Bioactive Saponins and Glycosides. II.[1] Senegae Radix. (2): Chemical Structures, Hypoglycemic Activity, and Ethanol Absorption-Inhibitory Effect of *E*-Senegasaponin c, *Z*-Senegasaponin c, and *Z*-Senegins II, III, and IV

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Received January 18, 1996; accepted February 26, 1996

Following the characterization of *E*-senegasaponins a and b and *Z*-senegasaponins a and b, new bioactive saponins named *E*-senegasaponin c and *Z*-senegasaponin c were isolated from Senegae Radix, the root of *Polygala senega* L. var. *latifolia* TORREY *et* GRAY., together with *Z*-senegins II, III, and IV. The chemical structures of *E* and *Z*-senegasaponins c and *Z*-senegins II, III, and IV were elucidated on the basis of chemical and physicochemical evidence, and the geometrical isomeric structures of the 4″-methoxycinnamoyl and 3″,4″-dimethoxycinnamoyl groups in these saponins were found to show tautomer-like behavior under irradiation with fluorescent lamps.

***E* and *Z*-Senegasaponins c and *E* and *Z*-senegins II, III, and IV were found to exhibit hypoglycemic activity in the oral D-glucose tolerance test. (*E*) and (*Z*)-Senegins II also showed an inhibitory effect on alcohol absorption in rats.**

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

In the course of our studies on bioactive constituents of natural medicines,[2] we have recently found that the methanolic extract of Japanese Senegae Radix, the root of *Polygala senega* L. var. *latifolia* TORREY *et* GRAY, inhibits ethanol absorption in rats. By bioassay-guided separation, *E*-senegasaponins a (**16**) and b (**7**), *Z*-senegasaponins a (**17**) and b (**8**), and *Z*-senegins II (**5**), III (**10**), and IV (**13**) were isolated from Japanese Senegae Radix, together with senegins II (**6**), III (**11**), and IV (**14**), and the chemical structures of *E*-senegasaponins a (**16**) and b (**7**) and *Z*-senegasaponins a (**17**) and b (**8**) were hitherto characterized.[3] Furthermore, *E* and *Z*-senegasaponins a (**16**, **17**) and b (**7**, **8**) were found to exhibit not only an inhibitory effect on ethanol absorption, but also hypoglycemic activity in the oral D-glucose tolerance test in rats.[1] As a continuation of our studies to find bioactive saponins,[4] we have isolated new saponins named *E*-senegasaponin c (**1**) and *Z*-senegasaponin c (**2**) from Japanese Senegae Radix. In this paper, we present the structure elucidation of *E* and *Z*-senegasaponins c (**1**, **2**) and *Z*-senegins II (**5**), III (**10**), and IV (**13**). In addition, we describe their hypoglycemic activity and inhibitory effect on ethanol absorption.[5]

***E* and *Z*-Senegasaponins c (1, 2)** *E*-Senegasaponin c (**1**) was isolated as colorless fine crystals of mp 230—232 °C. The UV spectrum of **1** showed absorption maxima at 226 nm (log ε, 4.0) and 310 nm (log ε, 4.3), which suggested the presence of an (*E*)-4″-methoxycinnamoyl group.[1] The IR spectrum of **1** showed absorption bands due to ester, carboxyl, and aromatic ring at 1750, 1719, 1707, 1638, 1605, and 1516 cm^{-1} and strong absorption bands at 3453 and 1076 cm^{-1} suggestive of oligoglycosidic structures. The molecular formula $C_{77}H_{114}O_{37}$ of **1** was clarified from the quasimolecular ion peaks observed in the positive-mode and negative-mode FAB-MS and by high-resolution MS measurement. Namely, quasimolecular ion peaks were observed at *m/z* 1653 $(M+Na)^+$ and *m/z* 1675 $(M+2Na-H)^+$ in the positive-mode FAB-MS, while the negative-mode FAB-MS showed a quasimolecular ion peak at *m/z* 1629 $(M-H)^-$.

Treatment of **1** with 1% sodium methoxide in methanol at room temperature furnished methyl (*E*)-4-methoxycinnamate and desacylsenegasaponin c (**3**), whose molecular formula $C_{65}H_{104}O_{34}$ was determined by high-resolution MS measurement of the quasimolecular ion peaks in the positive-mode and negative-mode FAB-MS. The UV spectrum of **3** showed no absorption maximum above 210 nm. Methanolysis of **3** with 9% hydrogen chloride in dry methanol liberated methyl glycosides of D-glucose, D-fucose, L-rhamnose, D-xylose, and D-galactose. Alkaline hydrolysis of **3** with 5% aqueous sodium hydroxide under reflux afforded tenuifolin (3-*O*-β-D-glucopyranosylpresenegenin, **4**), which is the common prosapogenol of senegasaponins (**7**, **8**, **16**, **17**)[1] and senegins (**6**, **11**, **14**).[6] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **3**, which were completely assigned by NMR analytical methods,[7] showed signals assignable to the tenuifolin part [δ 4.58 (1H, m, 3-H), 5.02 (1H, d, *J*=7.6 Hz, Glu-1-H)] and the 28-*O*-pentaglycoside moiety composed from β-D-fucopyranosyl, α-L-rhamnopyranosyl, β-D-glucopyranosyl, β-D-xylopyranosyl, and β-D-galactopyranosyl parts [δ 6.02 (1H, d, *J*=7.9 Hz, Fuc-1-H), 6.30 (1H, br s, Rha-1-H), 5.09 (1H, d, *J*=7.6 Hz, Glu-1′-H), 4.92 (2H, d, *J*=7.6 Hz, Xyl-1-H, Gal-1-H)]. The pentaglycoside structure bonding to the 28-carboxyl group of the tenuifolin 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; Fuc-1-H and 28-C; Rha-1-H and Fuc-2-C; Glu-1′-H and Fuc-3-C;

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Xyl-1-H and Rha-4-C; Gal-1-H and Xyl-4-C).

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[7] of **1** showed signals assignable to an (*E*)-4″-methoxycinnamoyl group [δ 6.48, 7.84 (1H each, both d, J=15.8 Hz, 2′,3′-H), 6.98, 7.39 (2H each, both d, J=8.6 Hz, 3″,5″,2″,6″-H), and 3.69 (3H, s, 4″-OCH$_3$)] and an acetyl group [δ 2.08 (3H, s)] together with the tenuifolin part and 28-*O*-pentaglycoside moiety [δ 6.20 (1H, d-like, Fuc-1-H), 6.32 (1H, br s, Rha-1-H), 5.04 (1H, d, J=7.3 Hz, Glu-1′-H), 4.98 (1H, d, J=7.9 Hz, Xyl-1-H), and 4.95 (1H, d, J=7.5 Hz, Gal-1-H)]. Comparison of the ^{13}C-NMR data for **1** with those for **3** revealed two acylation shifts around the 4-position of the D-fucopyranosyl moiety and the 6-position of the D-glucopyranosyl moiety in the pentaglycoside part of **1**. In the HMBC experiment on **1**, long-range correlations were observed between the 4-proton of the D-fucopyranosyl moiety [δ 6.00 (1H, br s)] and the carbonyl carbon (1′-C) of the (*E*)-4″-methoxycinnamoyl group and between the 6-protons of the D-glucopyranosyl moiety [δ 4.74, 5.01 (1H each, both m)] and the acetyl carbonyl carbon 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; Glu-1′-H and Fuc-3-C; Xyl-1-H and Rha-4-C; Gal-1-H and Xyl-4-C). Consequently, the structure of *E*-senegasaponin c has been elucidated as 3-*O*-β-D-glucopyranosylpresenegenin 28-*O*-[β-D-galactopyranosyl (1→4)-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)] [6-*O*-acetyl-β-D-glucopyranosyl (1→3)] [4-*O*-(*E*)-4″-methoxycinnamoyl]-β-D-fucopyranoside (**1**).

