ELATOSIDE E, A NEW HYPOGLYCEMIC PRINCIPLE FROM THE ROOT CORTEX OF *ARALIA ELATA* SEEM. : STRUCTURE-RELATED HYPOGLYCEMIC ACTIVITY OF OLEANOLIC ACID GLYCOSIDES

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A new inhibitor named elatosides E (which was shown to affect the elevation of plasma glucose level by oral sugar tolerance test in rats) was isolated from the root cortex of *Aralia elata* SEEM. together with elatoside F. The structures of elatosides E and F were elucidated on the basis of chemical and physicochemical evidence. The hypoglycemic activities of oleanolic acid and nine oleanolic acid glycosides obtained from the root cortex of *Aralia elata* have been examined, and some structure-activity relationships have been found.

KEYWORDS elatoside E; *Aralia elata*; hypoglycemic activity; oral sugar tolerance test; elatoside F

The root cortex of *Aralia elata* SEEM. (Japanese name "Taranoki", Araliaceae) is said to be useful for treating diabetes in Japanese folk medicine, and it has been used as a tonic, anti-arthritic, and anti-diabetic agent in Chinese traditional medicine. In regard to the biologically active constituents of *Aralia elata*, many saponins were isolated from the leaves of this plant and were reported to have a cytoprotective effect on carbon tetrachloride-induced hepatic injury.[1] Recently, we have isolated new inhibitors of ethanol absorption named elatosides A and B from the bark of *Aralia elata* together with elatosides C and D; by examination of the structure-activity relationships, oleanolic acid 3-*O*-monodesmoside structure was found to be essential to the activity.[2] In the course of our screening to find an anti-diabetic principle from natural medicine, the MeOH extract and saponin fraction from the root cortex of *Aralia elata* was found to exhibit potent hypoglycemic effect on oral sucrose and D-glucose tolerance tests in rats. This communication deals with characterization of the active principles in the root cortex by monitoring the inhibitory activity on the elevation of plasma glucose level and the structure-activity relationships of oleanolic acid glycoside.[3]

The MeOH extract of root cortex collected in Kyoto prefecture was partitioned into a AcOEt-water mixture, and the water soluble portion was further extracted with 1-BuOH. The 1-BuOH-soluble portion (so-called saponin fraction) of hypoglycemic activity was subjected to ordinary and reversed-phase SiO_2 column and HPLC to afford elatosides E(**2**, 0.012 % from the natural medicine) and F(**4**, 0.015 %) together with elatoside A[2](**1**, 0.004 %) and C[2](**3**, 0.015 %), oleanolic acid 3-*O*-glucuronide[4](**6**, 0.0001 %), tarasaponin VI[4](**7**, 0.019 %), chikusetsusaponin IV[4](**9**, 0.017 %), stipuleanoside R_1[5](**8**, 0.005 %), and R_2[5](**10**, 0.007 %).

