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Bioactive Saponins and Glycosides. VI.[1] Elatosides A and B, Potent Inhibitors of Ethanol Absorption, from the Bark of *Aralia elata* SEEM. (Araliaceae): The Structure-Requirement in Oleanolic Acid Glucuronide-Saponins for the Inhibitory Activity

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Potent inhibitors of ethanol absorption, elatosides A and B, were isolated from the bark of *Aralia elata* SEEM. through bioassay-guided separation together with elatosides C and D and four known oleanolic acid glucuronide-saponins, spinasaponin A, spinasaponin A 28-*O*-glucoside, and stipuleanosides R_1 and R_2. The structures of elatosides A, B, C, and D were determined on the basis of chemical and physicochemical evidence as oleanolic acid 3-*O*-{[β-D-xylopyranosyl (1→2)] [β-D-galactopyranosyl (1→3)]}-β-D-glucopyranosiduronic acid, oleanolic acid 3-*O*-{[β-D-galactopyranosyl (1→2)] [β-D-galactopyranosyl (1→3)]}-β-D-glucopyranosiduronic acid, and their 28-*O*-glucopyranosyl esters, respectively.

The inhibitory effect of various oleanolic acid 3, 28-*O*-bisdesmosides, oleanolic acid 3-*O*-monodesmosides, and oleanolic acid on ethanol absorption was examined and it was found that the 3-*O*-glucuronide moiety and the 28-carboxyl group in oleanolic acid glucuronide-saponin were required to exert the inhibitory activity.

Key words ethanol absorption inhibitor; oleanolic acid oligoglycoside; elatoside A; elatoside B; *Aralia elata*; activity structure-requirement

Excessive consumption of alcohol (ethanol) is known to profoundly affect nearly every organ in the body. In order to relieve ethanol toxicity in heavy drinking, a couple of methods using accelerators of ethanol metabolism (*e.g.*, clofibrate,[2] methyl γ-linolenate,[3] and ginseng extract[4]) and sequesterings of acetaldehyde (*e.g.*, D-penicillamine and L-cysteine)[5] have been reported. In addition, dehydrogenase inhibitors (*e.g.*, cyanamide and disulfiram) have been used clinically for chronic alcoholics. The dehydrogenase inhibitors are known to force alcoholics to quit drinking based on the fear of unpleasant reaction elicited after ethanol intake, but these drugs are also reported to show many strong side effects.[6]

Many Chinese traditional medicines, however, are known to have preventive and therapeutic effects for various levels of ethanol intoxication,[7] but their active components have not yet been identified.[8] In the course of our screening to identify biologically active principles from natural medicines,[9] we found that the extract of several natural medicines shows inhibitory activity on ethanol absorption in rats. By monitoring the inhibitory effect on ethanol absorption, we characterized the active principles of elatosides, sapindosides, escins, camelliasaponins, senegins, and senegasaponins from the bark of *Aralia elata* SEEM. (Araliaceae),[10] the pericarps of *Sapindus mukurossi* (Japanese soapnut tree, Sapindaceae),[11] the seeds of *Aesculus hippocastanum* L. (horse chestnut tree, Hippocastanaceae),[12] the seeds of *Camellia japonica* L. (Camellia, Theaceae),[1,13] and the roots of *Polygala senega* L. var. *latifolia* TORREY *et* GRAY (Japanese senega snakeroot, Polygalaceae).[14] Furthermore, by examination of the structure requirement for the inhibitory activity on ethanol absorption, the active triterpene oligoglycosides could be classified into the following three types of structure: 1) Olean-12-en-28-oic acid 3-*O*-monodesmoside (elatosides and sapindosides); 2) Acylated polyhydroxyolean-12-ene 3-*O*-glucuronide (escins and camelliasaponins); 3) Olean-12-ene 3, 28-*O*-acylated-bisdesmoside (senegins and senegasaponins).[11]

This paper offers a full account of the isolation of inhibitors on ethanol absorption from the bark of *Aralia elata* SEEM. through bioassay-guided separation and the structure elucidation of elatosides A (**1**), B (**2**), C (**3**), and D (**4**). We also describe the structure-requirement in oleanolic acid glucuronide-saponins for the inhibitory activity.[15]

The bark and root cortex of *Aralia elata* SEEM. (Japanese angelica tree, "taranoki" in Japanese) have been used for tonic, antiarthritic, and antidiabetic purposes in Chinese traditional medicine. In regard to the biologically active constituents of *Aralia elata*, eleven oleanolic acid and hederagenin oligoglycosides have been isolated from the leaves of this plant and their cytoprotective effects reported on carbon tetrachloride-induced hepatic injury.[16] We recently isolated elatosides E, F, G, H, I, J, and K from the root cortex[17] and young shoot[18] of *Aralia elata* and clarified their structures and hypoglycemic activity in an oral sugar tolerance test in rats. We also found that the methanolic extract of the fresh bark of this plant inhibits ethanol absorption in rats after a single oral administration of 500 mg/kg. Through the procedures shown in Chart 1, the methanolic extract was subjected to bioassay-guided separation and purification. Thus, this extract was partitioned into an ethyl acetate-water mixture to furnish the ethyl acetate-soluble portion (the ethyl acetate extract) and water phase. The water phase was further extracted with 1-butanol to give the 1-butanol-soluble portion (the 1-butanol extract) and the water-soluble portion (the water extract). The 1-butanol-soluble portion showed inhibitory activity on ethanol absorption

