Bioactive Saponins and Glycosides. I. Senegae Radix. (1): *E*-Senegasaponins a and b and *Z*-Senegasaponins a and b, Their Inhibitory Effect on Alcohol Absorption and Hypoglycemic Activity

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E-Senegasaponins a, b, and c were isolated from Senegae Radix, the root of *Polygala senega* L. var. *latifolia* TORREY *et* GRAY, together with their Z-isomers of the 4″-methoxycinnamoyl moiety, Z-senegasaponins a, b, and c. The structures of E-senegasaponins a and b and Z-senegasaponins a and b have been elucidated on the basis of chemical and physicochemical evidence, and the geometrical isomeric structure of the 4″-methoxycinnamoyl group in each saponin was found to show tautomer-like behavior in methanol solution or under irradiation with fluorescent lamps.

E and Z-Senegasaponins a and b were found to exhibit potent inhibitory effects on alcohol absorption and hypoglycemic activity in the oral D-glucose tolerance tests in rats, and some structure–activity relationships of the acylated bisdesmoside-type saponins were clarified.

Key words *E*-senegasaponin; *Z*-senegasaponin; Senegae Radix; *Polygala senega* var. *latifolia*; alcohol absorption inhibitor; hypoglycemic activity

Senegae Radix, which is an occidental natural medicine prepared from the root of *Polygala senega* L. (Polygalaceae), has been used clinically as an expectorant. In Japan, *Polygala senega* L. var. *latifolia* TORREY *et* GRAY (Hirohasenega in Japanese) has been cultured and its root has been used medicinally as Senegae Radix. The chemical constituents of Senegae Radix, the roots of *Polygala senega* L. and *P. senega* L. var. *latifolia* TORREY *et* GRAY, have been pursued extensively.[1] The saponin constituents, which are the principal ingredients of Senegae Radix, have been the target of many investigations and three bisdesmoside-type saponins having *E*-methoxycinnamoyl groups, senegins II (**7**), III (**8**), and IV (**9**), have been characterized.[2]

In the course of our studies on bioactive principles in natural medicines,[3] we have isolated several saponins from *Aralia elata* SEEM (root cortex, bark, and young shoot, Araliaceae),[4] *Camellia japonica* L. (seed, Theaceae),[5] and *Aesculus hippocastanum* L. (seed, Hippocastanaceae),[6] and elucidated their structures. Furthermore, these saponins were found to exhibit potent inhibitory activity on ethanol and sugar absorption in rats, and some structure–activity relationships of oleanolic acid monodesmosides and acylated polyhydroxyoleanene monodesmosides were clarified.[4−6] As a continuation of our screening program to find saponin constituents with inhibitory activity on ethanol absorption, we have isolated bisdesmoside-type inhibitors named *E*-senegasaponins a (**1**), b (**4**), and c, *Z*-senegasaponins a (**2**), b (**5**), and c, *Z*-senegins II, III, and IV from Japanese Senegae Radix, the root of *Polygala senega* L. var. *latifolia* TORREY *et* GRAY, together with senegins II (**7**), III (**8**), and IV (**9**) through bioassay-guided separation. These bisdesmoside saponins from Senegae Radix were also found to exhibit hypoglycemic activity in the oral D-glucose tolerance test in rats. In this paper, we present a full account of the structure elucidation of *E* and *Z*-senegasaponins a (**1**, **2**) and b (**4**, **5**), and their inhibitory effect on ethanol absorption and hypoglycemic activity.[7]

The air-dried root of *Polygala senega* L. var. *latifolia* TORREY *et* GRAY, cultivated for one year in Hyogo Prefecture, was extracted with methanol under reflux. Since the methanol extract was found to inhibit ethanol absorption in rats, it was subjected to bioassay-guided separation and purification through the procedures shown in Chart 1. Thus, the methanol extract was partitioned into an ethyl acetate–water mixture to furnish the ethyl acetate-soluble portion and the water phase. The water phase was further extracted with 1-butanol to give the 1-butanol-soluble portion and the water-soluble portion. The 1-butanol-soluble portion showed potent inhibitory activity on ethanol absorption, while the ethyl acetate-soluble and water-soluble portions were found to exhibit little activity (Table 1). The 1-butanol-soluble portion was subjected to normal silica gel column chromatography to give the saponin fraction with the inhibitory activity. The saponin fraction was further separated by normal silica gel column chromatography and then repeated HPLC with a YMC-Pack (D-ODS-5) to afford *E* and *Z*-mixtures of senegasaponins a, b, and c, and senegins II, III, and IV. Finally, each *E* and *Z*-mixture was subjected to HPLC separation with a YMC-Pack Ph to give nine new saponins, *E*-senegasaponins a (**1**), b (**4**), and c, *Z*-senegasaponins a (**2**), b (**5**), and c, and *Z*-senegins II, III, and IV, together with known senegins II (**7**), III (**8**), and IV (**9**).

***E* and *Z*-Senegasaponins a (1, 2)** *E*-Senegasaponin a (**1**) was obtained as colorless fine crystals of mp 228—231 °C. In the IR spectrum of **1**, it showed absorption bands at 1750, 1717, 1637, 1605, and 1514 cm^{-1} ascribable to ester, carboxyl, and aromatic ring, and strong absorption bands at 3432 and 1073 cm^{-1} suggestive of oligoglycosidic structure. The UV spectrum of **1** showed absorption maxima at 226 nm (log ε, 4.2) and 309 nm (log ε, 4.5), which suggested the presence of a cinnamoyl