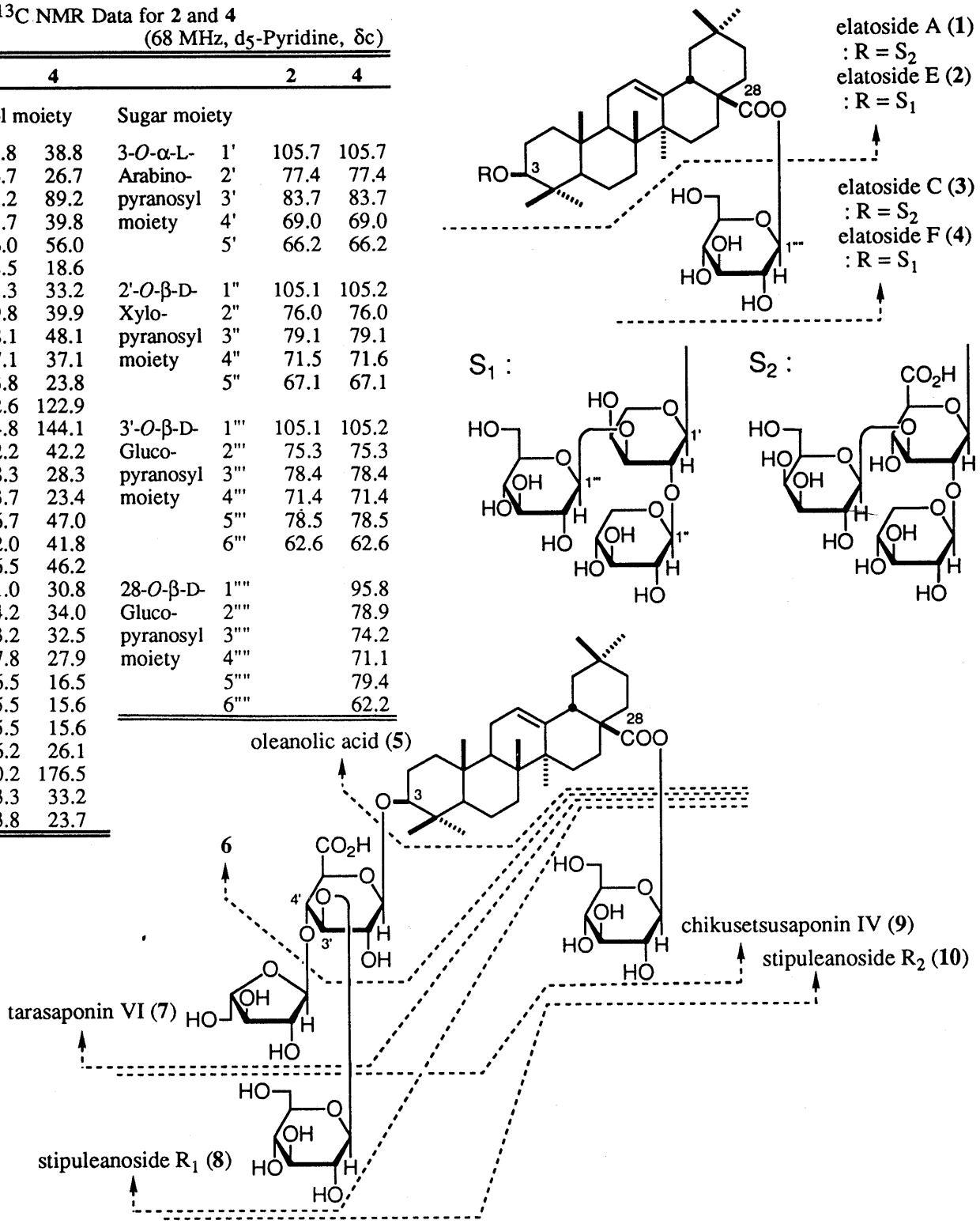
Elatoside E(**2**), colorless fine crystals, mp 192.5 - 194.0°C, $[\alpha]_D$ +43.6° (MeOH), $C_{46}H_{74}O_{26}$, IR(KBr) : 3432, 1698, 1076 cm^{-1}, positive FAB-MS(m/z) : 905(M+Na)$^+$, 889(M+Li)$^+$, gave oleanolic acid(**5**) together with methyl L-arabinoside, methyl D-glucoside, and methyl D-xyloside in a 1:1:1 ratio upon methanolysis(9% HCl-MeOH). The ^1H NMR(d_5-pyridine, J in Hz) and ^{13}C NMR (Table I) data for **2** were completely assigned by the COSY(^1H-^1H, ^1H-^{13}C), DEPT, HMBC, and HOHAHA(^1H-^1H, ^1H-^{13}C) experiment : δ 4.77(d, J = 7.3, 1'-H), 5.40(d, J = 7.3, 1"-H), 5.31(d, J = 7.9, 1-'"H). HMBC correlations were observed between the following carbons and protons in the oligosaccharide moiety of **2** : 3-C&1'-H, 2'-C&1"-H, 3'-C&1'"-H. Based on the ^{13}C NMR comparison for **2** with those for known oleanolic acid glycosides and examination of the ROESY data for **2**, the structure of elatoside E has been determined as **2**.