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in a concentration dependent manner as shown in Table 1, while the ethyl acetate-soluble and water-soluble portions were found to lack the activity. The 1-butanol-soluble portion was subjected to reversed-phase silica-gel column chromatography to give three fractions [fr. 1 (sugars), fr. 2 (phenolic glycosides), and fr. 3 (saponins)]. The saponin fraction (fr. 3), which inhibited ethanol absorption after a single oral administration of 200 mg/kg, was further separated by ordinary-phase silica-gel column chromatography to afford five fractions (frs. 3-1—3-5). Fractions 3-1 and 3-2 with the inhibitory activity were purified by normal-phase silica-gel column chromatography and finally HPLC separation to give elatosides A[10] (**1**, 0.0093%) and B[10] (**2**, 0.0018%) together with spinasaponin A[19] (**6**, 0.0041%) and stipuleanoside R_1[20] (**7**, 0.0104%). Ordinary-phase silica-gel column chromatography and HPLC separation of the inactive fractions (frs. 3-4 and 3-5) yielded elatosides C (**3**, 0.0392%) and D (**4**, 0.0332%), spinasaponin A 28-*O*-glucoside[19] (**11**, 0.0224%), and stipuleanoside R_2[20] (**12**, 0.0301%).

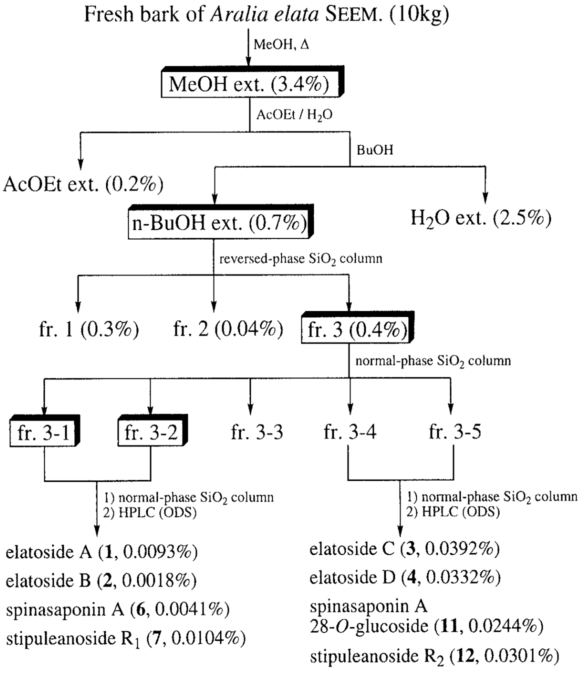


Chart 1

Chemical Structures of Elatosides A (1), B (2), C (3), and D (4) Elatoside A (**1**) was isolated as colorless fine crystals of mp 198.5—200.5 °C from chloroform–methanol solution. In the IR spectrum of **1**, it showed absorption bands at 1698 cm^{-1} ascribable to carboxyl group and strong broad bands at 3420 and 1076 cm^{-1} suggestive of glycosidic structure. The molecular formula $C_{47}H_{74}O_{18}$ was determined from the positive-mode FAB-MS and by high-resolution MS measurement. Thus, the positive-mode FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 949 $(M+Na)^+$, while two quasimolecular ion peaks were observed at *m/z* 933 $(M+Li)^+$ and 939 $(M+2Li-H)^+$ in the positive-mode FAB-MS adding lithium chloride. Methanolysis of **1** with 9% hydrogen chloride in dry methanol furnished oleanolic acid (**5**) and methyl glycosides of glucuronic acid, xylose, and galactose in a 1 : 1 : 1 ratio.[21] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) of **1**, which were assigned by various NMR analytical methods,[22] showed signals assignable to an oleanolic acid part [δ 3.26 (dd-like, 3-H), 5.45 (br s, 12-H)], β-D-glucuronic acid moiety [δ 4.95 (d-like, 1′-H)],[23] β-D-xylopyranosyl moiety [δ 5.54 (d, $J=7.2$ Hz, 1″-H)], and β-D-galactopyranosyl moiety [δ 5.32 (d, $J=7.6$ Hz, 1‴-H)]. Comparison of the ^{13}C-NMR data for **1** with those for several known oleanolic acid 3-*O*-glucuronide-saponins[17—20] led us to presume the oligoglycosidic structure at the 3-position of **5**. In the heteronuclear multiple bond correlation (HMBC) experiment of **1**, long-range correlations were observed between the anomeric proton (1′-H) of the glucuronic acid 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 glucuronic acid moiety, and between the anomeric proton (1‴-H) of the galactopyranosyl moiety and the 3′-carbon of the glucuronic acid

Table 1. Inhibitory Activity of Methanol Extract and Fractions from the Bark of *Aralia elata* SEEM. on Ethanol Absorption