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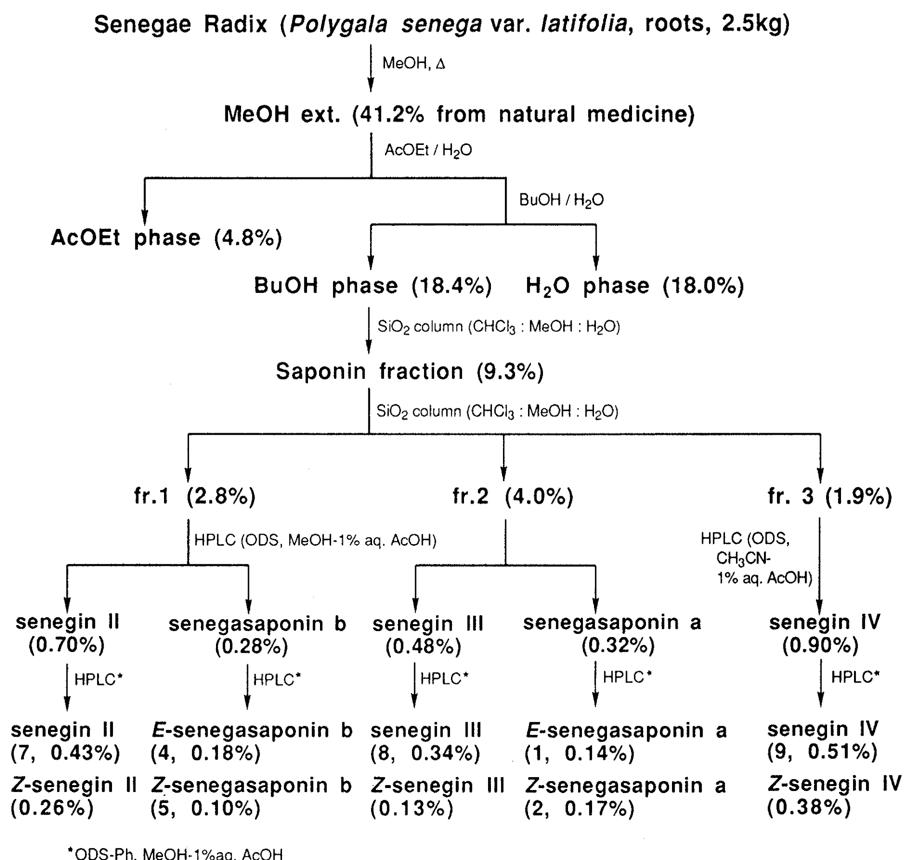

Chart 1

group. In the negative-mode FAB-MS of **1**, a quasi-molecular ion peak was observed at m/z 1557 $(M-H)^-$, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{74}H_{110}O_{35}$.

Treatment of **1** with 1% sodium methoxide at room temperature furnished methyl (E)-4-methoxycinnamate and desacylsenegasaponin a (**3**). In the UV spectrum of **3**, no absorption maximum was seen above 210 nm. Methanolysis of **3** with 9% hydrogen chloride in dry methanol liberated methyl glycosides of D-glucose, D-fucose, L-rhamnose, D-apiose, D-xylose, and D-galactose. On the other hand, alkaline hydrolysis of **3** with 5% aqueous sodium hydroxide under reflux afforded tenuifolin (**10**).[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra of **3**, which were completely assigned by NMR analytical methods,[9] showed signals assignable to a β-D-glucopyranoside moiety and a pentaglycoside moiety which was composed from β-D-fucopyranosyl, α-L-rhamnopyranosyl, β-D-apiofuranosyl, β-D-xylopyranosyl, and β-D-galactopyranosyl parts [δ 5.03 (d, J=7.6 Hz, Glu.-1), 6.04 (d, J=8.6 Hz, Fuc.-1), 6.22 (br s, Rha.-1), 6.01 (d, J=5.0 Hz, Api.-1), 5.22 (d, J=7.6 Hz, Xyl.-1), and 4.90 (d, J=7.6 Hz, Gal.-1). The pentaglycoside structure bonding to the 17-carboxyl group of the tenuifolin (**10**) moiety in **3** was characterized by means of a heteronuclear multiple bond correlation (HMBC) experiment. Namely, long-range correlations were observed between the following protons and carbons [Glu.-1-H and 3-C (δ 86.0); Fuc.-1-H and 28-C (δ 176.6); Rha.-1-H and Fuc.-2-C (δ 75.0); Api.-1-H and Rha.-3-C (δ 82.1); Xyl.-1-H and Rha.-4-C (δ 78.7); Gal.-1-H and Xyl.-4-C (δ 78.6)].

Table 1. Inhibitory Activity of the Fractions from Senegae Radix on Ethanol Absorption

	Dose (mg/kg, $p.o.$)	Ethanol concentration in blood (mg/ml, 1 h)
Control		0.70 ± 0.06
MeOH ext.	200	0.56 ± 0.08
	500	0.12 ± 0.06**
AcOEt phase	200	0.71 ± 0.03
BuOH phase	200	0.11 ± 0.03**
H_2O phase	200	0.67 ± 0.01
Saponin fraction	200	0.01 ± 0.01**

** $p<0.01$.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[9] of **1** showed signals due to an (E)-4-methoxycinnamoyl group at δ 6.51, 7.94 (1H each, both d, J= 15.8 Hz, 2′, 3′-H), 7.00, 7.44 (2H each, both d, J=8.9 Hz, 3″, 5″, 2″, 6″-H) and 3.67 (3H, s, 4″-OCH_3). Comparison of the ^{13}C-NMR data for **1** with those for **3** revealed an acylation shift around the 4-position of the D-fucopyranosyl moiety in **1**. Furthermore, a long-range correlation was observed between the 4-proton of the D-fucopyranosyl moiety [δ 5.73 (d-like)] and the carbonyl carbon (1′-C) of the (E)-4″-methoxycinnamoyl group in the HMBC experiment on **1**. Consequently, the structure of E-senegasaponin a has been elucidated as 3-O-β-D-glucopyranosylpresenegenin 28-O-{[β-D-apiofuranosyl (1—3)] [β-D-galactopyranosyl (1—4)-β-D-xylopyranosyl