Z-Senegasaponin c (**2**) was also obtained as colorless fine crystals of mp 229—232 °C. In the positive-mode and negative-mode FAB-MS of **2**, quasimolecular ion peaks were observed at m/z 1653 $(M+Na)^+$, 1675 $(M+2Na-H)^+$ and 1629 $(M-H)^-$, and high-resolution MS analysis revealed the molecular formula of **2** to be $C_{77}H_{114}O_{37}$, which was the same as that of *E*-senegasaponin c (**1**). The IR spectrum of **2** was found to be similar to that of **1**, while the UV spectrum of **2** showed an absorption maximum at 308 nm (log ε, 4.1), suggesting the presence of a (*Z*)-4″-methoxycinnamoyl group.[1] The treatment of **2** with 1% sodium methoxide furnished methyl (*Z*)-4-methoxycinnamate and desacylsenegasaponin c (**3**). The carbon signals in the ^{13}C-NMR (Table 1) spectrum[7] of **2** were 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 5.91, 6.80 (1H each, both d, J=13.2 Hz, 2′,3′-H), 6.98, 7.95 (2H each, both d, J=8.6 Hz, 3″,5″,2″,6″-H), and 3.64 (3H, s, 4″-OCH$_3$) and an acetyl group at δ 2.08 (3H, s). Furthermore, the HMBC experiment on **2** showed long-range correlations between the 4-proton of the D-fucopyranosyl moiety [δ 6.00 (1H, br s)] and the carbonyl carbon (1′-C) of the (*Z*)-4″-methoxycinnamoyl group and between the 6-proton of the D-glucopyranosyl moiety [δ 4.74, 5.01 (1H each, both m)] and the acetyl carbonyl carbon.

Finally, the structure of *Z*-senegasaponin c (**2**) was substantiated by chemical correlation with *E*-senegasaponin c (**1**). Previously, we have reported that the geometrical isomeric structure of the 4″-methoxycinnamoyl group in *E*-senegasaponins a (**16**) and b (**7**) and *Z*-senegasaponins a (**17**) and b (**8**) shows tautomer-like behavior in methanol solution or under irradiation with fluorescent lamps.[1] This time, under irradiation with fluorescent lamps for 1 d i) in methanolic solution or ii) on standing in the powder state, it was found that **1** was changed to **2** to yield a mixture of **1** and **2** [i) *ca.* 3 : 1, ii) *ca.* 1 : 1], while **2** was changed to **1** to furnish a mixture of **1** and **2** [i) *ca.* 1 : 3, ii) *ca.* 1 : 2]. Furthermore, **1** and **2** were also found to be stable

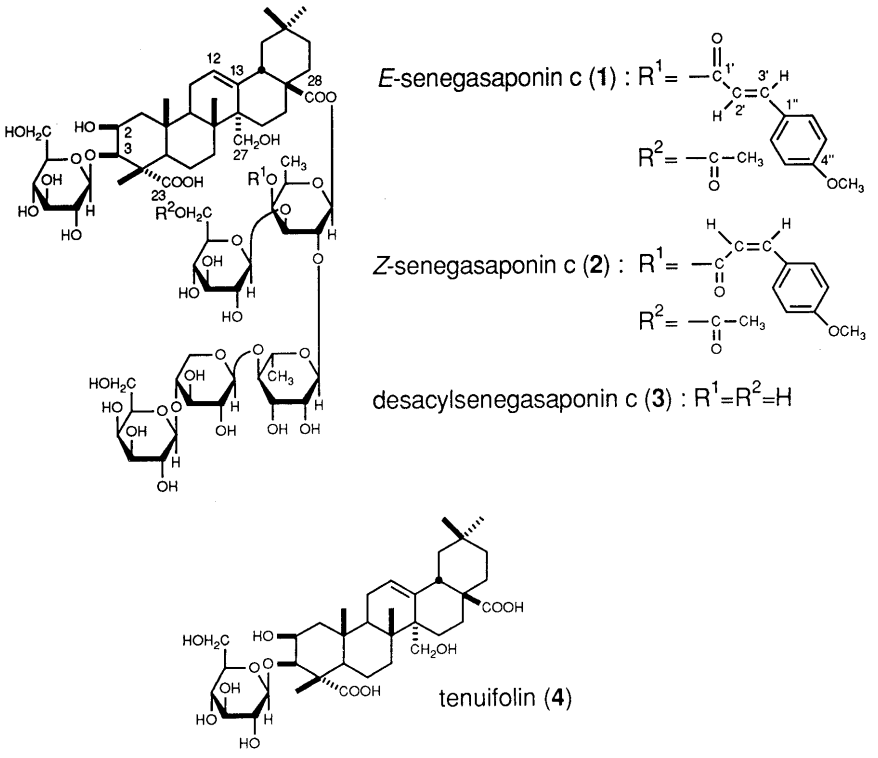

Chart 1

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

	1	2	3		1	2	3
C-1	44.2	44.3	44.3	Rha-1	101.6	101.6	101.4
C-2	70.3	70.3	70.3	2	71.8	71.8	71.9
C-3	85.9	85.9	85.9	3	72.3	72.3	72.3
C-4	52.9	52.9	52.9	4	84.8	84.8	84.8
C-5	52.5	52.9	52.4	5	68.5	68.4	68.4
C-6	21.4	21.6	21.4	6	18.7	18.7	18.4
C-7	33.6	33.6	33.6	Glu-1′	105.5	105.7	105.4
C-8	41.2	41.2	41.2	2′	75.2	75.2	75.0
C-9	49.3	49.3	49.3	3′	78.3	78.3	78.5
C-10	37.0	37.0	37.0	4′	71.0	71.1	71.6
C-11	23.6	23.6	23.5	5′	75.2	75.2	78.5
C-12	127.8	127.8	127.9	6′	64.0	64.0	62.8
C-13	138.9	138.9	139.0	Xyl-1	106.9	106.8	106.8
C-14	47.1	47.1	47.0	2	75.6	75.6	75.6
C-15	24.5	24.5	24.5	3	76.6	76.6	76.6
C-16	24.0	24.0	24.1	4	78.2	78.2	78.2
C-17	48.0	48.0	48.1	5	65.0	65.0	65.0
C-18	42.0	42.0	42.0	Gal-1	104.5	104.5	104.4
C-19	45.3	45.4	45.3	2	71.7	71.7	71.6
C-20	30.8	30.7	30.7	3	75.0	75.0	75.0
C-21	33.9	33.9	33.9	4	70.1	70.1	70.1
C-22	32.4	32.3	32.3	5	77.3	77.3	77.2
C-23	180.8	180.7	180.7	6	62.2	62.2	62.2
C-24	14.2	14.2	14.2	Cinnamoyl			
C-25	17.5	17.5	17.5	1′	167.1	165.9	
C-26	18.9	18.8	18.8	2′	116.2	117.1	
C-27	64.4	64.4	64.4	3′	144.9	143.3	
C-28	176.8	176.8	176.7	1″	127.6	127.8	
C-29	33.0	33.0	33.0	2″	130.4	133.1	
C-30	24.0	24.0	24.0	3″	114.8	114.0	
3-Glu-1	105.4	105.4	105.3	4″	162.0	161.0	
2	75.0	75.0	75.3	5″	114.8	114.0	
3	78.1	78.1	78.3	6″	130.4	133.1	
4	71.6	71.6	71.6	4″-OCH_3	55.4	55.2	
5	78.3	78.3	78.3	Ac-1	171.0	170.9	
6	62.7	62.7	62.7	2	21.0	20.9	
28-Fuc-1	94.6	94.6	94.8				
2	73.1	73.5	73.3				
3	82.8	82.6	85.4				
4	74.0	74.2	72.1				
5	71.0	71.1	71.6				
6	17.0	16.9	16.8				

in weakly alkaline solution such as pyridine under photo-irradiation. On the basis of these findings, *Z*-senegasaponin c (**2**) was determined to be the *Z*-isomer of **1** at the 4″-methoxycinnamoyl group.