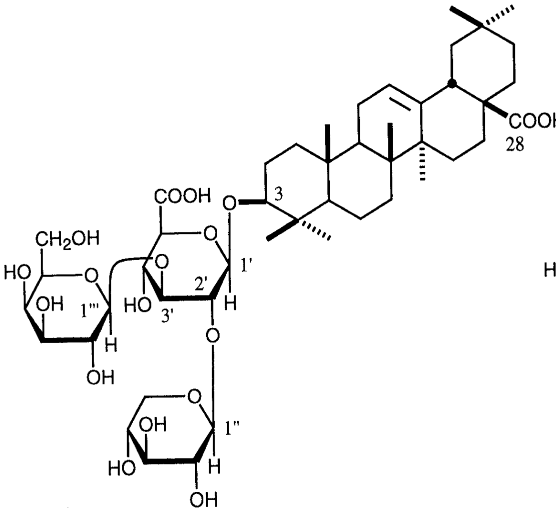
	Dose (mg/kg, *p.o.*)	*n*	Ethanol concentration in blood (mg/ml)		
			1 h	2 h	3 h
Control		5	0.60±0.01	0.24±0.02	0.08±0.01
MeOH extract	500	5	0.05±0.02**	0.08±0.02**	0.04±0.01
AcOEt extract	25	5	0.58±0.01	0.27±0.02	0.04±0.01
n-BuOH extract	50	5	0.51±0.02	0.19±0.03	0.02±0.01
	100	5	0.18±0.12	0.20±0.05	0.03±0.01
	200	5	0.02±0.00**	0.02±0.00**	0.02±0.00
H_2O extract	350	5	0.56±0.02	0.25±0.01**	0.04±0.00
Control		5	0.61±0.03	0.25±0.02	0.06±0.04
Fr. 1	200	5	0.62±0.04	0.27±0.01	0.02±0.01
Fr. 2	200	5	0.51±0.05	0.22±0.02	0.02±0.00
Fr. 3	200	5	0.02±0.00**	0.06±0.01**	0.03±0.01
Control		5	0.66±0.02	0.31±0.02	0.08±0.01
Fr. 3-1	200	5	0.06±0.01**	0.10±0.02**	0.08±0.01
Fr. 3-2	200	5	0.04±0.01**	0.07±0.01**	0.10±0.01
Fr. 3-3	200	5	0.34±0.11*	0.27±0.03	0.08±0.01
Fr. 3-4	200	5	0.65±0.02	0.31±0.02	0.09±0.01
Fr. 3-5	200	5	0.64±0.03	0.26±0.04	0.08±0.00

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

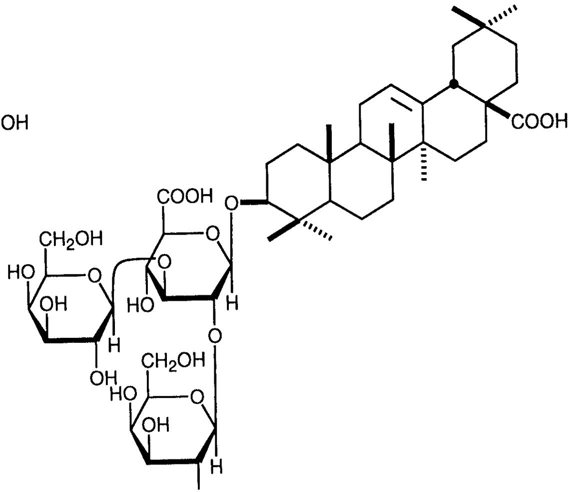
moiety. Furthermore, a correlation peak was observed between the 1′-H and the 1″-H in the rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectrum of **1**. On the basis of this evidence, the structure of elatoside A was determined to be oleanolic acid 3-*O*-{[*β*-D-xylopyranosyl (1→2)] [*β*-D-galactopyranosyl (1→3)]}-*β*-D-glucopyranosiduronic acid (**1**).

Elatoside B (**2**) was also isolated as colorless fine crystals of mp 186.0—187.0 °C from chloroform–methanol mixture and its IR spectrum showed absorption bands due to hydroxyl and carboxyl groups. The positive-mode FAB-MS of **2** showed quasimolecular ion peaks at *m*/*z* 979 $(M+Na)^+$ and 1001 $(M+2Na-H)^+$ and the molecular formula $C_{48}H_{76}O_{19}$ was determined by high-resolution MS measurement. Upon methanolysis with 9% hydrogen chloride-dry methanol, **2** liberated **5** together with methyl glucuronide and methyl galactoside in a 1:2 ratio.[21] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[22] of **2** showed the presence of an oleanolic acid part, a *β*-D-glucuronic acid moiety [δ 4.94 (d, J=7.3 Hz, 1′-H)], and two *β*-D-galactopyranosyl moieties [δ 5.53, 5.31 (both d, J=7.6 Hz, 1″, 1‴-H)]. Finally, in the HMBC experiment of **2**, long-range correlations were observed between the 1′-proton and the 3-carbon, between the 1″-proton and the 2′-carbon, and between the 1‴-proton and the 3′-carbon. These findings and comparison of the ^{13}C-NMR data for **2** with those for **1** and known oleanolic acid glucuronide-saponins[17-20] led us to formulate the structure of elatoside B as oleanolic acid 3-*O*-{[*β*-D-galactopyranosyl (1→2)] [*β*-D-galactopyranosyl (1→3)]}-*β*-D-glucopyranosiduronic acid (**2**).