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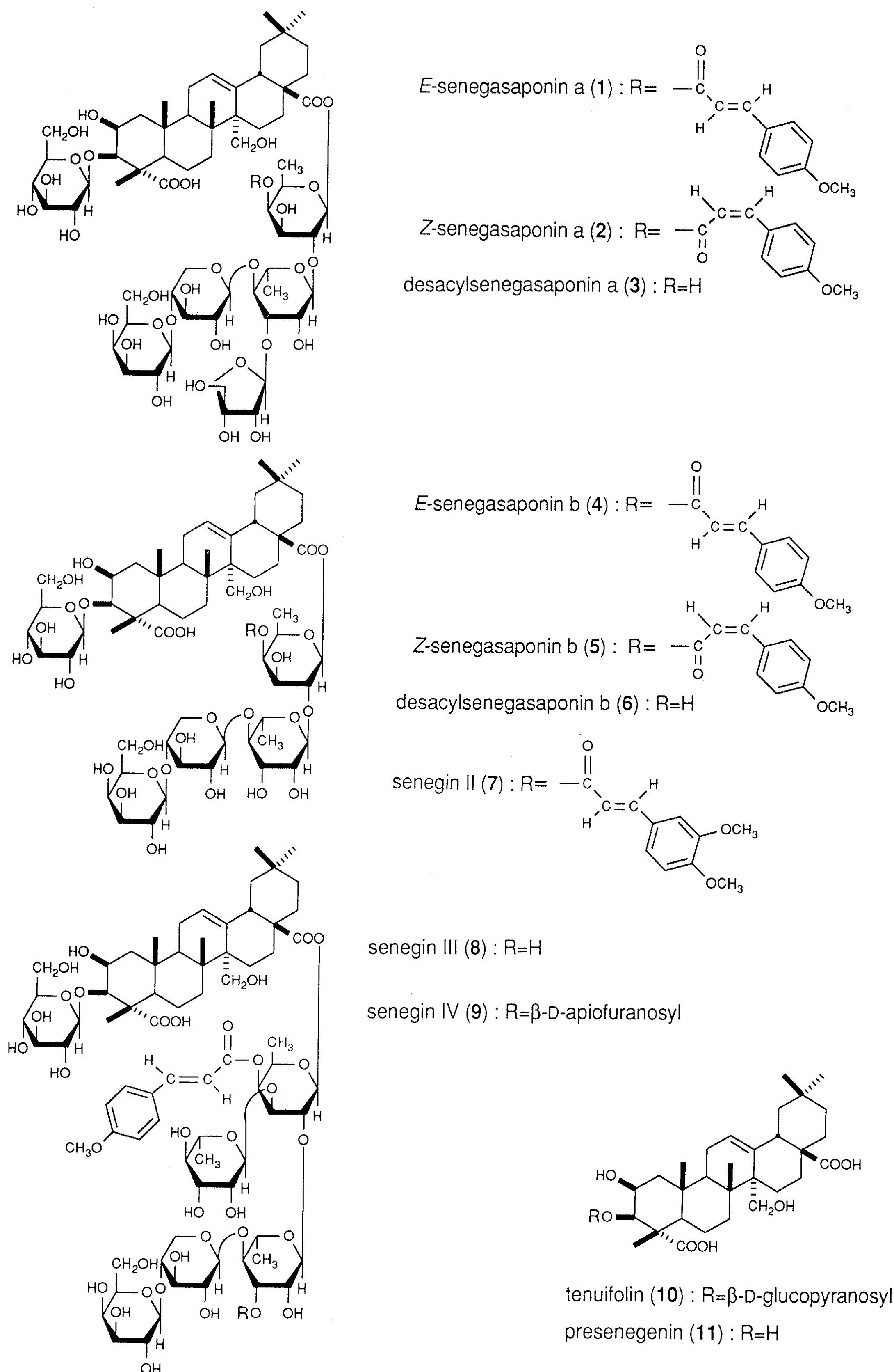

Chart 2

(1—4)]-α-L-rhamnopyranosyl (1—3)} {4-*O*-(*E*)-4″-methoxycinnamoyl}-*β*-D-fucopyranoside (**1**).

Z-Senegasaponin a (**2**), isolated as colorless fine crystals of mp 237—240 °C, furnished methyl (*Z*)-4-methoxycinnamate and desacylsenegasaponin a (**3**) upon alkaline treatment. The molecular formula $C_{74}H_{110}O_{35}$, which was the same as that of *E*-senegasaponin a (**1**), was determined from the negative mode and positive mode FAB-MS and by high-resolution MS measurement. Thus, in the negative-mode FAB-MS of **2**, a quasimolecular ion

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peak was observed at m/z 1557 $(M-H)^-$, while the positive-mode FAB-MS showed a quasimolecular ion peak at m/z 1603 $(M+2Na-H)^+$. The carbon signals in the ^{13}C-NMR (Table 2) spectrum[9] of **2** were shown to be superimposable on those of **1** except for some signals due to the 4″-methoxycinnamoyl group. The ^{1}H-NMR (pyridine-d_5) spectrum of **2** showed signals assignable to a (*Z*)-4″-methoxycinnamoyl group at δ 6.00, 6.85 (1H each, both d, $J=12.9$ Hz, 2′, 3′-H), 6.94, 7.99 (2H each, both d, $J=8.9$ Hz, 3″, 5″, 2″, 6″-H), and 3.66 (3H, s, 4″-OCH_3). Furthermore, a correlation peak was observed between the 4-proton of the D-fucopyranosyl moiety [δ 5.67 (d-like)] and the carbonyl carbon (1′-C) of the *Z*-4″-methoxycinnamoyl group in the HMBC spectrum.

Finally, the structure of *Z*-senegasaponin a (**2**) was substantiated by chemical correlation with *E*-senegasaponin a (**1**). Thus, it was found that, i) on standing in aqueous methanolic solution or ii) under irradiation with fluorescent lamps, **1** was changed to **2** to yield a mixture of *E* (**1**) and *Z*-form (**2**) (i: *ca.* 1.0 : 1.1; ii: 1.0 : 1.0).[10]

Similarly, **2** was changed to **1** by the same treatments (i, ii) to furnish a mixture of **1** and **2** (i: *ca.* 1.0 : 2.0; ii: 1.0 : 1.0).[10] On the other hand, **1** and **2** were found to be stable in weakly alkaline solution, such as pyridine. Consequently, the geometrical isomeric structures of the 4″-methoxycinnamoyl group in **1** and **2** were found to show tautomer-like behavior in aqueous methanol solution under photo-irradiation. Based on these findings, *Z*-senegasaponin a was determined to be the *Z*-isomer of **1** at the 4″-methoxycinnamoyl group, *i.e.*, 3-*O*-β-D-glucopyranosylpresenegenin 28-*O*-{[β-D-apiofuranosyl (1—3)] [β-D-galactopyranosyl (1—4)-β-D-xylopyranosyl (1—4)]-α-L-rhamnopyranosyl (1—3)} {4-*O*-(*Z*)-4″-methoxycinnamoyl}-β-D-fucopyranoside (**2**).

***E* and *Z*-Senegasaponins b (4, 5)** *E*-Senegasaponin b (**4**) and *Z*-senegasaonin b (**5**) were isolated as colorless fine crystals of mp 251—254 °C and 252—255 °C, respectively. *E* and *Z*-Senegasaponins b (**4**, **5**) were found to have the same molecular formula, $C_{69}H_{102}O_{31}$, which was determined from the quasimolecular ion peaks in their negative-mode FAB-MS [m/z 1425 $(M-H)^-$] and positive-mode FAB-MS [m/z 1449 $(M+Na)^+$] and by

Table 2. ^{13}C-NMR Data for **1**, **2**, **3**, **4**, **5**, and **6** (68 MHz, Pyridine-d_5, δ_C)

