ELATOSIDES A AND B, POTENT INHIBITORS OF ETHANOL ABSORPTION IN RATS FROM THE BARK OF *ARALIA ELATA* SEEM.: THE STRUCTURE-ACTIVITY RELATIONSHIPS OF OLEANOLIC ACID OLIGOGLYCOSIDES

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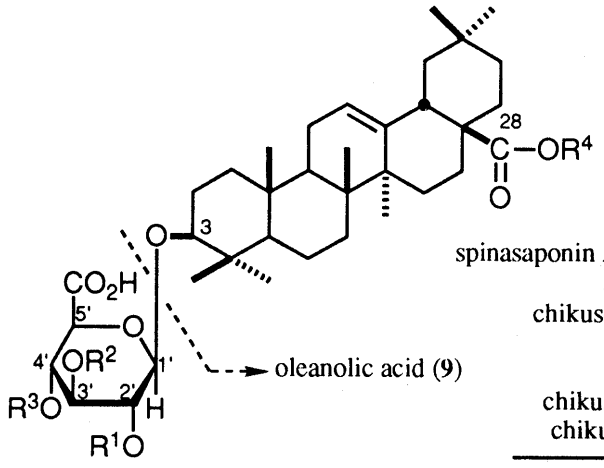
By monitoring the inhibitory effect on ethanol absorption in rats, new active saponins named elatosides A and B were isolated from the bark of *Aralia elata* SEEM. together with elatosides C and D. The structures of elatosides A, B, C, and D were elucidated on the basis of chemical and physicochemical evidence. The inhibitory effects of several oleanolic acid oligoglycosides on ethanol absorption have been examined and some structure-activity relationships have been found.

KEYWORDS ethanol absorption inhibitory effect; structure-activity relationship; oleanolic acid oligoglycoside; elatoside A; elatoside B; *Aralia elata*

Alcoholism, which is a major health problem in the world, causes as much trouble physiologically as it does socially. Excessive consumption of ethanol is known to affect profoundly nearly every organ. The hitherto reported methods to relieve ethanol toxicity in acute alcohol ingestion are classified into two categories: 1) methods by sequestering of ethanol and ethanol-induced acetoaldehyde in blood (*e.g.* D-penicillamine)[1]; 2) methods using accelerator of ethanol metabolism (*e.g.* clofibrate, γ-linolenic acid).[2] Recently, it has been reported that several plant extracts possessed the inhibitory activity on ethanol absorption,[3] but their active components have not been identified so far. In the course of our screening to find biologically active principles contained in Japanese and Chinese traditional medicine,[4] several crude drugs were found to contain inhibitors of ethanol absorption. This communication mainly deals with the results of studies on new inhibitors, elatosides A(**1**) and B(**2**), isolated from the bark of *Aralia elata* SEEM. (Araliaceae) and the structure-activity relationships in oleanolic acid oligoglycosides.[5]

The bark and root cortex of *Aralia elata* SEEM. (Taranoki in Japanese) has been used in Japanese and Chinese traditional medicine as a tonic, anti-arthritic, and anti-diabetes agent. In regard to the chemical constituents of this plant, many saponins have been isolated from the roots[6] and leaves.[7] We have found that the MeOH extract of the bark of *Aralia elata* SEEM. collected in Kyoto Prefecture has an inhibitory effect on ethanol absorption in rats. The MeOH extract of the bark was partitioned into AcOEt-water, and the water-soluble portion was further extracted with 1-BuOH. The 1-BuOH-soluble portion of the inhibitory activity was subjected to ordinary and reversed phase SiO_2 column and HPLC to afford elatosides A (**1**, 0.0093% from the crude drug) and B(**2**, 0.0018%), spinasaponin A[9](**5**, 0.0041%) and stipuleanoside R_1[10](**6**, 0.0104%) from the active fractions. On the other hand, elatosides C(**3**, 0.0392%) and D(**4**, 0.0332%) and 7[9](0.0244%) and stipuleanoside R_2[10](**8**, 0.0301%) were isolated from the inactive fractions.



	R^1	R^2	R^3	R^4
elatoside A (**1**):	β-D-Xyl	β-D-Gal	H	H
elatoside B (**2**):	β-D-Gal	β-D-Gal	H	H
elatoside C (**3**):	β-D-Xyl	β-D-Gal	H	β-D-Glu
elatoside D (**4**):	β-D-Gal	β-D-Gal	H	β-D-Glu
spinasaponin A (**5**):	H	β-D-Glu	H	H
stipuleanoside R_1 (**6**):	H	β-D-Glu	α-L-Ara(f)	H
spinasaponin A 28-O-glucoside (**7**):	H	β-D-Glu	H	β-D-Glu
stipuleanoside R_2 (**8**):	H	β-D-Glu	α-L-Ara(f)	β-D-Glu
chikusetsusaponin IVa (**10**):	H	H	H	H
11 :	H	H	α-L-Ara(f)	H
12 :	β-D-Glu	H	H	H
chikusetsusaponin IV (**13**):	H	H	α-L-Ara(f)	β-D-Glu
chikusetsusaponin V (**14**):	β-D-Glu	H	H	β-D-Glu