Z-Senegins II (5), III (10), and IV (13) *Z*-Senegin II (**5**) was isolated as colorless fine crystals of mp 238—242 °C. The UV spectrum of **5** showed an absorption maximum at 321 nm (log ε, 4.2), while its IR spectrum showed absorption bands due to hydroxyl, ester, and carbonyl groups, and aromatic ring. The positive-mode and negative-mode FAB-MS of **5** showed quasimolecular ion peaks at *m/z* 1479 $(M+Na)^+$ and 1455 $(M-H)^-$, respectively, and the molecular formula $C_{70}H_{104}O_{32}$, which was the same as that of senegin II (**6**), was determined by high-resolution MS measurement. The treatment of **5** with sodium methoxide gave methyl (*Z*)-3,4-dimethoxycinnamate and desacylsenegasaponin b (**9**).[1] The ^{1}H-NMR (pyridine-d_5) spectrum of **5** showed signals assignable to a (*Z*)-3″,4″-dimethoxycinnamoyl group at δ 5.97, 6.86 (1H, both d, J=12.5 Hz, 2′,3′-H), 6.90 (1H, d, J=8.3 Hz, 5″-H), 7.46 (1H, dd-like, 6″-H), 8.03 (1H, br s, 2″-H), and 3.73, 3.81 (3H each, both s, 3″,4″-OCH_3). The carbon signals in the ^{13}C-NMR (Table 2) spectrum[7] of **5** were found to be almost superimposable on those of **6**, *E* and *Z*-senegasaponin b (**7**, **8**), except for some signals due to the cinnamoyl group. In the HMBC experiment on **5**, a long-range correlation was observed between the 4-proton of the D-fucopyranosyl moiety and the 1″-carbonyl carbon of the (*Z*)-3″,4″-dimethoxycinnamoyl group. Finally, photo-irradiation of **5** in methanol solution (i) or in the powder state (ii) furnished a mixture of **5** and **6** [i) *ca.* 1.0:1.1, ii) *ca.* 1.0:1.3]. Consequently, *Z*-senegin II was characterized as the *Z*-isomer of senegin II (**6**) at the 3″,4″-dimethoxycinnamoyl group, *i.e.*, 3-*O*-β-D-glucopyranosylpresenegenin 28-*O*-[β-D-galactopyranosyl (1→4)-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)] [4-*O*-(*Z*)-3″,4″-dimethoxycinnamoyl]-β-D-fucopyranoside (**5**).

Z-Senegin III (**10**), isolated as colorless fine crystals of mp 243—246 °C, was found to have the same molecular formula $C_{75}H_{112}O_{35}$ as that of senegin III (**11**), which was determined from the quasimolecular ion peaks in the positive-mode FAB-MS [*m/z* 1595 $(M+Na)^+$] and negative-mode FAB-MS [*m/z* 1571 $(M-H)^-$] and by high-resolution MS measurement. The treatment of **10** with sodium methoxide furnished methyl (*Z*)-4-methoxycinnamate and desacylsenegin III (**12**), which was identical with the desacyl derivative obtained from **11**. The carbon signals in the ^{13}C-NMR (Table 2) spectrum[7] of **10** were almost superimposable on those of **11**, except for a few signals due to the 4″-methoxycinnamoyl group, and the ^{1}H-NMR spectrum of **10** showed signals assignable to a (*Z*)-4″-methoxycinnamoyl group. In the HMBC experiment on **10**, long-range correlations were observed between the following protons and carbons : Glu-1-H and 3-C; Fuc-1-H and 28-C; Rha-1-H and Fuc-2-C; Rha-1′-H and Fuc-3-C; Fuc-4-H and 4″-methoxycinnamoylcarbonyl-C; Xyl-1-H and Rha-4-C; Gal-1-H and Xyl-4-C. Furthermore, photo-irradiation of **10** in methanol solution (i) or in the powder state (ii) yielded a mixture of **10** and **11** [i) *ca.* 1.0 : 1.6, ii) *ca.* 1.0:3.9]. On the basis of the above-mentioned evidence and a comparison of the ^{13}C-NMR data for **10** with those for **12**, the structure of *Z*-senegin III was determined to be 3-*O*-β-D-glucopyranosylpresenegenin 28-*O*-[β-D-galactopyranosyl (1→4)-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)] [α-L-rhamnopyranosyl (1→3)] [4-*O*-(*Z*)-4″-methoxycinnamoyl]-β-D-fucopyranoside (**10**).

Z-Senegin IV (**13**) was also obtained as colorless fine crystals of mp 247—248 °C and its molecular formula $C_{80}H_{120}O_{39}$, which was the same as that of senegin IV (**14**), was clarified from the quasimolecular ion peaks observed in the positive-mode FAB-MS [*m/z* 1727 $(M+Na)^+$, 1749 $(M+2Na-H)^+$] and negative-mode FAB-MS [*m/z* 1703 $(M-H)^-$] and by high-resolution MS measurement. The sodium methoxide treatment of **13** furnished methyl (*Z*)-4-methoxycinnamate and desacylsenegin IV (**15**), which was identical with the desacyl derivative obtained by the sodium methoxide treatment of **14**. The ^{1}H-NMR and ^{13}C-NMR (Table 2) spectra[7] of **13** showed signals due to a (*Z*)-4″-methoxycinnamoyl group and the HMBC experiment on **13** showed long-