	1	2	3	4	5	6
C-1	44.3	44.3	44.3	44.2	44.3	44.3
C-2	70.3	70.3	70.1	70.3	70.3	70.1
C-3	85.9	85.9	86.0	85.9	85.9	86.0
C-4	52.9	52.9	52.9	52.8	52.9	52.9
C-5	52.6	52.6	52.6	52.5	52.5	52.5
C-6	21.5	21.3	21.3	21.6	21.5	21.5
C-7	33.8	33.8	33.9	33.5	33.5	33.6
C-8	41.1	41.1	41.2	41.1	41.1	41.2
C-9	49.3	49.3	49.4	49.3	49.3	49.4
C-10	37.1	37.0	37.1	37.0	37.0	37.0
C-11	23.6	23.6	23.7	23.6	23.6	23.5
C-12	127.8	127.9	127.8	127.8	127.9	127.9
C-13	139.0	139.0	139.1	138.9	138.9	139.0
C-14	47.0	47.0	47.0	46.9	47.0	46.9
C-15	24.5	24.5	24.5	24.5	24.5	24.5
C-16	24.1	24.1	24.0	24.1	24.0	24.0
C-17	47.9	47.9	48.0	48.0	48.0	48.1
C-18	42.0	42.0	42.0	42.0	42.0	42.0
C-19	45.4	45.4	45.5	45.3	45.4	45.4
C-20	30.8	30.8	30.8	30.8	30.8	30.8
C-21	33.8	33.8	33.9	33.8	33.9	33.9
C-22	32.4	32.4	32.4	32.4	32.4	32.4
C-23	180.9	180.9	180.9	180.7	180.8	180.7
C-24	14.3	14.3	14.2	14.2	14.2	14.2
C-25	17.5	17.5	17.5	17.5	17.5	17.5
C-26	18.9	18.9	18.8	18.7	18.7	18.8
C-27	64.4	64.4	64.6	64.4	64.4	65.0
C-28	176.7	176.7	176.6	176.8	176.8	176.7
C-29	33.0	33.0	33.1	33.0	33.0	33.1
C-30	24.1	24.1	24.1	24.0	24.0	24.0
Glu.-1	105.4	105.4	105.3	105.4	105.5	105.4
2	75.0	75.2	75.3	75.2	75.2	75.2
3	78.4	78.4	78.3	78.3	78.4	78.3
4	71.6	71.6	71.4	71.5	71.6	71.5
5	78.4	78.4	78.3	78.3	78.4	78.3
6	62.7	62.7	62.7	62.7	62.7	62.6
Fuc.-1	94.5	94.5	94.8	94.6	94.6	94.8
2	75.9	75.9	75.0	74.6	74.6	76.6
3	74.2	73.9	76.3	74.4	74.2	73.2
4	74.7	74.5	73.1	74.8	74.7	72.4
5	70.8	70.6	72.3	70.9	70.7	70.8
6	16.6	16.6	16.9	16.6	16.6	16.9

	1	2	3	4	5	6
Rha.-1	102.3	102.3	101.5	101.9	101.9	101.9
2	71.8	71.6	71.6	71.8	71.7	71.7
3	82.4	82.4	82.1	72.5	72.5	72.4
4	78.8	78.8	78.7	85.2	85.2	85.1
5	68.4	68.4	68.3	68.4	68.4	68.1
6	19.1	19.0	18.6	18.7	18.7	18.3
Xyl.-1	104.8	104.8	104.8	107.0	107.0	106.9
2	75.0	75.1	75.1	75.1	75.1	75.7
3	76.6	76.6	76.2	76.7	76.7	76.6
4	78.6	78.6	78.6	78.3	78.3	78.3
5	64.7	64.7	64.6	65.0	65.0	64.9
Gal.-1	104.5	104.5	104.4	104.5	104.5	104.4
2	71.8	71.8	71.8	71.8	71.8	71.7
3	75.0	75.1	75.0	75.7	75.7	75.0
4	70.1	70.1	70.1	70.1	70.1	70.0
5	77.4	77.4	77.3	77.3	77.3	77.2
6	62.3	62.3	62.3	62.2	62.2	62.2
Api.-1	111.8	111.7	111.7			
2	77.5	77.5	77.6			
3	79.6	79.6	79.6			
4	74.5	74.5	74.6			
5	64.6	64.5	64.6			
Cinnamoyl						
1′	167.7	166.8		167.6	166.7	
2′	116.2	117.1		116.1	117.1	
3′	145.2	143.9		145.2	144.0	
1″	127.5	127.9		127.4	127.9	
2″	130.4	133.2		130.4	133.2	
3″	114.8	113.9		114.7	113.9	
4″	161.9	161.0		161.9	161.1	
5″	114.8	113.9		114.7	113.9	
6″	130.4	133.2		130.4	133.2	
4″-OMe	55.3	55.2		55.0	55.2	

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high-resolution MS measurement. *E*-Senegasaponin b (**4**) showed UV absorption maxima at 226 nm (log ε, 4.2) and 311 nm (log ε, 4.4), like *E*-senegasaponin a (**1**). The alkaline treatment of **4** with sodium methoxide liberated methyl (*E*)-4-methoxycinnamate and desacylsenegasaponin b (**6**) which was found to be identical with the desacyl derivative obtained from senegin II (**7**) by alkaline treatment. On the other hand, *Z*-senegasaponin b (**5**) furnished methyl (*Z*)-4-methoxycinnamate and **6** upon alkaline treatment.

The carbon signals in the ^{13}C-NMR (Table 2) spectra[9] of **4** and **5** were superimposable on those of **7** except for a few signals due to the methoxycinnamoyl group. The ^{1}H-NMR spectra of **4** and **5** showed signals assignable to an (*E*)-4″-methoxycinnamoyl group for **4** and a (*Z*)-4″-methoxycinnamoyl group for **5**. Furthermore, in HMBC experiments on **4** and **5**, long-range correlations were observed between the 4-proton of the D-fucopyranosyl moiety and the 1′-carbonyl carbon of the 4″-methoxycinnamoyl group together with the following protons and carbons [Glu.-1-H and 3-C; Fuc.-1-H and 28-C; Rha.-1-H and Fuc.-2-C; Xyl.-1-H and Rha.-4-C; Gal.-1-H and Xyl.-4-C]. Finally, 4 was changed to **5** upon standing for 24h in aqueous methanolic solution to yield a mixture of **4** and **5** (*ca.* 4.5 : 1), while **5** was also changed to **4** by the same treatment to furnish a mixture of **5** and **4** (*ca.* 2.5 : 1). Consequently, the structures of *E*-senegasaponin b and *Z*-senegasaponin b were characterized as 3-*O*-β-D-glucopyranosylpresenegenin 28-*O*-{β-D-galactopyranosyl (1—4)-β-D-xylopyranosyl (1—4)-α-L-rhamnopyranosyl (1—2)} {4-*O*-(*E*)-4″-methoxycinnamoyl}-β-D-fucopyranoside (**4**) and its *Z*-isomer (**5**) of the 4″-methoxycinnamoyl group.