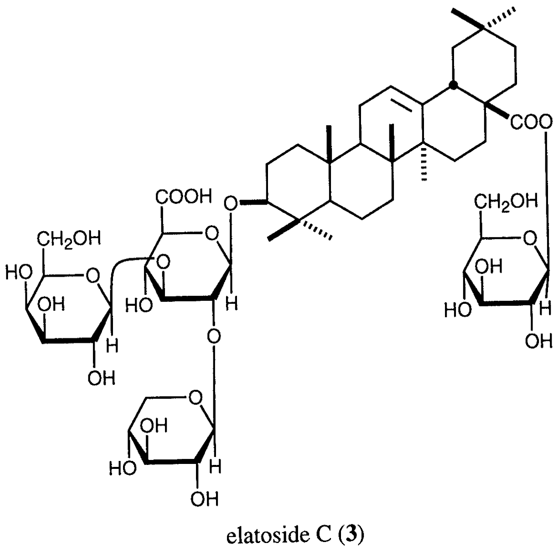
Elatoside C (**3**), obtained as colorless fine crystals of mp 208.5—209.5 °C, liberated **5** and methyl glycosides of glucuronic acid, xylose, galactose, and glucose in a 1:1:1:1 ratio upon methanolysis,[21] while alkaline hydrolysis of **3** with 5% aqueous sodium hydroxide yielded elatoside A (**1**). The IR spectrum of **3** showed absorption bands due to hydroxyl, ester, and carboxyl groups and the molecular formula $C_{53}H_{84}O_{23}$ was clarified from the quasimolecular ion peak in the positive-mode FAB-MS at *m*/*z* 1111 $(M+Na)^+$ and by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[22] of **3** showed signals assignable to an elatoside A part [δ 4.93 (d-like, 1′-H),[23] 5.54 (d, J=7.6 Hz, 1″-H), 5.31 (d, J=7.6 Hz, 1‴-H)] and 28-*O*-*β*-D-



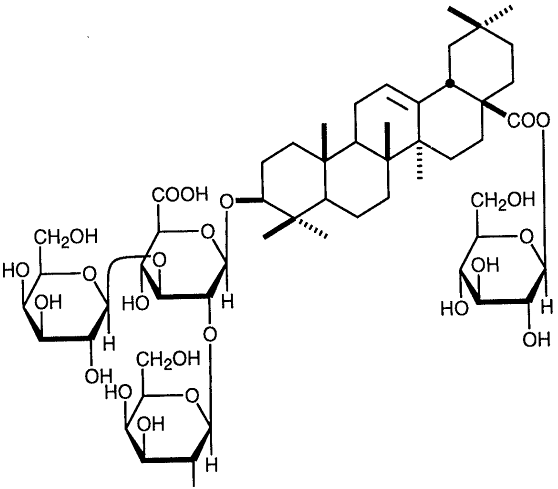
elatoside A (**1**)



elatoside B (**2**)



elatoside C (**3**)



elatoside D (**4**)

Chart 2

Table 2. ^{13}C-NMR Data of Elatosides A (**1**), B (**2**), C (**3**), and D (**4**)

	1	**2**	**3**	**4**
C-1	38.6	38.6	38.6	38.6
C-2	26.6	26.6	26.6	26.6
C-3	89.8	89.6	89.8	89.6
C-4	39.7	39.6	39.7	39.6
C-5	55.8	55.8	55.8	55.7
C-6	18.5	18.5	18.5	18.5
C-7	33.2	33.2	33.1	33.2
C-8	39.7	39.7	39.9	39.9
C-9	48.0	47.9	48.0	48.0
C-10	36.9	36.9	36.9	36.9
C-11	23.8	23.8	23.6	23.8
C-12	122.5	122.6	122.9	122.9
C-13	144.8	144.8	144.1	144.1
C-14	42.1	42.2	42.1	42.2
C-15	28.3	28.3	28.2	28.2
C-16	23.8	23.8	23.4	23.5
C-17	46.6	46.7	47.0	47.0
C-18	42.0	42.0	41.7	41.8
C-19	46.5	46.5	46.2	46.2
C-20	31.0	31.0	30.8	30.8
C-21	34.2	34.2	34.0	34.0
C-22	33.2	33.2	32.5	32.6
C-23	27.7	28.1	27.7	27.9
C-24	16.4	16.7	16.4	16.7
C-25	15.4	15.4	15.5	15.5
C-26	17.4	17.4	17.4	17.5
C-27	26.2	26.2	26.1	26.1
C-28	180.2	180.2	176.4	176.3
C-29	33.3	33.3	33.1	33.2
C-30	23.8	23.8	23.6	23.6
3-GlcA-1'	105.3	105.3	105.3	105.3
2'	79.0	79.3	79.0	79.3
3'	87.8	87.6	88.1	87.7
4'	71.8	71.9	71.9	71.9
5'	77.2	77.1	77.2	77.2
6'	171.8	172.2	171.8	172.2
2'-Xyl or Gal-1''	104.7	104.6	104.7	104.7
2''	76.1	73.7	76.1	73.8
3''	78.9	75.3	79.0	75.4
4''	71.3	69.8	71.3	69.8
5''	67.1	76.6	67.1	76.6
6''		61.6		61.6
3'-Gal-1'''	105.2	105.2	105.3	105.2
2'''	72.9	72.9	72.9	73.0
3'''	75.3	75.3	75.3	75.4
4'''	70.1	70.2	70.1	70.2
5'''	77.3	77.3	77.5	77.4
6'''	62.0	62.0	61.9	62.0
28-Glc-1''''			95.7	95.8
2''''			74.1	74.2
3''''			78.9	78.9
4''''			71.1	71.1
5''''			79.3	79.3
6''''			62.2	62.2