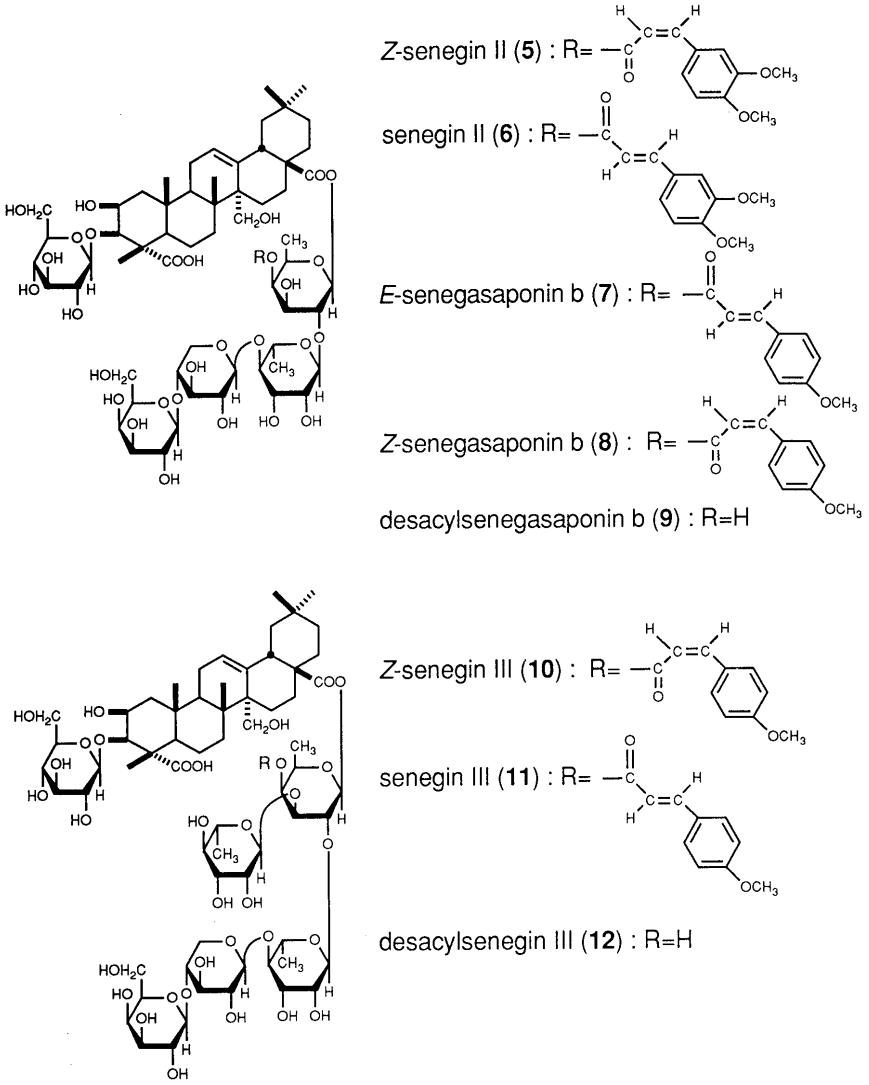

Chart 2

range correlations between the 4-proton of the D-fucopyranosyl moiety and the 1′-carbonyl carbon of the (*Z*)-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; Rha-1′-H and Fuc-3-C; Api-1-H and Rha-3-C; Xyl-1-H and Rha-4-C; Gal-1-H and Xyl-4-C. Furthermore, photo-irradiation of **13** in methanolic solution (i) or in the powder state (ii) afforded a mixture of **13** and **14** [i) *ca.* 3.3 : 1.0, ii) *ca.* 3.6 : 1.0]. Comparison of the ^{13}C-NMR data for **13** with those for **14** and **15** led us to formulate the structure of *Z*-senegin IV as 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→2)}{α-L-rhamnopyranosyl (1→3)}{4-*O*-(*Z*)-4″-methoxycinnamoyl}-*β*-D-fucopyranoside (**13**).

Hypoglycemic Activity of the Saponin Fraction, *E* and *Z*-Senegasaponins c (1, 2), *E* and *Z*-Senegins II (5, 6), III (10, 11), and IV (13, 14), and Their Desacyl Derivatives (3, 12, 15) As described in the previous paper,[1] *E* and *Z*-mixtures (*ca.* 1 : 1) of senegasaponin c and senegins II, III, and IV were used for the bioassays, since the geometrical isomeric structures of the methoxycinnamoyl group in each saponin were expected to show tautomer-like behavior during the bioassay experiment. The saponin fraction[1] from Senegae Radix showed hypoglycemic activity at the dose of 200 and 500 mg/kg.

The hypoglycemic effects of the saponin fraction, *E* and *Z*-senegasaponins c (**1**, **2**), *E* and *Z*-senegins II (**5**, **6**), III (**10**, **11**), and IV (**13**, **14**) and their desacyl derivatives (**3**, **12**, **15**) are summarized in Table 3. *E* and *Z*-Senegasaponins c (**1**, **2**), and *E* and *Z*-senegins II (**5**, **6**), III (**10**, **11**), and IV (**13**, **14**) were found to inhibit the elevation of plasma glucose level in the oral D-glucose tolerance test in rats after a single oral administration of each *E* and *Z*-saponin mixture (100 mg/kg). In the same bioassay, desacylsenegasaponin c (**3**), desacylsenegins III (**12**) and IV (**15**) lacked the activity. Previously, we have reported that *E* and *Z*-senegasaponins a (**16**, **17**) and b (**7**, **8**) show hypoglycemic activity, while desacylsenegasaponins a (**18**) and b (**9**) show weak activity.[1] In this experiment, it was confirmed that the methoxycinnamoyl group of senegasaponins and senegins is required for their hypoglycemic activities.

Inhibitory Activity of *E* and *Z*-Senegins II (5, 6), III (10, 11), and IV (13, 14) and Desacylsenegins III (12) and IV (15) on Ethanol Absorption The inhibitory effects of *E* and *Z*-senegins II (**5**, **6**), III (**10**, **11**), and IV (**13**, **14**) and

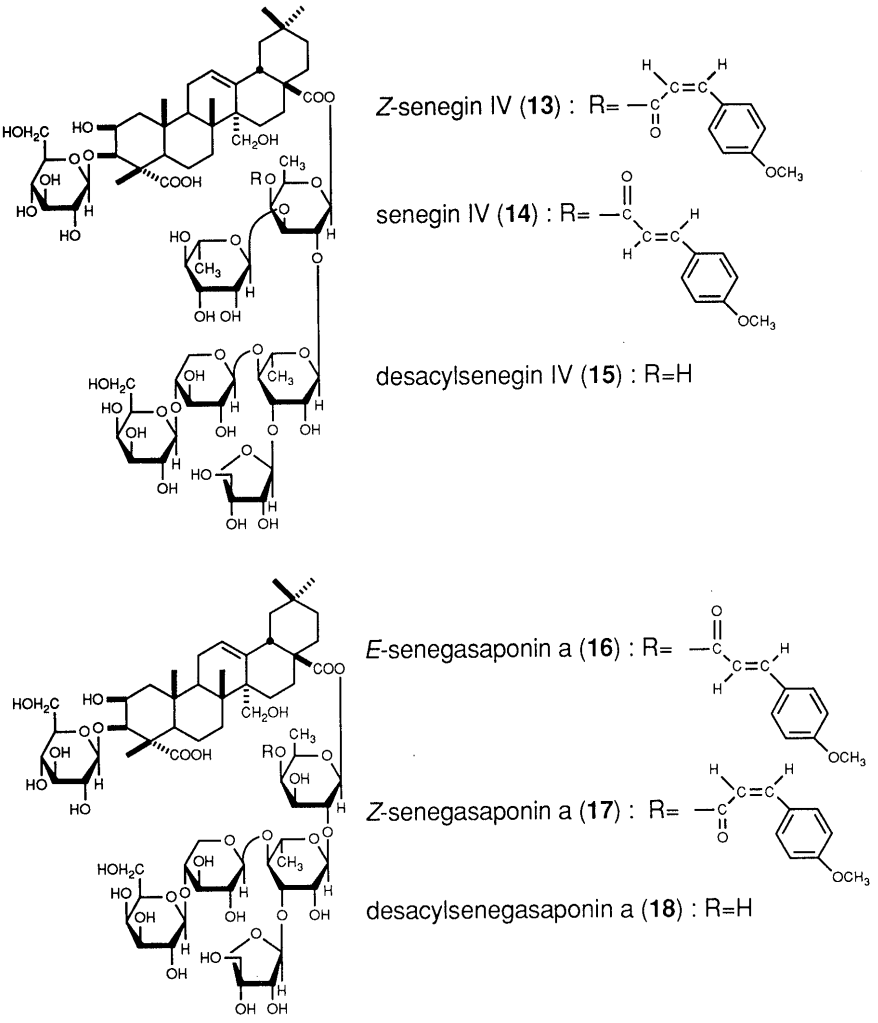

Chart 3

desacylsenegins III (**12**) and IV (**15**) on ethanol absorption in rats were examined and the results are shown in Table 4. *E* and *Z*-Senegins II (**5**, **6**) were found to exhibit potent inhibitory activity on single oral administration of 100 mg/kg, like *E* and *Z*-senegasaponins a (**16**, **17**) and b (**7**, **8**) in the previous report.[1] *E* and *Z*-Senegins III (**10**, **11**) also showed some activity, but it was much weaker than those of *E* and *Z*-senegins II (**5**, **6**), *E* and *Z*-senegasaponins a (**16**, **17**) and b (**7**, **8**) and desacylsenegasaponins a (**18**) and b (**9**). *E* and *Z*-Senegins IV (**13**, **14**) and desacylsenegins III (**12**) and IV (**15**) lacked the inhibitory activity.

We have reported that the 4″-methoxycinnamoyl group in *E* and *Z*-senegasaponins a (**16**, **17**) and b (**7**, **8**) is required for the inhibitory activity on ethanol absorption.[1] This time, detailed comparison of the 28-oligoglycoside structures and the inhibitory activities of *E* and *Z*-senegins II (**5**, **6**) and *E* and *Z*-senegasaponins a (**16**, **17**) and b (**7**, **8**) with those for inactive *E* and *Z*-senegins III (**10**, **11**) and IV (**13**, **14**) led us to presume that the α-L-rhamnopyranosyl group linked to the 3-hydroxyl group of the fucopyranosyl moiety in *E* and *Z*-senegins III (**10**, **11**) and IV (**13**, **14**) decreased the activity.