Inhibitory Activity of *E* and *Z*-Senegasaponins a (1, 2) and b (4, 5) on Ethanol Absorption Since the geometrical isomeric structures of the 4″-methoxycinnamoyl group in each senegasaponin were presumed to show tautomer-like behavior in acidic aqueous solution, *E* and *Z*-mixtures (*ca.* 1 : 1) of senegasaponins a and b were used for the bioassays. As shown in Table 3, the inhibitory effect of *E* and *Z*-senegasaponins a (**1**, **2**) and b (**4**, **5**), and desacylsenegasaponins a (**3**) and b (**6**) on ethanol absorption in rats was examined. In addition, in order to obtain data on the structure–activity relationship, the inhibitory activity of tenuifolin (**10**) and presenegenin (**11**), the common prosapogenol and the genuine sapogenol of senegasaponins and senegins, was also examined. *E* and *Z*-Senegasaponins a (**1**, **2**) and b (**4**, **5**) showed potent activity on single oral administration of 100 mg/kg. Desacylsenegasaponins a (**3**) and b (**6**) also showed activity, but their activities were weaker than those of senegasaponins a and b. Furthermore, tenuifolin (**10**) and presenegenin (**11**) lacked the activity. These results indicated that the 28-*O*-oligoglycoside moiety with the 4″-methoxycinnamoyl group was essential for the inhibition of ethanol absorption.

**Hypoglycemic Activity of *E* and *Z*-Senegasaponins a (1,

Table 3. Inhibitory Activity of *E* and *Z*-Senegasaponins a (**1**, **2**) and b (**4**, **5**) and Desacylsenegasaponins a (**3**) and b (**6**) on Ethanol Absorption

	Dose (mg/kg, *p.o.*)	*n*	Ethanol concentration in blood (mg/ml)		
			1 h	2 h	3 h
Control		10	0.50±0.02	0.19±0.03	0.03±0.01
E,*Z*-Senegasaponin a (**1**, **2**)	50	4	0.43±0.08	0.18±0.03	0.02±0.01
	100	4	0.06±0.02**	0.07±0.03**	0.03±0.01
Desacylsenegasaponin a (**3**)	100	5	0.22±0.08	0.22±0.04	0.03±0.01
E,*Z*-Senegasaponin b (**4**, **5**)	50	4	0.45±0.04	0.20±0.02	0.03±0.01
	100	4	0.07±0.01**	0.02±0.01**	0.03±0.01
Desacylsenegasaponin b (**6**)	100	5	0.26±0.12	0.10±0.05	0.05±0.01
Tenuifolin (**10**)	100	4	0.49±0.01	0.11±0.02	0.01±0.00
Presenegenin (**11**)	100	4	0.51±0.01	0.15±0.02	0.01±0.00

** $p<0.01$.

Table 4. Inhibitory Effect of *E* and *Z*-Senegasaponins a (**1**, **2**) and b (**4**, **5**) and Desacylsenegasaponins a (**3**) and b (**6**) on the Elevation of Plasma Glucose Level in the Oral Glucose Tolerance Test

	Dose (mg/kg, *p.o.*)	*n*	Glucose concentration in blood (mg/ml)		
			0.5 h	1.0 h	2.0 h
Control (normal)		6	85.3±2.3**	93.7±5.2**	86.0±6.2
Control		7	150.4±3.4	132.7±3.7	108.1±3.5
(Glucose tolerance)			(65.1±3.4)	(39.0±3.7)	(22.1±3.5)
E,*Z*-Senegasaponin a (**1**, **2**)	100	5	107.5±7.0**	117.1±5.7*	104.4±6.2
			(22.2±7.0**)	(23.4±5.7*)	(18.4±6.2)
Desacylsenegasaponin a (**3**)	100	5	132.3±8.7*	133.6±4.3	95.6±6.0
			(47.0±8.7*)	(39.9±4.3)	(9.6±6.0)
E,*z*-Senegasaponin b (**4**, **5**)	100	5	122.9±4.1**	132.6±5.3	113.7±4.4
			(37.6±4.1**)	(38.9±5.3)	(27.7±4.4)
Desacylsenegasaponin b (**6**)	100	5	141.3±8.4	159.5±10.7	142.0±1.5
			(56.8±8.4)	(58.2±10.7)	(46.8±1.5)

* $p<0.05$, ** $p<0.01$. Values in parenthesis show the difference in plasma glucose concentration between the normal control and each sample treatment.

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2) and b (4, 5) and Desacylsenegasaponins a (3) and b (6) Inhibitory effects of *E* and *Z*-senegasaponins a (**1, 2**), *E* and *Z*-senegasaponins b (**4, 5**), and desacylsenegasaponins a (**3**) and b (**6**) on the elevation of plasma glucose level in the oral D-glucose tolerance test in rats are summarized in Table 4. *E* and *Z*-Senegasaponins a (**1, 2**) and b (**4, 5**) were found to exhibit potent inhibitory activity. Desacylsenegasaponins a (**3**) and b (**6**) also showed inhibitory activity but their activities were weaker than those of senegasaponins. Here again, it is noteworthy that the 4″-methoxycinnamoyl group in senegasaponins is required for potent activity. Furthermore, it was found that *E* and *Z*-senegasaponins a (**1, 2**) and desacylsenegasaponin a (**3**) showed more potent activity than *E* and *Z*-senegasaponin b (**4, 5**) and desacylsenegasaponin b (**6**), respectively.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[3)]

Isolation of *E*-Senegasaponins a (1) and b (4), *Z*-Senegasaponins a (2) and b (5), *Z*-Senegins II, III, and IV Senegae Radix (2.5 kg, purchased from Koshiro Seiyaku, Osaka) was cut finely and extracted with MeOH under reflux three times. The extracted solution, after evaporation *in vacuo*, gave a residue (1.03 kg) and 560 g of the residue was partitioned into AcOEt–H_2O (1 : 1) solution. The aqueous layer was extracted with 1-BuOH. Removal of the solvent *in vacuo* from the AcOEt-soluble portion, 1-BuOH-soluble portion and aqueous phase yielded 64.7 g (4.8%), 250.6 g (18.4%), and 244.7 g (18.0%) of residue, respectively. The *n*-BuOH extract was subjected to silica gel column chromatography (3 kg) with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer) to give the saponin fraction (126.3 g, 9.3%). The saponin fraction was further subjected to silica gel column chromatography [3 kg, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)] to furnish three fractions [Fractions 1 (38.7 g, 2.8%), 2 (55.4 g, 4.0%), and 3 (26.5 g, 1.9%)]. Fraction 1 (1.0 g) was subjected to HPLC [YMC-Pack ODS-5 (250 × 20 mm i.d.), MeOH–1% AcOH (3 : 1)] to give a mixture (253.1 mg, 0.70%) of *Z*-senegin II and senegin II (**7**) and a mixture (99.2 mg, 0.28%) of **4** and **5**. HPLC [YMC-Pack ODS-5 (250 × 20 mm i.d.), MeOH–1% AcOH (3 : 1)] separation of fraction 2 (500 mg) furnished a mixture (39.7 mg, 0.32%) of **1** and **2** and a mixture (60.0 mg, 0.48%) of *Z*-senegin III and senegin III (**8**). Fraction 3 (2.0 g) was separated by HPLC [YMC-Pack ODS-5 (250 × 20 mm i.d.), CH_3CN–H_2O–AcOH (40 : 60 : 0.4)] to yield a mixture (100.3 mg, 0.90%) of *Z*-senegin IV and senegin IV (**9**).