68 MHz, pyridine-d_5.

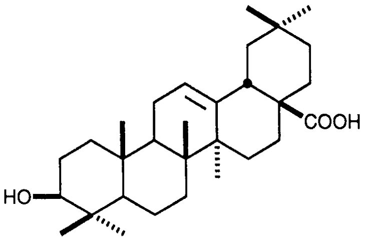
glucopyranosyl ester glycoside moiety [δ 6.33 (d, $J=7.9$ Hz, 1''''-H)]. Consequently, the structure of elatoside C was characterized as 28-*O*-β-D-glucopyranosyloleanolic acid 3-*O*-{[β-D-xylopyranosyl (1→2)] [β-D-galactopyranosyl (1→3)]}-β-D-glucopyranosiduronic acid (**3**).

Elatoside D (**4**) was also obtained as colorless fine crystals of mp 188.5—189.5 °C and its IR spectrum showed the presence of hydroxyl, ester, and carboxyl group. Here again, the molecular formula $C_{54}H_{86}O_{24}$ was determined from the positive-mode FAB-MS [*m/z* 1141 $(M+Na)^+$] and by high-resolution MS measurement. The methanolysis of **4** furnished **5** and methyl glycosides of glucuronic acid, galactose, and glucose in a 1 : 2 : 1 ratio,[21] while **4** liberated elatoside B (**2**) upon alkaline hydrolysis. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[22] of **4** showed the presence of an elatoside B part [δ 4.82 (d, $J=7.3$ Hz, 1'-H), 5.39, 5.16 (both d, $J=7.6$ Hz, 1'', 1'''-H)] and 28-*O*-β-D-glucopyranosyl ester glycoside moiety [δ 6.19 (d, $J=7.6$ Hz, 1''''-H)]. On the basis of this evidence, the structure of elatoside D was determined to be 28-*O*-β-D-glucopyranosyl oleanolic acid 3-*O*-{[β-D-galactopyranosyl (1→2)] [β-D-galactopyranosyl (1→3)]}-β-D-glucopyranosiduronic acid (**4**).

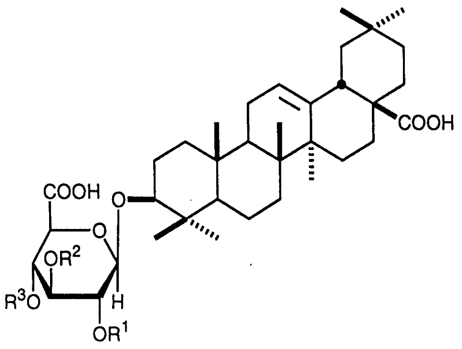
Inhibitory Activity of Elatosides A (1), B (2), C (3), and D (4) and Other Oleanolic Acid Oligoglycosides (6, 7, 11, 12) from the Bark of *Aralia elata* on Ethanol Absorption All oleanolic acid oligoglycosides (**1**, **2**, **6**, **7**) isolated from the fractions with inhibitory activity on ethanol absorption were found to possess a glycosyl residue at the 3-hydroxyl group of oleanolic acid (**5**), and they are chemically classified as oleanolic acid 3-*O*-monodesmoside. On the other hand, **3**, **4**, **11**, **12** obtained from the inactive fraction have two glycosyl residues at the 3 and 28-positions of **5** (oleanolic acid 3,28-*O*-bisdesmoside). These oleanolic acid oligoglycosides were dissolved in water and were orally administered to male Wistar rats. As the control, the same value of water instead of the saponin solution was orally administered to the rats. The blood was collected at 1, 2, and 3 h after ethanol administration and their ethanol concentrations were assayed by the enzyme method.

Oleanolic acid 3-*O*-monodesmosides (**1**, **2**, **6**, **7**) were found to show potent inhibitory activity on ethanol absorption after a single oral administration at the dose of 100 mg/kg. Particularly, elatoside A (**1**) and spinasaponin A (**6**) inhibited ethanol absorption in a dose dependent manner at 25—100 mg/kg. Based on the detailed pharmacological assessment, these oleanolic acid 3-*O*-monodesmosides showed more potent inhibitory activity than the acylated polyhydroxyolean-12-ene 3-*O*-glucuronide-saponins escins[12] and camelliasaponins[13] and the olean-12-ene 3,28-*O*-acylated bisdesmosides senegins and senegasaponins.[14] Oleanolic acid 3,28-*O*-bisdesmosides (**3**, **4**, **11**, **12**), however, lacked the inhibitory activity. This evidence reveals that oleanolic acid 3-*O*-monodesmoside structure is essential to the inhibitory activity in ethanol absorption.

