcOEt-H₂O

mixture and the H₂O-soluble portion was further extracted with *n*-BuOH. Removal of the solvent from the AcOEt-soluble, the *n*-BuOH-soluble, and the H₂O-soluble portions under reduced pressure yielded the AcOEt extract, the *n*-BuOH extract (64.4 g, 3.8%), and the H₂O extract.

The *n*-BuOH extract (62.2 g) was subjected to reversed-phase silica-gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical, Ltd., 500 g), H₂O→MeOH-H₂O (1:2→1:1)→MeOH] followed by evaporation of the solvent under reduced pressure to furnish four fractions [fr. 1 (0.1 g), fr. 2 (2.7 g), fr. 3 (27.6 g), fr. 4 (16.6 g)]. Fraction 3 (20 g) was separated by normal-phase silica-gel column chromatography {1 kg, CHCl₃-MeOH-H₂O [7:3:1 (lower layer)→65:35:10 (lower layer)]→MeOH} and HPLC [YMC-Pack ODS-5 (250×20 mm i.d.), MeOH-1% aqueous AcOH (70:30, v/v)] to give elatoside C (**7**, 487 mg, 0.04%), stipuleanoside R₂ (**8**, 85 mg, 0.007%), and chikusetsusaponins IV (**9**, 207 mg, 0.017%) and IVa (**10**, 24 mg, 0.002%). Fraction 4 (10 g) was purified by normal-phase silica-gel column chromatography [BW-200 (Fuji Silysia Chemical, Ltd., 500 g), CHCl₃-MeOH-H₂O (7:3:1, lower layer)→MeOH] and HPLC [YMC-Pack ODS-5 (YMC Co. Ltd., 250×20 mm i.d.), MeOH-1% aqueous AcOH (85:15, v/v)] to give elatosides E (**1**, 113 mg, 0.012%) and F (**2**, 139 mg, 0.015%), elatoside A (**3**, 38 mg, 0.004%), stipuleanoside R₁ (**4**, 47 mg, 0.005%), oleanolic acid 3-*O*-α-L-arabinofuranosyl (1→4)-β-D-glucopyranosiduronic acid (**5**, 19 mg, 0.002%), and oleanolic acid 3-*O*-β-D-glucopyranosiduronic acid (**6**, 10 mg, 0.001%). Eight known oleanolic acid oligoglycosides (**3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**) were identified by comparison of their physical data with the reported values.

Elatoside E (**1**): Colorless fine crystals from CHCl₃-MeOH-aqueous

AcOH, mp 192.5–194.0 °C, $[\alpha]_D^{25} +43.6^\circ$ ($c=1.0$, MeOH). High-resolution positive-mode FAB-MS Calcd for $C_{46}H_{74}NaO_{16}$ ($M+Na$)⁺: 905.4874. Found: 905.4868. IR (KBr): 3432, 2943, 1698, 1076 cm^{-1} . ¹H-NMR (pyridine-*d*₅) δ : 0.85, 0.97, 0.99, 1.02, 1.10 (3H each, all s, 25, 26, 29, 30, 24-H₃), 1.30 (6H, s, 23, 27-H₃), 3.28 (2H, dd-like, 3, 18-H), 4.77 (1H, d, $J=7.3$ Hz, 1'-H), 5.31 (1H, d, $J=7.9$ Hz, 1'''-H), 5.40 (1H, d, $J=7.3$ Hz, 1''-H), 5.47 (1H, brs, 12-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS m/z : 905 ($M+Na$)⁺, 889 ($M+Li$)⁺, 771, 743.

Elatoside F (2): Colorless fine crystals from $CHCl_3$ -MeOH-aqueous AcOH, mp 212.5–214.0 °C, $[\alpha]_D^{25} +24.3^\circ$ ($c=1.0$, MeOH). High-resolution positive-mode FAB-MS Calcd for $C_{52}H_{84}NaO_{21}$ ($M+Na$)⁺: 1067.5403. Found: 1067.5393. IR (KBr): 3432, 2944, 1734, 1638, 1076 cm^{-1} . ¹H-NMR (pyridine-*d*₅) δ : 0.88, 0.90, 0.92, 1.26, 1.28 (3H each, all s, 25, 30, 29, 27, 23-H₃), 1.10 (6H, s, 24, 26-H₃), 3.24 (2H, dd-like, 3, 18-H), 4.75 (1H, d, $J=7.9$ Hz, 1'-H), 5.29 (1H, d, $J=7.6$ Hz, 1'''-H), 5.40 (1H, d, $J=7.6$ Hz, 1''-H), 5.43 (1H, brs, 12-H), 6.32 (1H, d, $J=7.6$ Hz, 1'''-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS m/z : 1067 ($M+Na$)⁺, 905, 859.

Methanolysis of Elatosides E (1) and F (2) A solution of elatosides (1 mg each of 1 and 2) in 9% HCl-dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. The sapogenol constituent of each product, which was obtained from the filtrate by removal of the solvent under reduced pressure, was shown to be identical with an authentic oleanolic acid (11) by TLC [$CHCl_3$ -MeOH (10:1), benzene-acetone (3:1), and hexane-AcOEt (1:2)] comparisons. The sugar composition of the product was analyzed by GLC. A solution of each product in pyridine (0.01 ml) was treated with *N,O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA, 0.02 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify trimethylsilyl (TMS) derivatives of methyl glycosides [methyl arabinoside (i), methyl xyloside (ii), and methyl glucoside (iii)] from 1 and 2; GLC conditions: CBR1-M25-025, 0.25 mm (i.d.) $\times$ 25 m capillary column, column temperature 140–280 °C, 5 °C/min, He flow rate 15 ml/min, t_R : i (12.5, 12.7 min), ii (15.3, 15.7 min), iii (20.6, 20.7 min).

Alkaline Hydrolysis of Elatoside F (2) with 5% Aqueous NaOH Giving Elatoside E (1) A solution of 2 (100 mg) in 5% aqueous NaOH (5 ml) was stirred under reflux for 2 h. The reaction mixture was neutralized with Dowex HCR W $\times$ 2 (H⁺ form) and the resin was removed by filtration. Evaporation of the solvent from filtrate *in vacuo* yielded a product which was subjected to reversed-phase silica-gel column chromatography (2 g, $H_2O \rightarrow MeOH$) to give elatoside E (1, 83 mg). 1 was identified on the basis of TLC, $[\alpha]_D$, and ¹H- and ¹³C-NMR spectra comparisons with an authentic sample.

Bioassay for the Hypoglycemic Activity in Rats Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighing 125–155 g were starved for 20–24 h but allowed water *ad libitum*. The test samples were dissolved in water (5 ml/kg) and orally administered to the rats at each dose. At 30 min thereafter, a water solution (5 ml/kg) of sucrose (0.5 g/kg) was orally administered. Blood (0.4 ml) was collected from the carotid at 0.5, 1.0, and 2.0 h after D-glucose administration and the plasma glucose concentration was assayed by the enzymatic glucose oxidase method. Statistical significance of differences was estimated by analysis of variance (ANOVA) followed by Dunnett's test.¹⁵ Results were expressed as the mean $\pm$ S. E. (Tables 1, 3, and 4).

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References and Notes

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- This work was reported in our preliminary communication.⁷⁾ Recently, elatoside E (1) was isolated from the root bark of this plant as the methyl ester derivative (called tarasaponin III methyl ester) and elatoside F (2) was also reported as tarasaponin IV.¹⁶⁾
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- The proportions of carbohydrates were determined from the peak areas in GLC analysis.
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