Each geometrical isomeric mixture (*E* and *Z*-isomers of the methoxycinnamoyl moiety) was separated by HPLC [YMC-Pack Ph (250 × 20 mm i.d.), MeOH–1% AcOH (3 : 1)]: senegin II (**7**, 26.6 mg, 0.43%) and *Z*-senegin II (16.2 mg, 0.26%) from the mixture (44 mg), **4** (25.5 mg, 0.18%) and **5** (13.8 mg, 0.10%) from the mixture (40 mg), **1** (13.2 mg, 0.14%) and **2** (15.4 mg, 0.17%) from the mixture (29 mg); senegin III (**8**, 38.0 mg, 0.34%) and *Z*-senegin III (15.0 mg, 0.13%) from the mixture (54 mg), and senegin IV (**9**, 27.1 mg, 0.51%) and *Z*-senegin IV (20.5 mg, 0.38%) from the mixture (49 mg). Senegins II (**7**), III (**8**), and IV (**9**) were identified by comparison of their physical data with the reported values and from their FAB-MS data (negative mode and positive mode), as well as NMR data, which were completely assigned by various methods.[9)]

E-Senegasaponin a (**1**): Colorless fine crystals, mp 228—231 °C, $[\alpha]_D^{28}$ −12.9° (*c* = 1.0, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{74}H_{109}O_{35}$ $(M-H)^-$: 1557.6749; Found: 1557.6643. UV λ_{max}^{MeOH} nm (log ε): 226 (4.2), 309 (4.5). IR (KBr): 3432, 1750, 1717, 1637, 1605, 1514, 1073 cm^{-1}. ^{1}H-NMR (pyridine-d_5, δ): 0.79, 0.98, 1.13, 1.57, 2.02 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.38 (3H, d-like, Fuc.-6), 1.78 (3H, d, *J* = 6.3 Hz, Rha.-6), 3.28 (1H, dd-like, 18-H), 3.67 (3H, s, OCH_3), 4.96 (1H, d, *J* = 7.9 Hz, Gal.-1), 5.10 (1H, d, *J* = 7.6 Hz, Glu.-1), 5.31 (1H, d, *J* = 7.6 Hz, Xyl.-1), 5.73 (1H, d-like, Fuc.-4), 5.82 (1H, br s, 12-H), 6.08 (1H, d-like, Api.-1), 6.21 (1H, d, *J* = 8.3 Hz, Fuc.-1), 6.31 (1H, br s, Rha.-1), 6.51, 7.94 (1H each, both d, *J* = 15.8 Hz, 2′, 3′-H), 7.00, 7.44 (2H each, both d, *J* = 8.9 Hz, 3″, 5″, 2″, 6″-H). ^{13}C-NMR: see Table 2. Negative-mode FAB-MS (*m/z*): 1557 $(M-H)^-$.

Z-Senegasaponin a (**2**): Colorless fine crystals, mp 237—240 °C, $[\alpha]_D^{27}$ −22.0° (*c* = 1.0, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{74}H_{109}O_{35}$ $(M-H)^-$: 1557.6749; Found : 1557.6639. UV λ_{max}^{MeOH} nm (log ε): 307 (4.0). IR (KBr): 3432, 1750, 1719, 1638, 1605, 1512, 1071 cm^{-1}. ^{1}H-NMR (pyridine-d_5, δ): 0.80, 0.98, 1.12, 1.57, 2.03 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.34 (3H, d-like, Fuc.-6), 1.77 (3H, d-like, Rha.-6), 3.27 (1H, dd-like, 18-H), 3.66 (3H, s, OCH_3), 4.96 (1H, d, *J* = 7.9 Hz, Gal.-1), 5.10 (1H, d, *J* = 7.9 Hz, Glu.-1), 5.30 (1H, d, *J* = 7.9 Hz, Xyl.-1), 5.67 (1H, d-like, Fuc.-4), 5.80 (1H, br s, 12-H), 6.08 (1H, d, *J* = 5.0 Hz, Api.-1), 6.16 (1H, d, *J* = 7.9 Hz, Fuc.-1), 6.26 (1H, br s, Rha.-1), 6.00, 6.85 (1H each, both d, *J* = 12.9 Hz, 2′, 3′-H), 6.94, 7.99 (2H each, both d, *J* = 8.9 Hz, 3″, 5″, 2″, 6″-H). ^{13}C-NMR: see Table 2. Negative-mode FAB-MS (*m/z*): 1557 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1603 $(M+2Na-H)^+$.

E-Senegasaponin b (**4**): Colorless fine crystals, mp 251—254 °C, $[\alpha]_D^{27}$ +7.4° (*c* = 1.0, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{69}H_{102}O_{31}Na$ $(M+Na)^+$: 1449.6303; Found: 1449.6342. UV λ_{max}^{MeOH} nm (log ε): 226 (4.2), 311 (4.4). IR (KBr): 3432, 1750, 1717, 1636, 1605, 1512, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5, δ): 0.78, 0.95, 1.16, 1.55, 1.99 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.35 (3H, d, *J* = 5.9 Hz, Fuc.-6), 1.80 (3H, d, *J* = 6.3 Hz, Rha.-6), 3.27 (1H, dd-like, 18-H), 3.68 (3H, s, OCH_3), 4.99 (1H, d, *J* = 7.9 Hz, Gal.-1), 5.02 (1H, d-like, Xyl.-1), 5.09 (1H, d, *J* = 7.6 Hz, Glu.-1), 5.75 (1H, d-like, Fuc.-4), 5.85 (1H, br s, 12-H), 6.21 (1H, d, *J* = 8.3 Hz, Fuc.-1), 6.40 (1H, br s, Rha.-1), 6.50, 7.92 (1H each, both d, *J* = 15.8 Hz, 2′, 3′-H), 7.00, 7.43 (2H each, both d, *J* = 8.6 Hz, 3″, 5″, 2″, 6″-H). ^{13}C-NMR: see Table 2. Negative-mode FAB-MS (*m/z*): 1425 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1449 $(M+Na)^+$.