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) spectrometer and JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) spectrometer and JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., 100—200 mesh); TLC, pre-coated TLC plate with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 $60F_{254}$ (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plate with Silica gel RP-18 $60WF_{254S}$ (Merck, 0.25 mm); Detection was done by spraying 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 and heating.

Isolation of *E*-Senegasaponin c (1) and *Z*-Senegasaponin c (2) Fraction 2 (55.4 g, 4.0%), obtained from Senegae Radix (2.5 kg, cultivated for one year in Hyogo Prefecture and purchased from Koshiro Seiyaku, Osaka) as reported previously,[1] was subjected to HPLC [YMC-Pack ODS-5 (250 × 20 mm, i.d.), MeOH–1% AcOH (3 : 1, v/v)] separation to furnish the senegasaponin c fraction (64.5 mg, 0.53%) together with a mixture (0.32%) of *E* and *Z*-senegasaponins a (**16**, **17**) and a mixture (0.49%) of *Z*-senegin III (**10**) and senegin III (**11**). HPLC [YMC-Pack ODS-5 (250 × 20 mm, i. d.), CH_3CN–1% AcOH (45 : 55, v/v)] separation of the senegasaponin c fraction gave a mixture (23.3 mg, 0.19%) of **1** and **2**. Finally, a geometrical isomeric mixture (**1**, **2**) was separated by HPLC [YMC-Pack Ph (250 × 20 mm, i.d.), MeOH–1% AcOH (3 : 1, v/v)] to afford *E*-senegasaponin c (**1**, 18.6 mg, 0.09%) and *Z*-senegasaponin c (**2**, 19.8 mg, 0.10%).

Table 2. ^{13}C-NMR Data for **5**, **6**, **10**, **11**, **12**, **13**, **14**, and **15** (68 MHz, Pyridine-d_5)

	5	**6**	**10**	**11**	**12**	**13**	**14**	**15**		**5**	**6**	**10**	**11**	**12**	**13**	**14**	**15**
C-1	44.2	44.2	44.3	44.2	44.3	44.2	44.2	44.3	Rha-1	101.9	101.8	102.0	102.0	101.7	102.2	102.2	101.9
C-2	70.3	70.3	70.3	70.3	70.1	70.3	70.3	70.2	2	71.8	71.8	71.6	71.6	71.6	71.6	71.6	71.5
C-3	85.9	85.9	85.9	86.0	86.0	86.0	85.9	86.0	3	72.5	72.5	72.5	72.6	72.4	82.7	82.7	82.4
C-4	52.9	52.9	52.9	52.8	52.9	52.9	52.8	52.9	4	85.1	85.2	84.8	84.7	85.0	78.4	78.4	78.5
C-5	52.5	52.5	52.5	52.4	52.4	52.6	52.6	52.6	5	68.4	68.4	68.6	68.6	68.3	68.8	68.8	68.4
C-6	21.5	21.5	21.4	21.5	21.5	21.3	21.3	21.3	6	18.7	18.7	18.7	18.9	18.6	18.7	18.7	18.7
C-7	33.5	33.5	33.6	33.6	33.6	33.9	33.9	33.9	Rha-1′			105.1	105.0	104.8	105.0	105.0	104.7
C-8	41.1	41.1	41.1	41.1	41.2	41.2	41.2	41.2	2′			72.3	72.3	72.4	72.1	72.2	72.3
C-9	49.3	49.3	49.3	49.3	49.4	49.3	49.3	49.4	3′			73.6	73.6	73.8	73.6	73.7	73.8
C-10	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.1	4′			72.6	72.6	72.6	72.5	72.5	72.6
C-11	23.6	23.6	23.8	23.8	23.5	23.6	23.5	23.6	5′			70.9	71.0	70.4	70.9	70.9	70.3
C-12	127.8	127.7	127.7	127.7	127.8	127.7	127.7	127.9	6′			18.6	18.7	18.5	18.6	18.7	18.6
C-13	139.0	138.9	138.9	138.9	139.0	139.0	139.0	139.0	Xyl-1	106.9	107.0	106.8	106.8	106.8	104.7	104.7	104.8
C-14	47.0	47.0	47.1	47.1	47.0	47.0	47.0	46.8	2	75.7	75.7	75.5	75.5	75.6	75.0	75.0	75.0
C-15	24.5	24.5	24.5	24.5	24.5	24.6	24.5	24.5	3	76.7	76.7	76.6	76.6	76.6	76.4	76.5	76.5
C-16	24.0	24.0	24.0	24.0	24.0	24.1	24.1	23.8	4	78.4	78.4	78.4	78.3	78.3	78.0	78.0	78.3
C-17	48.0	48.0	48.0	48.0	48.1	48.0	47.9	48.0	5	65.0	65.0	64.4	65.0	65.0	64.7	64.7	64.5
C-18	42.0	42.0	42.0	42.0	42.0	42.0	42.1	42.1	Gal-1	104.5	104.5	104.5	104.5	104.5	104.4	104.4	104.4
C-19	45.4	45.3	45.5	45.4	45.5	45.7	45.6	45.6	2	71.8	71.8	71.8	71.8	71.8	71.8	71.7	71.8
C-20	30.8	30.8	30.8	30.8	30.8	30.8	30.9	30.8	3	75.1	75.1	75.1	75.1	75.1	75.0	75.0	75.0
C-21	33.9	33.8	33.9	33.9	33.6	33.9	33.9	33.9	4	70.1	70.1	70.1	70.1	70.3	70.1	70.2	70.2
C-22	32.4	32.5	32.3	32.3	32.3	32.2	32.2	32.2	5	77.3	77.3	77.3	77.3	77.2	77.4	77.5	77.4
C-23	180.8	180.8	180.7	180.8	180.7	180.9	180.8	180.8	6	62.2	62.2	62.2	62.2	62.3	62.3	62.3	62.3
C-24	14.2	14.2	14.2	14.2	14.2	14.3	14.3	14.2	Api-1						111.9	111.9	111.7
C-25	17.5	17.5	17.5	17.5	17.5	17.6	17.6	17.5	2						77.3	77.3	77.6
C-26	18.7	18.7	18.9	18.6	18.8	19.2	19.1	19.0	3						79.8	79.8	79.7
C-27	64.4	64.4	64.3	64.3	65.0	63.8	63.8	64.7	4						74.3	74.3	74.6
C-28	176.8	176.7	176.7	176.7	176.7	176.6	176.3	176.4	5						64.4	64.5	64.6
C-29	33.0	33.0	33.1	33.0	33.1	33.1	33.1	33.1	Cinnamoyl								
C-30	24.0	24.0	24.0	24.0	24.0	24.1	24.1	24.0	1′	166.8	167.7	166.3	167.2		166.3	167.2	
3-Glu-1	105.4	105.4	105.4	105.4	105.3	105.3	105.4	105.3	2′	116.9	116.1	116.4	115.7		116.4	115.8	
2	75.2	75.2	75.2	75.2	75.3	75.2	75.2	75.3	3′	144.6	145.8	144.8	145.6		144.8	145.6	
3	78.4	78.4	78.4	78.3	78.3	78.4	78.4	78.3	1″	128.2	127.7	127.7	127.4		127.7	127.4	
4	71.6	71.6	71.6	71.5	71.7	71.5	71.5	71.6	2″	114.8	110.9	133.3	130.5		133.3	130.5	
5	78.4	78.4	78.4	78.3	78.3	78.4	78.4	78.3	3″	150.5	150.0	114.1	114.7		114.1	114.8	
6	62.7	62.7	62.7	62.7	62.8	62.7	62.7	62.8	4″	151.2	152.1	161.1	162.0		161.1	162.0	
28-Fuc-1	94.6	94.6	94.8	94.8	94.9	95.0	95.0	95.1	5″	111.4	111.9	114.1	114.7		114.1	114.8	
2	74.6	74.6	74.9	74.7	73.4	76.7	76.5	74.8	6″	125.8	123.4	133.3	130.5		133.3	130.5	
3	74.1	74.4	80.9	81.2	85.0	79.8	80.1	83.9	3″-OCH_3	55.8	56.0						
4	74.8	74.8	73.4	73.6	72.3	73.2	73.4	72.2	4-″OCH_3	55.7	55.8	55.2	55.4		55.2	55.4	
5	70.7	70.9	70.8	71.0	72.3	70.6	70.8	72.2									
6	16.6	16.6	16.9	17.0	16.9	16.9	17.0	16.9									