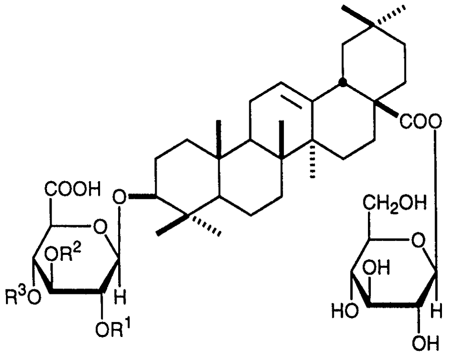
Inhibitory Activity of Oleanolic Acid (5), Oleanolic Acid 3-*O*-Monodesmosides (8, 9, 10) and Oleanolic Acid 3, 28-*O*-Bisdesmosides (13, 14) on Ethanol Absorption To ob-



oleanolic acid (**5**)



	R^1	R^2	R^3
spinasaponin A (**6**)	H	β-D-Glc	H
stipuleanoside R_1 (**7**)	H	β-D-Glc	α-L-Ara (f)
oleanolic acid 3-*O*-glucuronide (**8**)	H	H	H
9	H	H	α-L-Ara (f)
10	β-D-Glc	H	H



	R^1	R^2	R^3
spinasaponin A 28-*O*-glucoside (**11**)	H	β-D-Glc	H
stipuleanoside R_2 (**12**)	H	β-D-Glc	α-L-Ara (f)
chikusetsusaponin IV (**13**)	H	H	α-L-Ara (f)
chikusetsusaponin V (**14**)	β-D-Glc	H	H

β-D-Glc : β-D-glucopyranosyl
α-L-Ara (f) : α-L-arabinofuranosyl

Chart 3

Table 3. Inhibitory Activity of Elatosides A (**1**), B (**2**), C (**3**), and D (**4**) and Other Oleanolic Acid Oligoglycosides (**6**, **7**, **11**, **12**) from the Bark of *Aralia elata* Seem. on Ethanol Absorption

	Dose (mg/kg *p.o.*)	*n*	Ethanol concentration in blood (mg/ml)		
			1 h	2 h	3 h
Control		8	0.57±0.01	0.19±0.01	0.03±0.02
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±0.09*	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 (**6**)	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 (**7**)	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 (**11**)	100	3	0.58±0.01	0.21±0.00	0.00±0.00
Stipuleanoside R_2 (**12**)	100	5	0.56±0.02	0.23±0.02	0.04±0.00

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

Table 4. Inhibitory Activity of Oleanolic Acid (**5**), Prosapogenols (**8**, **9**, **10**), and Chikusetsusaponins IV (**13**) and V (**14**) on Ethanol Absorption

	Dose (mg/kg *p.o.*)	*n*	Ethanol concentration in blood (mg/ml)		
			1 h	2 h	3 h
Control[a]		10	0.61±0.02	0.18±0.02	0.05±0.01
Oleanolic acid (**5**)	100	4	0.59±0.06	0.22±0.01	0.02±0.00
Oleanolic acid 3-*O*-glucuronide (**8**)	25	5	0.18±0.09*	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
9	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
10	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
Chikusetsusaponin IV (**13**)	100	5	0.55±0.01	0.21±0.02	0.01±0.00
Chikusetsusaponin V (**14**)	100	4	0.64±0.02	0.21±0.02	0.02±0.00
Control[b]		5	0.40±0.03	0.12±0.01	0.00±0.00
8	100	5	0.40±0.00	0.10±0.01	0.00±0.00

The ethanol solution was administered orally *a*) or intraperitoneally *b*). * $p<0.05$, ** $p<0.01$.

tain further evidence for the structure–activity relationships of oleanolic acid oligoglycosides, we examined the inhibitory activity of oleanolic acid (**5**), oleanolic acid 3-*O*-monodesmosides (**8**, **9**, **10**), and oleanolic acid 3,28-*O*-bisdesmosides [chikusetsusaponins IV (**13**) and V (**14**)] on ethanol absorption. As shown in Table 4, oleanolic acid 3-*O*-monodesmosides (**8**, **9**, **10**) exhibited potent inhibitory activity similar to **1**, **2**, **6**, and **7**, while oleanolic acid 3,28-*O*-bisdesmosides (**13**, **14**) were confirmed to lack the activity, and the common aglycone oleanolic acid (**5**) was also found to show little activity. These findings led us to corroborate that the 3-*O*-glycoside moiety and 28-carboxyl group in oleanolic acid oligoglycoside are essential for exertion of the inhibitory activity on ethanol absorption.

We further found that when 20% aqueous ethanol was intraperitoneally administered to rats, oleanolic acid 3-*O*-glucuronide (**8**) did not decrease the ethanol level in the blood. Although the inhibition mechanism of oleanolic acid 3-*O*-monodesmosides on ethanol absorption is not yet understood, we have deduced that these monodesmosides decrease the ethanol concentration in the blood by inhibiting absorption across the cell membranes of the digestive tract.