Z-Senegasaponin b (**5**): Colorless fine crystals, mp 252—255 °C, $[\alpha]_D^{28}$ −13.2° (*c* = 1.0, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{69}H_{101}O_{31}$ $(M-H)^-$: 1425.6327; Found: 1425.6388. UV λ_{max}^{MeOH} nm (log ε): 307 (4.2). IR (KBr): 3432, 1750, 1717, 1636, 1605, 1512, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5, δ): 0.79, 0.95, 1.15, 1.55, 1.99 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.32 (3H, d, *J* = 6.3 Hz, Fuc.-6), 1.78 (3H, d, *J* = 5.9 Hz, Rha.-6), 3.27 (1H, dd-like, 18-H), 3.66 (3H, s, OCH_3), 4.99 (1H, d, *J* = 8.3 Hz, Gal.-1), 5.02 (1H, d-like, Xyl.-1), 5.10 (1H, d, *J* = 7.9 Hz, Glu.-1), 5.68 (1H, d-like, Fuc.-4), 5.84 (1H, br s, 12-H), 6.16 (1H, d, *J* = 8.2 Hz, Fuc.-1), 6.34 (1H, br s, Rha.-1), 5.94, 6.84 (1H each, both d, *J* = 12.9 Hz, 2′, 3′-H), 6.95, 8.00 (2H each, both d, *J* = 12.9 Hz, 3″, 5″, 2″, 6″-H). ^{13}C-NMR: see Table 2. Negative-mode FAB-MS (*m/z*): 1425 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1449 $(M+Na)^+$.

Alkaline Treatment of *E*-Senegasaponin a (1) Giving Methyl (*E*)-4-Methoxycinnamate and Desacylsenegasaponin a (3) A solution of **1** (20 mg) in 1% NaOMe–MeOH (2 ml) was stirred for 1 h at room temperature (24 °C). 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 silica gel column chromatography [2g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give methyl (*E*)-4-methoxycinnamate (3 mg) and desacylsenegasaponin a (**3**, 17 mg). Methyl (*E*)-4-methoxycinnamate [^{1}H-NMR (pyridine-d_5, δ): 3.76 (3H, s, 1′-OCH_3), 3.96 (3H, s, 4″-OCH_3), 6.51, 7.90 (1H each, both d, *J* = 15.8 Hz, 2′, 3′-H), 7.08, 7.59 (2H each, both d, *J* = 8.6 Hz, 3′, 5′, 2′, 6′-H)] was identical with a commercial authentic sample.

Desacylsenegasaponin a (**3**): Colorless fine crystals, mp 228—229 °C, $[\alpha]_D^{29}$ −5.8° (*c* = 1.0, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{64}H_{101}O_{33}$ $(M-H)^-$: 1397.6225; Found : 1397.6265. IR (KBr): 3432, 1750, 1719, 1632, 1071 cm^{-1}. ^{1}H-NMR (pyridine-d_5, δ): 0.79, 0.94, 1.12, 1.56, 1.98 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.50 (3H, d, *J* = 6.3 Hz, Fuc.-6), 1.62 (3H, d, *J* = 5.6 Hz, Rha.-6), 3.22 (1H, dd-like, 18-H), 4.90 (1H, d, *J* = 7.6 Hz, Gal.-1), 5.03 (1H, d, *J* = 7.6 Hz, Glu.-1), 5.22 (1H, d, *J* = 7.6 Hz, Xyl.-1), 6.01 (1H, d, *J* = 5.0 Hz, Api.-1), 6.02 (1H, br s, 12-H), 6.04 (1H, d, *J* = 8.6 Hz, Fuc.-1), 6.22 (1H, br s, Rha.-1). ^{13}C-NMR: see Table 2. Negative-mode FAB-MS (*m/z*): 1397 $(M-H)^-$. Positive-mode FAB-MS (*m/z*): 1443 $(M+2Na-H)^+$.

Methanolysis of Desacylsenegasaponin a (3) A solution of **3** (5 mg) 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. After removal of the solvent *in vacuo* from the filtrate, the residue was dissolved in pyridine (0.01 ml) and the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 ml) for 1 h. The reaction solution was then

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subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i), methyl fucoside (ii), methyl rhamnoside (iii), methyl apioside (iv), methyl xyloside (v), and methyl galactoside (vi). GLC conditions: CBR-M25-025, 0.25 mm (i.d.) × 25 m capillary column; column temperature 140—280 °C; He flow rate 15 ml/min; t_R: i, 17.8, 18.2, 19.2. ii, 12.9, 14.0. iii, 11.5, 13.9. iv, 14.3, 15.3. v, 15.8, 16.2. vi, 18.9, 19.4.

Alkaline Hydrolysis of Desacylsenegasaponin a (3) by 5% Aqueous NaOH, Giving Tenuifolin (10) A solution of **1** (100 mg) in 5% aqueous NaOH (2 ml) was heated under reflux for 2 h in an N_2 atmosphere. The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and filtered. After removal of the solvent *in vacuo* from the filtrate, a product (103 mg) was purified on a silica gel column [20 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give tenuifolin (**10**, 7 mg), which was identical with an authentic sample obtained from senegin III by TLC, ^{1}H-NMR (pyridine-d_5), and ^{13}C-NMR (pyridine-d_5).

Alkaline Treatment of *Z*-Senegasaponin a (2), Giving Methyl (*Z*)-4-Methoxycinnamate and 3 A solution of **2** (20 mg) in 1% NaOMe–MeOH (2 ml) was stirred for 1 h at room temperature (24 °C). The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and then treated as described for the alkaline treatment of **1**. The product was separated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give **3** (17 mg) and methyl (*Z*)-4-methoxycinnamate (2 mg) [^{1}H-NMR (pyridine-d_5, δ): 3.07 (3H, s, 1'-OCH_3), 3.68 (3H, s, 4''-OCH_3), 5.98, 6.93 (1H each, both d, J = 12.9 Hz, 2', 3'-H), 6.97, 7.95 (2H each, both d, J = 8.9 Hz, 3'', 5'', 2'', 6''-H)] which was identified by comparison of its physical data with the reported values.[11]