Table 3. Inhibitory Effect of *E* and *Z*-Senegasaponins c (**1**, **2**), *E* and *Z*-Senegins II (**5**, **6**), III (**10**, **11**), and IV (**13**, **14**), and Their Derivatives (**3**, **12**, **15**) 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	—	10	75.5±4.1**		101.8±4.1**		96.8±4.2	
Control (glucose tolerance)	—	10	138.6±3.8	(63.1±3.8)	142.5±2.6	(40.7±2.6)	107.1±3.2	(10.3±3.2)
Saponin fraction	200	4	117.6±7.7	(42.1±7.7)	132.9±6.3	(31.1±6.3)	120.4±6.6	(23.6±6.6)
	500	5	99.5±3.3**	(24.0±3.3**)	120.7±4.6*	(18.9±4.6*)	110.5±3.9	(13.7±3.9)
Control	—	5	70.6±3.7**		90.6±6.1**		73.9±4.7**	
Control (glucose tolerance)	—	5	137.5±2.3	(66.9±2.3)	125.0±4.8	(34.4±4.8)	97.9±2.3	(24.0±2.3)
E, *Z*-Senegasaponin c (**1**, **2**)	100	5	128.0±3.9	(57.0±2.3)	128.6±3.6	(38.0±3.6)	103.9±7.4	(30.0±7.4)
Desacylsenegasaponin c (**3**)	100	4	135.0±4.3	(64.4±4.3)	126.6±8.1	(126.6±8.1)	97.8±5.9	(23.9±5.9)
Control	—	12	88.6±4.2**		103.6±3.3**		100.8±4.8	
Control (glucose tolerance)	—	16	145.6±2.3	(57.0±2.3)	139.5±2.3	(35.9±2.3)	113.1±2.5	(12.3±4.5)
E, *Z*-Senegin II (**5**, **6**)	100	12	120.9±4.8**	(32.3±4.8**)	135.3±3.4	(31.7±3.4)	128.0±4.0**	(27.2±4.0**)
E, *Z*-Senegin III (**10**, **11**)	100	13	127.3±3.8**	(38.7±3.8**)	139.3±3.6	(35.7±3.6)	119.5±3.0	(18.7±3.0)
E, *Z*-Senegin IV (**13**, **14**)	100	7	133.1±5.6	(44.5±5.6)	150.4±4.8	(46.8±4.8)	127.1±6.9	(26.3±6.9)
Control	—	6	76.6±6.0**		94.3±4.6**		86.6±6.1*	
Control (glucose tolerance)	—	9	153.1±4.4	(76.5±4.4)	136.9±3.7**	(42.6±3.7**)	103.7±2.9	(17.1±2.9)
Desacylsenegin III (**12**)	100	5	157.7±5.9	(81.1±5.9)	152.8±4.2	(58.5±4.2)	121.7±5.7	(35.1±5.7)
Desacylsenegin IV (**15**)	100	6	160.5±2.5	(83.9±2.5)	147.0±5.0	(52.7±5.0)	115.0±7.5	(28.1±7.5)

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

Table 4. Inhibitory Effects of *E* and *Z*-Senegins II (**5**, **6**), III (**10**, **11**), and IV (**13**, **14**) and Their Derivatives (**12**, **15**) on Ethanol Absorption

	Dose (mg/kg, *p.o.*)	*n*	1 h	2 h	3 h
Control	—	3	0.560±0.025	0.223±0.018	0.041±0.006
E, *Z*-Senegin II (**5**, **6**)	100	2	0.049±0.021**	0.091±0.047*	0.008±0.008*
Control	—	5	0.517±0.013	0.146±0.017	0.046±0.022
E, *Z*-Senegin III (**10**, **11**)	100	5	0.458±0.022	0.116±0.021	0.012±0.001
E, *Z*-Senegin IV (**13**, **14**)	100	4	0.498±0.021	0.203±0.021	0.010±0.003
Desacylsenegin III (**12**)	100	4	0.509±0.010	0.156±0.022	0.010±0.003
Desacylsenegin IV (**15**)	100	5	0.492±0.013	0.146±0.011	0.014±0.002

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

E-Senegasaponin c (**1**) : Colorless fine crystals, mp 230—232 °C, $[\alpha]_D^{29}$ +11.4° ($c=0.1$, MeOH). High-resolution negative-mode FAB-MS : Calcd for $C_{77}H_{113}O_{37}$ $(M-H)^-$: 1629.6961; Found: 1629.6995. High-resolution positive-mode FAB-MS : Calcd for $C_{77}H_{114}NaO_{37}$ $(M+Na)^+$: 1653.6937; Found: 1653.7035. UV λ_{max}^{MeOH} nm (log ε): 226 (4.0), 310 (4.3). IR (KBr) cm^{-1}: 3453, 1750, 1719, 1707, 1638, 1605, 1516, 1076. ^{1}H-NMR (pyridine-d_5) δ: 0.77, 0.92, 1.15, 1.54, 1.97 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.32 (3H, d-like, Fuc-6), 1.74 (3H, d-like, Rha-6), 2.08 (3H, s, OAc), 3.24 (1H, dd-like, 18-H), 3.69 (3H, s, 4″-OCH_3), 4.60 (1H, m, 3-H), 4.74, 5.01 (1H each, both m, Glu-6′), 4.95 (1H, d, $J=7.5$ Hz, Gal-1), 4.98 (1H, d, $J=7.9$ Hz, Xyl-1), 5.04 (1H, d, $J=7.3$ Hz, Glu-1), 5.04 (1H, d, $J=7.3$ Hz, Glu-1′), 5.85 (1H, br s, 12-H), 6.00 (1H, br s, Fuc-4), 6.20 (1H, d-like, Fuc-1), 6.32 (1H, br s, Rha-1), 6.48, 7.84 (1H each, both d, $J=15.8$ Hz, 2′,3′-H), 6.98, 7.39 (2H each, both d, $J=8.6$Hz, 3″,5″,2″,6″-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (*m/z*): 1629 $(M-H)^-$, positive-mode FAB-MS (*m/z*): 1653 $(M+Na)^+$, 1675 $(M+2Na-H)^+$.

Z-Senegasaponin c (**2**): Colorless fine crystals, mp 229—232 °C, $[\alpha]_D^{29}$ −4.1° ($c=0.1$, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{77}H_{113}O_{37}$ $(M-H)^-$: 1629.6961; Found: 1629.6980. UV λ_{max}^{MeOH} nm (log ε): 308 (4.1). IR (KBr) cm^{-1}: 3435, 1750, 1719, 1707, 1637, 1605, 1518, 1075. ^{1}H-NMR (pyridine-d_5) δ: 0.79, 0.93, 1.15, 1.54, 1.98 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.32 (3H, d-like, Fuc-6), 1.74 (3H, d-like, Rha-6), 2.08 (3H, s, OAc), 3.21 (1H, dd-like, 18-H), 3.64 (3H, s, 4″-OCH_3), 4.62 (1H, m, 3-H), 4.74, 5.01 (1H each, both m, Glu-6′), 4.95 (1H, d, $J=7.6$ Hz, Gal-1), 4.98 (1H, d, $J=7.9$ Hz, Xyl-1), 5.04 (1H, d, $J=7.3$ Hz, Glu-1), 5.04 (1H, d, $J=7.3$ Hz, Glu-1′), 5.82 (1H, br s, 12-H), 6.00 (1H, br s, Fuc-4), 6.12 (1H, d-like, Fuc-1), 6.42 (1H, br s, Rha-1), 5.91, 6.80 (1H each, both d, $J=13.2$ Hz, 2′,3′-H), 6.98, 7.95 (2H each, both d, $J=8.6$ Hz, 3″,5″,2″,6″-H). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (*m/z*): 1629 $(M-H)^-$, Positive-mode FAB-MS (*m/z*): 1653 $(M+Na)^+$, 1675 $(M+2Na-H)^+$.