Alcoholism is a major health problem in the world and is known to cause as much trouble physiologically as it does socially. Recently, acute alcoholism in young people induced by the excessive intake of such alcohol as "Ikkinomi" has caused public distress in Japan. Inhibitors of alcohol absorption such as elatosides may have a potential preventive effect for acute alcoholism and may also be available for chronic alcoholics as an antialcohol drug.

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 A (1), B (2), C (3), and D (4) and Known Oleanolic Acid Oligoglycosides (6, 7, 11, 12) from the Bark of *Aralia elata* Seem. The fresh bark of *Aralia elata* (10 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 (340 g, 3.4% from natural medicine). The MeOH extract was partitioned into AcOEt–H_2O mixture and the water-soluble portion was further extracted with *n*-BuOH. Removal of the solvent from the AcOEt-soluble, the *n*-BuOH-soluble, and the H_2O-soluble portions under reduced pressure yielded the AcOEt extract (20 g, 0.2%), the *n*-BuOH extract (70 g, 0.7%), and the H_2O extract (250 g, 2.5%).

The *n*-BuOH extract was subjected to Chromatorex ODS silica-gel column chromatography [2 kg, MeOH–H_2O (v/v, 1:2→1:1)→MeOH] followed by evaporation of the solvent under reduced pressure to furnish three fractions [fr. 1 (31 g, sugar fraction), fr. 2 (4.0 g, phenolic glycoside fraction), and fr. 3 (35 g, saponin fraction)]. Fraction 3 (30 g) was purified by normal-phase silica-gel column chromatography [1 kg, $CHCl_3$–MeOH–H_2O (7:3:1, lower layer)] to give five fractions [fr. 3-1 (1.67 g), fr. 3-2 (6.40 g), fr. 3-3 (3.78 g), fr. 3-4 (13.94 g), and fr. 3-5 (0.62 g)]. Fractions 3-2 and 3-3 (10.0 g) were subjected to silica-gel column chromatography [300 g, $CHCl_3$–MeOH–H_2O (65:35:10, lower layer)] and HPLC [YMC-Pack ODS-5 (250 × 20 mm i.d.), MeOH–1% aqueous AcOH (v/v, 4:1)] to give elatoside A (**1**, 0.0093%), elatoside B (**2**, 0.0018%), spinasaponin A (**6**, 0.0041%), and stipuleanoside R_1 (**7**, 0.0104%). Fractions 3-4 and 3-5 (10.0 g) were subjected to normal-phase silica-gel column chromatography [300 g, $CHCl_3$–MeOH–H_2O (6:4:1)] and HPLC [MeOH–1% aqueous AcOH (v/v, 7:3)] to give elatoside C (**3**, 0.0392%), elatoside D (**4**, 0.0332%), spinasaponin A 28-*O*-glucoside (**11**, 0.0244%), and stipuleanoside R_2 (**12**, 0.0301%). Four known saponins (**6**, **7**, **11**, **12**) were identified by comparison of their physical data with the reported values.[19,20)]

Elatoside A (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 198.5—200.5 °C, $[\alpha]_D^{20}$ +14.1° (c=0.5, MeOH). High-resolution positive-mode FAB-MS Calcd for $C_{47}H_{74}NaO_{18}$ $(M+Na)^+$: 949.4773. Found: 949.4747. IR (KBr) cm^{-1}: 3420, 2944, 1698, 1636, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.79, 1.01, 1.06, 1.27, 1.32 (3H each, all s, 25, 30, 24, 23, 27-H_3), 0.97 (6H, s, 26, 29-H_3), 3.25 (1H, dd-like, 18-H), 3.26 (1H, dd-like, 3-H), 4.95 (1H, d-like, 1′-H), 5.32 (1H, d, J=7.6 Hz, 1‴-H), 5.45 (1H, br s, 12-H), 5.54 (1H, d, J=7.2 Hz, 1″-H). ^{13}C-NMR : given in Table 2. Positive-mode FAB-MS (m/z): 949 $(M+Na)^+$, 933 $(M+Li)^+$, 939 $(M+2Li-H)^+$.

Elatoside B (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 186.0—187.0 °C, $[\alpha]_D^{21}$ +15.3° (c=0.5, MeOH). High-resolution positive-mode FAB-MS Calcd for $C_{48}H_{76}NaO_{19}$ $(M+Na)^+$: 979.4879. Found : 979.4866, Calcd for $C_{48}H_{75}Na_2O_{19}$ $(M+2Na-H)^+$: 1001.4698. Found: 1001.4743. IR (KBr) cm^{-1}: 3430, 2946, 1698, 1638, 1078. ^{1}H-NMR (pyridine-d_5) δ: 0.77, 1.00, 1.11 (3H each, all s, 25, 30, 24-H_3), 0.96, 1.31 (6H each, both s, 26, 29, 23, 27-H_3), 3.27 (2H, dd-like, 3, 18-H), 4.94 (1H, d, J=7.3 Hz, 1′-H), 5.31 (1H, d, J=7.6 Hz, 1‴-H), 5.45 (1H, br s, 12-H), 5.53 (1H, d, J=7.6 Hz, 1″-H). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 979 $(M+Na)^+$, 1001 $(M+2Na-H)^+$.