Isomerization of *E*-Senegasaponins a (1) and *Z*-Senegasaponins a (2) in Aqueous MeOH Solution i) A solution of **1** (50 mg) in MeOH (10 ml) was left to stand for 3 d at room temperature. Removal of the solvent from the reaction solution gave a product (50 mg), which was separated by HPLC [YMC-Pack Ph (250 × 20 mm i.d.), MeOH–1% trifluoroacetic acid (TFA) (3 : 1)] to afford **1** (34 mg) and **2** (16 mg). Thus obtained **2** was shown to be identical with authentic *Z*-senegasaponin a by ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) comparisons.

ii) A solution of **2** (20 mg) in MeOH (5 ml) was allowed to stand for 3 d at room temperature. After work-up in the above mentioned manner, **1** (8.7 mg) and **2** (11.3 mg) were obtained and identified by ^{1}H-NMR and ^{13}C-NMR.

iii) A solution of **1** or **2** (5 mg each) in MeOH (10 ml) was allowed to stand for 24 h at room temperature. The ratio [*ca.* 1.0 : 1.1 from **1**, *ca.* 1.0 : 2.0 from **2**] of **1** and **2** in the solution was characterized by HPLC [YMC-Pack Ph (250 × 4.6 mm i.d.), MeOH–1% TFA (3 : 1), detection: UV 315 nm].

Isomerization of *E*-Senegasaponins a (1) and *Z*-Senegasaponin a (2) Under Irradiation with Fluorescent Lamps A 50% aqueous ethanol solution (10 ml) of **1** or **2** (5 mg each) in a Pyrex tube was irradiated (distance 5 cm) externally with five 20W fluorescent lamps for 5 h and the ratio of **1** : **2** (*ca.* 1.0 : 1.0) in the reaction mixture was determined by HPLC (as described above).

Alkaline Treatment of *E*-Senegasaponin b (4) Giving Methyl (*E*)-4-Methoxycinnamate and Desacylsenegasaponin b (6) A solution of **4** (50 mg) in 1% NaOMe–MeOH (10 ml) was stirred for 1 h at room temperature (25 °C). The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and then filtered. Removal of the solvent from the filtrate yielded a product (51 mg), which was subjected to silica gel column chromatography [3 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give methyl (*E*)-4-methoxycinnamate (5 mg) and desacylsenegasaponin b (**6**, 32 mg).

Desacylsenegasaponin b (**6**): Colorless fine crystals, mp 229—231 °C, $[\alpha]_D^{25}$ −8.6° (c = 1.0, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{59}H_{93}O_{29}$ $(M-H)^-$: 1265.5803; Found : 1265.5751. IR (KBr): 3432, 1750, 1719, 1638, 1071 cm^{-1}. ^{1}H-NMR (pyridine-d_5, δ): 0.80, 0.93, 1.12, 1.53, 1.93 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.48 (3H, d, J = 6.0 Hz, Fuc.-6), 1.61 (3H, d, J = 5.9 Hz, Rha.-6), 3.22 (1H, dd-like, 18-H), 4.90 (1H, d, J = 7.6 Hz, Gal.-1), 4.95 (1H, d, J = 7.3 Hz, Xyl.-1), 5.01 (1H, d, J = 7.6 Hz, Glu.-1), 5.80 (1H, br s, 12-H), 6.03 (1H, d, J = 8.3 Hz, Fuc.-1), 6.31 (1H, br s, Rha.-1). ^{13}C-NMR: see Table 2. Negative-mode FAB-MS (m/z): 1265 $(M-H)^-$. Positive-mode FAB-MS (m/z): 1289 $(M+Na)^+$.

Alkaline Treatment of Senegin II (7) with 1% NaOMe–MeOH Giving Desacylsenegasaponin b (6) A solution of **7** (200 mg) in 1% NaOMe–MeOH (40 ml) was stirred at room temperature (24 °C) for 15 min. The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and then filtered. After removal of the solvent from the filtrate, the crude product (213 mg) was purified by silica gel column chromatography [10 g, $CHCl_3$–MeOH–H_2O (65 : 35 : 10 and 5 : 5 : 1, lower layer)] to give **6** (188 mg), which was shown to be identical with desacylsenegasaponin b by TLC [$CHCl_3$–MeOH–H_2O (6 : 4 : 1), 1-BuOH–AcOH–H_2O (5 : 1 : 6, upper layer)], ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) comparisons.

Alkaline Treatment of *Z*-Senegasaponin b (5) with 1% NaOMe–MeOH Giving Methyl (*Z*)-4-Methoxycinnamate and Desacylsenegasaponin b (6) A solution of **5** (24 mg) in 1% NaOMe–MeOH (10 ml) was stirred at room temperature (25 °C) for 1 h. The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and then filtered. After evaporation of the solvent from the filtrate, the crude product (30 mg) was separated by silica gel column chromatography [1g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give methyl (*Z*)-4-methoxycinnamate (3 mg) and **6** (18 mg), both of which were identified by comparison with authentic samples obtained from **2** and **4**, respectively.

Isomerization of *E*-Senegasaponin b (4) and *Z*-Senegasponin b (5) in MeOH Solution A solution of **4** or **5** (5 mg each) in MeOH (10 ml) was allowed to stand for 24 h at room temperature (25 °C). The ratio (*ca.* 4.5 : 1.0 from **4**, *ca.* 1.0 : 2.5 from **5**) of **4** and **5** in the solution was characterized by HPLC (the same conditions as described for the characterization of geometrical ratio in the isomerization reaction of **1** and **2**).

Bioassay for Inhibitory Effect on Ethanol Absorption Male Wistar rats (Kiwa Laboratory Animals Ltd., Wakayama) weighing 170—180 g were fasted for 20—24 h but were given water *ad libitum*. The tested samples were dissolved in water (5 ml/kg), and then orally administered to the rats at each dose. At 1 h thereafter, 20% aqueous ethanol (5 ml/kg) was orally administered. Blood (*ca.* 0.4 ml) was collected from the carotid at 1, 2, and 3 h after 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. Results were expressed as the mean ± S.E. (Table 3).

Bioassay for Hypoglycemic Activity Male Wistar rats (Kiwa Laboratory Animals Ltd.) weighing 125—155 g were starved for 20—24 h, but given water *ad libitum*. The test samples were dissolved in water (5 ml/kg), and then orally administered to the rats. At 30 min thereafter, a water solution (5 ml/kg) of D-glucose (0.5 g/kg) was orally administered. Blood (*ca.* 0.4 ml) was collected from the carotid at 0.5, 1.0, and 2.0 h after D-glucose administration. The plasma glucose concentration was assayed by the enzymatic glucose oxidase method (glucose C-II test, Wako). Statistical significance of differences was estimated by the same method as in the case of inhibitory activity on absorption and results were expressed as the mean ± S.E. (Table 4).

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