Isolation of *Z*-Senegins II (5), III (10), and IV (13) *Z*-Senegins II (**5**), III (**10**), and IV (**13**) were isolated as described in our previous paper.[1)]

Z-Senegin II (**5**): Colorless fine crystals, mp 238—242 °C, $[\alpha]_D^{28}$ −24.0° ($c=0.1$, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{70}H_{103}O_{32}$ $(M-H)^-$: 1455.6433; Found: 1455.6326. UV λ_{max}^{MeOH} nm (log ε): 321 (4.2). IR (KBr) cm^{-1}: 3432, 1750, 1719, 1707, 1630, 1601, 1516, 1073. ^{1}H-NMR (pyridine-d_5) δ: 0.79, 0.95, 1.15, 1.54, 1.98 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.33 (3H, d, $J=5.9$ Hz, Fuc-6), 1.77 (3H, d-like, Rha-6), 3.26 (1H, dd-like, 18-H), 3.73, 3.81 (3H each, both s, 3″,4″-OCH_3), 4.63 (1H, m, 3-H), 4.98 (1H, d, $J=7.9$ Hz, Gal-1), 5.01 (1H, d-like, Xyl-1), 5.08 (1H, d, $J=7.9$ Hz, Glu-1), 5.83 (1H, br s, 12-H), 6.16 (1H, d, $J=8.2$ Hz, Fuc-1), 6.33 (1H, br s, Rha-1), 5.97, 6.86 (1H each, both d, $J=12.5$ Hz, 2′,3′-H), 6.90 (1H, d, $J=8.3$ Hz, 5″-H), 7.46 (1H, dd-like, 6″-H), 8.03 (1H, br s, 2″-H). ^{13}C-NMR: given in Table 2. Negative-mode FAB-MS (*m/z*): 1455 $(M-H)^-$, Positive-mode FAB-MS (*m/z*): 1479 $(M+Na)^+$.

Z-Senegin III (**10**): Colorless fine crystals, mp 243—246 °C, $[\alpha]_D^{27}$ −17.0° ($c=0.1$, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{75}H_{111}O_{35}$ $(M-H)^-$: 1571.6906; Found: 1571.6975. UV λ_{max}^{MeOH} nm (log ε): 309 (4.2). IR (KBr) cm^{-1}: 3453, 1750, 1719, 1707, 1637, 1605, 1509, 1069. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.97, 1.13, 1.56, 1.98 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.29 (3H, d-like, Fuc-6), 1.72 (3H, d-like, Rha-6), 1.72 (3H, d-like, Rha-6′), 3.34 (1H, dd-like, 18-H), 3.64 (3H, s, 4″-OCH_3), 4.62 (1H, m, 3-H), 4.98 (1H, d, $J=7.9$ Hz, Gal-1), 4.99 (1H, d-like, Xyl-1), 5.08 (1H, d, $J=7.6$ Hz, Glu-1), 5.70 (1H, br s, Rha-1′), 5.83 (1H, br s, 12-H), 5.91 (1H, br s, Rha-1), 6.09 (1H, d, $J=7.6$ Hz, Fuc-1), 5.91, 6.86 (1H each, both d, $J=12.9$ Hz, 2′,3′-H), 7.04, 8.01 (2H each, both d, $J=8.9$ Hz, 3″,5″,2″,6″-H). ^{13}C-NMR: given in Table 2. Negative-mode FAB-MS (*m/z*): 1571 $(M-H)^-$, positive-mode FAB-MS (*m/z*): 1595 $(M+Na)^+$.

Z-Senegin IV (**13**): Colorless fine crystals, mp 247—248 °C, $[\alpha]_D^{26}$ −23.1° ($c=0.1$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{80}H_{119}Na_2O_{39}$ $(M+2Na-H)^+$: 1749.7124; Found: 1749.7089, Calcd for $C_{80}H_{120}NaO_{39}$ $(M+Na)^+$: 1727.7304; Found: 1727.7269. UV λ_{max}^{MeOH} nm (log ε): 311 (4.3). IR (KBr) cm^{-1}: 3432, 1750, 1719, 1636, 1605, 1512, 1071. ^{1}H-NMR (pyridine-d_5) δ: 0.81, 1.05, 1.13, 1.63, 2.01 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.28 (3H, d-like, Fuc-6), 1.67 (3H, d-like, Rha-6′), 1.72 (3H, d-like, Rha-6), 3.19 (1H, dd-like, 18-H), 3.65 (3H, s, 4″-OCH_3), 4.65 (1H, m, 3-H), 4.94 (1H, d, $J=7.6$ Hz, Gal-1), 5.08 (1H, d, $J=7.6$ Hz, Glu-1), 5.23 (1H, d, $J=7.6$ Hz, Xyl-1), 5.53 (1H, br s, Rha-1′), 5.75 (1H, br s, Rha-1), 5.82 (1H, br s, 12-H), 6.04 (1H, d, $J=9.2$ Hz, Fuc-1), 6.12 (1H, br s, Api-1), 5.99, 6.91 (1H each, both d, $J=12.9$ Hz, 2′,3′-H), 7.06, 8.01 (2H each, both d, $J=8.9$ Hz, 3″,5″,2″,6″-H). ^{13}C-NMR: given in Table 2. Negative-mode FAB-MS (*m/z*): 1703 $(M-H)^-$, positive-mode FAB-MS (*m/z*): 1727 $(M+Na)^+$, 1749 $(M+2Na-H)^+$.

Alkaline Treatment of *E*-Senegasaponin c (1) Giving Methyl (*E*)-4-methoxycinnamate and Desacylsenegasaponin c (3) A solution of **1** (25 mg) in 1% NaOMe–MeOH (5 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 [1 g, $CHCl_3$–MeOH–H_2O (10:3:1, lower layer)] to give methyl (*E*)-4-methoxycinnamate (3 mg) and desacylsenegasaponin c (**3**, 20 mg). Methyl (*E*)-4-methoxycinnamate was identical with an authentic sample by TLC and ^{1}H-NMR comparisons.[1)]

Desacylsenegasaponin c (**3**): Colorless fine crystals, mp 236—239 °C, $[\alpha]_D^{22}$ +9.2° ($c=0.1$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{65}H_{103}Na_2O_{34}$ $(M+2Na-H)^+$: 1473.6127; Found: 1473.6154, Calcd for $C_{65}H_{104}NaO_{34}$ $(M+Na)^+$: 1451.6306; Found: 1451.6340. IR (KBr) cm^{-1}: 3432, 1750, 1719, 1707, 1638, 1075. ^{1}H-NMR (pyridine-d_5) δ: 0.79, 0.91, 1.14, 1.52, 1.94 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.39 (3H, d, $J=6.3$ Hz, Fuc-6), 1.68 (3H, d, $J=5.9$ Hz, Rha-6), 3.21 (1H, dd-like, 18-H), 4.58 (1H, m, 3-H), 4.92 (2H, d, $J=7.6$ Hz, Gal-1, Xyl-1), 5.02 (1H, d, $J=7.6$ Hz, Glu-1), 5.09 (1H, d, $J=7.6$ Hz, Glu-1′), 5.81 (1H, br s, 12-H), 6.02 (1H, d, $J=7.9$ Hz, Fuc-1), 6.30 (1H, br s, Rha-1). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (*m/z*): 1427 $(M-H)^-$, positive-mode FAB-MS (*m/z*): 1451 $(M+Na)^+$, 1473 $(M+2Na-H)^+$.

Methanolysis of Desacylsenegasaponin c (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 subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucoside (i), methyl fucoside (ii), methyl rhamnoside (iii), methyl xyloside (iv), and methyl galactoside (v). 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, 15.8, 16.2. v, 18.9, 19.4.