β-D-Xyl : β-D-xylopyranosyl β-D-Gal : β-D-galactopyranosyl
β-D-Glu : β-D-glucopyranosyl α-L-Ara(f) : α-L-arabinofuranosyl

Table I. Inhibitory Effects of Elatosides A(**1**), B(**2**), C(**3**), and D(**4**) and Other Oleanolic Acid Oligoglycosides (**5**, **6**, **7**, **8**) from the Bark of *Aralia elata* SEEM. on Ethanol Absorption

	Dose (mg /kg, p.o.)	n	Ethanol concentration in blood (mg / ml) 1h	2h	3h
Elatoside A (**1**)	25	4	0.11±0.02**	0.13±0.05	0.02±0.00
	50	4	0.01±0.01**	0.04±0.02**	0.01±0.00
	100	5	0.00±0.00**	0.00±0.01**	0.00±0.00
Elatoside B (**2**)	25	5	0.56±0.01	0.19±0.02	0.01±0.00
	50	5	0.50±0.04	0.19±0.02	0.02±0.01
	100	5	0.25±0.10*	0.18±0.02	0.02±0.00
Elatoside C (**3**)	100	5	0.57±0.02	0.24±0.02	0.04±0.00
Elatoside D (**4**)	100	4	0.57±0.01	0.23±0.02	0.04±0.00
Spinasaponin A (**5**)	25	5	0.26±0.07*	0.20±0.04	0.03±0.01
	50	5	0.03±0.01**	0.04±0.02**	0.02±0.01
	100	4	0.03±0.02**	0.02±0.01**	0.01±0.00
Stipuleanoside R_1 (**6**)	25	5	0.42±0.07	0.21±0.02	0.03±0.00
	50	4	0.34±0.09	0.18±0.03	0.01±0.00
	100	5	0.08±0.06**	0.09±0.04*	0.00±0.00
Spinasaponin A 28-O-glucoside(**7**)	100	3	0.58±0.01	0.21±0.00	0.00±0.00
Stipuleanoside R_2 (**8**)	100	5	0.56±0.02	0.23±0.02	0.04±0.00
Control		8	0.56±0.01	0.16±0.01	0.00±0.00

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

Table II. Inhibitory Effects of Chikusetsusaponins IVa(**10**), IV(**13**), and V (**14**), Prosapogenols (**11**, **12**), and Oleanolic Acid(**9**) on Ethanol Absorption

	Dose (mg / kg, p.o.)	n	Ethanol concentration in blood (mg / ml) 1h	2h	3h
Oleanolic acid (**9**)	100	4	0.59±0.06	0.22±0.01	0.02±0.00
Chikusetsu-saponin IVa (**10**)	25	5	0.18±0.10*	0.14±0.03	0.01±0.01
	50	5	0.06±0.04**	0.07±0.03**	0.00±0.00
	100	5	0.06±0.02**	0.03±0.01**	0.01±0.00
11	25	5	0.21±0.09*	0.11±0.04	0.00±0.00
	50	5	0.02±0.01**	0.03±0.01**	0.00±0.00
	100	5	0.00±0.00**	0.02±0.01**	0.01±0.00
12	25	5	0.60±0.02	0.21±0.01	0.09±0.02
	50	5	0.50±0.05*	0.16±0.03	0.09±0.02
	100	4	0.06±0.02**	0.04±0.02**	0.02±0.00
Chikusetsu-saponin IV (**13**)	100	5	0.55±0.01	0.21±0.02	0.01±0.00
Chikusetsu-saponin V (**14**)	100	4	0.64±0.02	0.21±0.02	0.02±0.00
Control		10	0.61±0.02	0.18±0.02	0.05±0.01

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

Elatoside A (**1**), colorless fine crystals mp 198.5-200.5°C, $[\alpha]_D$ +14.1° (MeOH), $C_{47}H_{74}O_{18}$, IR(KBr): 3420, 2944, 1698 cm^{-1}, positive FAB-MS(m/z): 949(M+Na)$^+$, gave oleanolic acid (**9**), methyl D-glucuronide, methyl D-galactoside, and methyl D-xyloside, upon methanolysis. The ^1H NMR (d_5-pyridine, J in Hz) and ^{13}C NMR (Table III) data for **1** were assigned by ^1H-^1H COSY, ^1H-^{13}C COSY, and Homonuclear Hartmann-Hahn spectroscopy (^1H-^1H HOHAHA, ^1H-^{13}C HOHAHA): 4.95(m,[11]) D-glucuronic acid 1'-H), 5.32(d, J=7.6, D-galactose 1'''-H), 5.54(d, J=7.2, D-xylose 1''-H). Comparison of the ^{13}C NMR data for **1** with those for known oleanolic acid glucuronide-saponins (*e.g.* **5**, **6**, **10**, **11**, **12**) led us to presume the oligosaccharide structure at 3-position in **1**. Furthermore, the ROE was observed between 1'-H and 1''-H in the ROESY spectrum, so that the D-xylopyranosyl moiety of **1** was shown directly linked to 2'-hydroxyl group of D-glucuronopyranosyl moiety. Consequently, the structure of elatoside A (**1**) has been determined.