Elatoside C (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp

208.5—209.5 °C, $[\alpha]_D^{21}$ −1.6° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{53}H_{84}NaO_{23}$ $(M+Na)^+$: 1111.5302. Found: 1111.5280. IR (KBr) cm^{-1}: 3389, 2946, 1734, 1076. ^1H-NMR (pyridine-d_5) δ: 0.82, 0.88, 0.92, 1.07, 1.08, 1.26, 1.29 (3H each, all s, 25, 30, 29, 24, 26, 23, 27-H_3), 3.19 (1H, dd-like, 18-H), 3.24 (1H, dd-like, 3-H), 4.93 (1H, d-like, 1'-H), 5.31 (1H, d, J=7.6 Hz, 1'''-H), 5.41 (1H, br s, 12-H), 5.54 (1H, d, J=7.6 Hz, 1''-H), 6.33 (1H, d, J=7.9 Hz, 1''''-H). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1111 $(M+Na)^+$.

Elatoside D (**4**): Colorless fine crystals from $CHCl_3$–MeOH, mp 188.5—189.5 °C, $[\alpha]_D^{22}$ +6.9° (c=0.5, MeOH). High-resolution positive-mode FAB-MS Calcd for $C_{54}H_{86}NaO_{24}$ $(M+Na)^+$: 1141.5407. Found: 1141.5432. IR (KBr) cm^{-1}: 3368, 2946, 1734, 1078. ^1H-NMR (pyridine-d_5) δ: 0.66, 0.75, 0.78, 0.94, 0.99, 1.13, 1.16 (3H each, all s, 25, 30, 29, 24, 26, 23, 27-H_3), 3.05 (1H, dd-like, 18-H), 3.14 (1H, dd-like, 3-H), 4.82 (1H, d, J=7.3 Hz, 1'-H), 5.16 (1H, d, J=7.6 Hz, 1'''-H), 5.27 (1H, br s, 12-H), 5.39 (1H, d, J=7.6 Hz, 1''-H), 6.19 (1H, d, J=7.6 Hz, 1''''-H). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1141 $(M+Na)^+$.

Methanolysis of Elatosides A (1), B (2), C (3), and D (4) A solution of elatosides (each 1 mg, **1**, **2**, **3**, **4**) 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 (**5**) by TLC [$CHCl_3$–MeOH (10 : 1), benzene–acetone (3 : 1), 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 glucuronide (i), methyl xyloside (ii), and methyl galactoside (iii) from **1**; i and iii from **2**; i, ii, iii, and methyl glucoside (iv) from **3**; i, iii, and iv from **4**; GLC conditions: CBR1-M25-025, 0.25 (i.d.) × 25 m capillary column, column temperature 140—280 °C, He flow rate 15 ml/min, t_R: i (18.0, 21.0, and 21.3 min), ii (15.6 and 16.0 min), iii (19.0, 19.7 min), and iv (20.6 min).

Alkaline Hydrolysis of Elatoside C (3) with 5% Aqueous NaOH Giving Elatoside A (1) A solution of **3** (50 mg) in 5% aqueous NaOH (3 ml) was stirred under reflux for 2 h. The reaction mixture was neutralized with Dowex HCR W×2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate *in vacuo* yielded a product, which was subjected to reversed-phase silica-gel column chromatography (1g, $H_2O \rightarrow$ MeOH) to give elatoside A (**1**, 41 mg). **1** was identified on the basis of TLC, $[\alpha]_D$, and ^1H- and ^{13}C-NMR spectra comparisons with an authentic sample.

Alkaline Hydrolysis of Elatoside D (4) with 5% Aqueous NaOH Giving Elatoside B (2) A solution of **4** (100 mg) in 5% aqueous NaOH (5 ml) was stirred under reflux for 2 h. The reaction mixture was neutralized with Dowex HCR W×2 (H^+ form) and the resin was removed by filtration. After work-up of the filtrate in the above mentioned manner, the product was subjected to reversed-phase silica-gel column chromatography (2 g, $H_2O \rightarrow$ MeOH) to give elatoside B (**2**, 83 mg), which was identified with an authentic sample by TLC, $[\alpha]_D$, and ^1H- and ^{13}C-NMR spectra comparisons.

Effects on Blood Ethanol Level Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama) weighing 130—170 g were fasted for 20—24 h but were given water *ad libitum*. The tested samples were orally administered to the rats at each dose as a suspension of 5% gum arabic solution (5 ml/kg). At 1 h thereafter, 20% aqueous ethanol (5 ml/kg) was orally (*p.o.*) or intraperitoneally (i.p.) administered. Blood (*ca.* 0.4 ml) was collected from the jugular vein at 1, 2, and 3 h after the ethanol administration. The ethanol concentration in the blood was assayed by the enzyme method (blood alcohol test "BMY", Boehringer–Mannheim Yamanouchi). Statistical significance was estimated by analysis of variance (ANOVA) followed by Dunnett's test.[24] Results were expressed as the mean ± S.E. (Tables 3, 4).

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