Elatoside F(**4**), colorless fine crystals, mp 212.5 - 214.0°C, $[\alpha]_D$ +24.3°(MeOH), $C_{52}H_{84}O_{21}$, IR(KBr) : 3432, 1734, 1076 cm^{-1}, ^1H NMR(d_5-pyridine, δ) : 4.75(d, J = 7.9, 1'-H), 5.40(d, J = 7.6, 1"-H), 5.29(d, J = 7.6, 1'"-H), 6.38(d, J = 7.6, 1""-H), positive FAB-MS(m/z) : 1067(M+Na)$^+$, liberated elatoside E(**2**) by alkaline hydrolysis(5% NaOH). Upon methanolysis(9% HCl-MeOH), **4** gave methyl L-arabinoside, methyl D-glucoside, and methyl D-xyloside in a 1:2:1 ratio and **5**. Based on detailed ^1H NMR and ^{13}C NMR (Table I) examination, the structure of elatoside F(**4**) was elucidated as shown.

Inhibitory effects of elatosides E(**2**) and F(**4**), oleanolic acid(**5**) and the other oleanolic acid glycosides(**1**, **3**, **6**, **7**, **8**, **9**, **10**) from the root cortex of *Aralia elata* on the elevation of plasma glucose level by oral sucrose tolerance test in rats are summarized in Table II and III. Not only oleanolic acid 3-*O*-monodesmosides(**1**, **2**, **6**, **7**, **8**) but also 3, 28-*O*-bisdesmosides

Table I. ^{13}C NMR Data for **2** and **4** (68 MHz, d_5-Pyridine, δc)

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



having 4'-*O*-arabinosyl moiety(**9**, **10**) exhibited hypoglycemic activity. It may be of interest that the above findings are different from those observed in ethanol absorption inhibitory activity of oleanolic acid glycosides.[2] The more potent activity is observed for elatoside E(**2**) and tarasaponin VI(**7**), while oleanolic acid 3, 28-*O*-bisdesmosides having 2', 3'-*O*-diglycoside moiety(**3**, **4**) and oleanolic acid(**5**) showed little activity.

Detailed examination of the hypoglycemic activity (Table III) of oleanolic acid glucuronides(**6**, **7**, **8**, **9**, **10**) from *Aralia elata* and oleanolic acid(**5**) led us to presume the following structure-activity relationships of oleanolic acid glycoside : 1) the 3-

Table II. Inhibitory Effects of Elatosides A(**1**), C(**3**), E(**2**), and F(**4**) on the Elevation of Plasma Glucose Level in Rats by Oral Sucrose Tolerance Test

	Dose (mg /kg, p.o.)	n	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 A (**1**)	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 (**3**)	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)
Elatoside E (**2**)	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 (**4**)	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)

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

Table III. Inhibitory Effects of Oleanolic acid(**5**) and its Glycosides(**6** - **10**) from the Root Cortex of *Aralia elata* on the Elevation of Plasma Glucose Level in Rats by Oral Sucrose Tolerance Test

	Dose (mg /kg, p.o.)	n	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)
Oleanolic acid (**5**)	100	5	161.6±5.8 (82.8±5.8)	152.0±6.1 (58.7±6.1)	115.2±3.9 (30.8±3.9)
Oleanolic acid 3-*O*-GluA (**6**)	100	6	105.7±6.4** (26.9±6.4**)	117.5±7.2** (24.2±7.2*)	113.0±4.4 (28.6±4.4)
Tarasaponin VI (**7**)	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)
Stipleanoside R_1 (**8**)	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)
Chikusetsu-saponin 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)
Stipuleanoside R_2 (**10**)	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)

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

Each sample was orally administered to rats 30 min before oral administration of sucrose(1g / kg). Numbers in parenthesis show the difference in plasma glucose concentration between normal control and each sample treatment.

O-glycoside moiety is essential to the activity; 2) the 28-ester glucoside moiety significantly reduces the activity; and 3) in the 3-*O*-oligoglycoside structure, the 3'-*O*-glucopyranosyl moiety tends to decrease the activity, while the 4'-*O*-arabinofuranosyl moiety tends to increase the activity. Since several hypoglycemic saponins(**2**, **7**, **9**) were more abundant in the root than the leaves and bark of this plant,[6] these saponins may be related to traditional effect of this crude drug.

We have also examined hypoglycemic effect of oleanolic acid glycosides from the root cortex and found a similar hypoglycemic effect of these glycosides to those on oral sucrose tolerance test. The detailed hypoglycemic activities of the above-mentioned oleanolic acid glycosides on various diabetic models are currently under investigation.

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