Elatoside B(**2**), colorless fine crystals, mp 186.0-187.0°C, $[\alpha]_D$ +15.3° (MeOH), $C_{48}H_{76}O_{19}$, IR(KBr): 3430, 2946, 1698, ^1H NMR (d_5-pyridine, δ): 4.94(d, J = 7.2, D-glucuronic acid 1'-H), 5.31(d, J=7.6, D-galactose 1'''-H), 5.53(d, J=7.2, D-galactose 1''-H), positive FAB-MS(m/z): 979 (M+Na)$^+$, gave **9** together with methyl D-glucuronide and methyl D-galactoside in a 1:2 ratio upon methanolysis. These findings together with the examination of ^{13}C NMR data for **2** (Table III) led us to elucidate the structure of elatoside B (**2**).

The structures of elatoside C(**3**)[12] and elatoside D(**4**)[13] have been elucidated in the same way. Based on the ^1H NMR and ^{13}C NMR (Table III) analysis, it was concluded that **3** and **4** had the same oligoglycoside structure at 3-position as **1** and **2**, respectively, regardless of the presence of 28-O-D-glucopyranosyl moiety. Upon methanolysis, **3** liberated **9**, methyl D-glucuronide, methyl D-galactoside, methyl D-xyloside, and methyl D-glucoside, while **4** liberated **9**, methyl D-glucuronide, methyl D-galactoside, and methyl D-glucoside. Alkaline hydrolysis of **3** and **4** with 5% aq. NaOH gave **1** and **2**, respectively. Consequently, the structures of elatosides C(**3**) and D(**4**) were elucidated as shown.

Table III. ^{13}C NMR Data for **1**, **2**, **3**, and **4** (68 MHz, d_5-Pyridine, δc)

		1	2	3	4
3-O-β-D-Glucurono-pyranosyl moiety	1'	105.3	105.3	105.3	105.1
	2'	79.0	79.3	79.0	79.1
	3'	88.0	87.6	88.0	87.5
	4'	71.9	71.9	71.9	71.7
	5'	77.2	77.1	77.2	77.0
	6'	171.8	172.2	171.9	172.2
2'-O-β-D-Xylo- or galacto-pyranosyl moiety	1"	104.7	104.6	104.7	104.5
	2"	76.3	73.7	76.2	73.6
	3"	79.0	75.3	79.0	75.2
	4"	71.4	69.8	71.4	69.6
	5"	67.5	76.6	67.2	76.4
	6"		61.6		61.4
3'-O-β-D-Galacto-pyranosyl moiety	1"'	105.3	105.2	105.3	105.0
	2"'	72.9	72.9	72.9	72.8
	3"'	75.3	75.3	75.3	75.2
	4"'	70.1	70.2	70.1	70.0
	5"'	77.4	77.3	77.4	77.2
	6"'	62.0	62.0	62.0	62.0
28-O-β-D-Gluco-pyranosyl moiety	1""			95.8	95.6
	2""			74.2	74.0
	3""			78.9	78.7
	4""			71.1	70.9
	5""			79.3	79.1
	6""			62.2	61.8

Inhibitory effects of elatosides A(**1**), B(**2**), C(**3**), and D(**4**) and other oleanolic acid oligoglycosides(**5**, **6**, **7**, **8**) from the bark of *Aralia elata* on ethanol absorption in rats were summarized in Table I. Among the compounds tested, oleanolic acid 3-O-monodesmosides(**1**, **2**, **5**, **6**) showed potent inhibitory activity on ethanol absorption; elatoside A(**1**) possessed the highest activity among them. On the other hand, oleanolic acid 3, 28-O-bisdesmosides(**3**, **4**, **7**, **8**) exhibited little inhibitory effect, indicating that oleanolic acid 3-O-monodesmoside was essential to the inhibition of ethanol absorption. In order to clarify this assumption, the inhibitory effects of chikusetsusaponins IVa(**10**), IV(**13**), and V(**14**) and prosapogenols(**11**, **12**),[14] and oleanolic acid(**9**) were examined. As shown in Table II, chikusetsusaponin IVa(**10**) and prosapogenols(**11**, **12**), which have a glycosyl residue at the 3-position of **9**, showed an inhibitory activity similar to elatosides A(**1**) and B(**2**), irrespective of the branching position or sugar composition linked to the D-glucuronic acid moiety of **10**. Furthermore, bisdesmosides(**13**, **14**) and sapogenol(**9**) eliminated the activity completely, again suggesting oleanolic acid 3-O-glucuronide structure of the molecule to be essential to the activity.

In alleviating alcohol-related problems, crude drug constituents having inhibitory effects on ethanol absorption may be of potential therapeutic value. We are currently surveying the inhibitory effects of various oligoglycosides on ethanol absorption.

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(Received September 21, 1993)