Alkaline Treatment of *Z*-Senegasaponin c (2) Giving Methyl (*Z*)-

4-Methoxycinnamate and 3 A solution of **2** (22 mg) in 1% NaOMe–MeOH (5 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 worked up as described for the alkaline treatment of **1**. The product was separated by silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give **3** (18 mg) and methyl (*Z*)-4-methoxycinnamate (2 mg). Methyl (*Z*)-4-methoxycinnamate was identical with an authentic sample[1] obtained from *Z*-senegasaponin a (**17**) on the basis of TLC and ^{1}H-NMR comparisons, and **3** was identical with desacylsenegasaponin c obtained from **1**, based on TLC, ^{1}H-NMR and ^{13}C-NMR comparisons.

Isomerization of *E*-Senegasaponin c (1) and *Z*-Senegasaponin c (2) i) An MeOH (5 ml) solution of **1** or **2** (20 mg each) in a Pyrex tube was left standing for 24 h under photo-irradiation with four 20 W fluorescent lamps at room temperature. The ratio [*ca.* 1 : 1 from **1**, *ca.* 1 : 2 from **2**) of **1** and **2** in the solution was characterized by HPLC [YMC-Pack Ph (250 × 4.6 mm i.d.), MeOH–1% AcOH (3 : 1, v/v), detection: UV 315 nm]. Removal of the solvent from the reaction solution gave a product, which was separated by HPLC (the same conditions as described above) to afford **1** and **2** [**1** (10 mg) and **2** (9 mg) from **1**, **1** (6 mg) and **2** (13 mg) from **2**]. Thus obtained **1** and **2** were shown to be identical with authentic samples by TLC, HPLC, ^{1}H- and ^{13}C-NMR comparisons.

ii) Powdered **1** or **2** (10 mg each) was left standing for 24 h under photo-irradiation with four 20 W fluorescent lamps at room temperature to yield a mixture of **1** and **2**. The ratio [*ca.* 3 : 1 from **1**, *ca.* 1 : 3 from **2**] of **1** and **2** was characterized by HPLC as described above.

Alkaline Treatment of *Z*-Senegin II (5) with 1% NaOMe–MeOH Giving Methyl (*Z*)-3,4-Dimethoxycinnamate and Desacylsenegasaponin b (9) A solution of **5** (24 mg) in 1% NaOMe–MeOH (6 ml) was stirred at room temperature (25 °C) for 1 h. The reaction mixture was neutralized with Dowex HCR W × 2 (H^+ form) and the resin was removed by filtration. After evaporation of the solvent from the filtrate *in vacuo*, the crude product (27 mg) was separated by silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give methyl (*Z*)-3,4-dimethoxycinnamate (3 mg) and **9** (20 mg). Methyl (*Z*)-3,4-dimethoxycinnamate [^{1}H-NMR (pyridine-d_5) δ: 3.69 (3H, s, 1′-OCH_3), 3.74, 3.85 (3H each, both s, 4″,3″-OCH_3), 6.00, 6.95 (1H each, both d, J = 13.2 Hz, 2′,3′-H), 6.92 (1H, d, J = 8.2 Hz, 5″-H), 7.43 (1H, dd-like, 5″-H), 8.06 (1H, d, J = 2.0 Hz, 2″-H)] was identical with an authentic sample, and **9** was identified as desacylsenegasaponin b[1] by TLC, ^{1}H- and ^{13}C-NMR comparisons with an authentic sample.

Alkaline Treatment of *Z*-Senegin III (10) with 1% NaOMe–MeOH Giving Methyl (*Z*)-4-Methoxycinnamate and Desacylsenegin III (12) A solution of **10** (25 mg) in 1% NaOMe–MeOH (5 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. Removal of the solvent from the filtrate yielded a product, which was subjected to silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to afford methyl (*Z*)-4-methoxycinnamate (3 mg) and **12** (20 mg). Methyl (*Z*)-4-methoxycinnamate was identical with an authentic sample[1] obtained from *Z*-senegasaponin c (**2**) by TLC, ^{1}H-, and ^{13}C-NMR comparisons.

Desacylsenegin III (**12**): Colorless fine crystals, mp 233—236 °C, $[\alpha]_D^{28}$ −6.0° (c = 0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{65}H_{103}Na_2O_{33}$ $(M+2Na-H)^+$: 1457.6177; Found: 1457.6167, Calcd for $C_{65}H_{104}NaO_{33}$ $(M+Na)^+$: 1435.6357; Found: 1435.6318. IR (KBr) cm^{-1}: 3411, 1750, 1719, 1638, 1071. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.92, 1.12, 1.52, 1.94 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.47 (3H, d, J = 6.3 Hz, Fuc-6), 1.57 (3H, d, J = 6.3 Hz, Rha-6′), 1.63 (3H, d, J = 5.9 Hz, Rha-6), 3.20 (1H, dd-like, 18-H), 4.58 (1H, m, 3-H), 4.93 (1H, d, J = 7.9 Hz, Gal-1), 4.94 (1H, d-like, Xyl-1), 5.02 (1H, d, J = 7.9 Hz, Glu-1), 5.62 (1H, br s, Rha-1′), 5.81 (1H, br s, 12-H), 5.93 (1H, br s, Rha-1), 6.05 (1H, d-like, Fuc-1). ^{13}C-NMR: given in Table 2. Negative-mode FAB-MS (m/z): 1411 $(M-H)^-$, positive-mode FAB-MS (m/z): 1435 $(M+Na)^+$, 1457 $(M+2Na-H)^+$.

Alkaline Treatment of *Z*-Senegin IV (13) with 1% NaOMe–MeOH Giving Methyl (*Z*)-4-Methoxycinnamate and Desacylsenegin IV (15) A solution of **13** (26 mg) in 1% NaOMe–MeOH (5 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. Removal of the solvent from the filtrate yielded a product, which was subjected to silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to yield methyl (*Z*)-4-methoxycinnamate (3 mg) and **15** (22 mg). Methyl (*Z*)-4-methoxycinnamate was identified by TLC, ^{1}H-, and ^{13}C-NMR comparisons with an authentic sample.

Desacylsenegin IV (**15**): Colorless fine crystals, mp 233—236 °C, $[\alpha]_D^{28}$ −15.0° (c = 0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{70}H_{111}Na_2O_{37}$ $(M+2Na-H)^+$: 1589.6600; Found: 1589.6587, Calcd for $C_{70}H_{112}NaO_{37}$ $(M+Na)^+$: 1567.6780; Found: 1567.6731. IR (KBr) cm^{-1}: 3432, 1750, 1719, 1638, 1071. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.95, 1.14, 1.58, 1.98 (3H each, all s, 29, 30, 26, 25, 24-H_3), 1.50 (3H, d, J = 6.3 Hz, Fuc-6), 1.51 (3H, d, J = 4.6 Hz, Rha-6′), 1.60 (3H, d, J = 4.3 Hz, Rha-6), 3.20 (1H, dd-like, 18-H), 4.60 (1H, m, 3-H), 4.90 (1H, d, J = 7.9 Hz, Gal-1), 5.04 (1H, d, J = 7.9 Hz, Glu-1), 5.19 (1H, d, J = 7.6 Hz, Xyl-1), 5.55 (1H, br s, Rha-1′), 5.77 (1H, br s, 12-H), 5.82 (1H, br s, Rha-1), 6.01 (1H, br s, Api-1), 6.05 (1H, d, J = 7.3 Hz, Fuc-1). ^{13}C-NMR: given in Table 1. Negative-mode FAB-MS (m/z): 1543 $(M-H)^-$, positive-mode FAB-MS (m/z): 1567 $(M+Na)^+$, 1589 $(M+2Na-H)^+$.

Isomerization of *Z*-Senegins II (5), III (10), and IV (13) i) An MeOH solution (10 ml) of **5**, **10**, or **13** (5 ml each) in a Pyrex tube was left standing for 24 h under photo-irradiation with four 20 W fluorescent lamps at room temperature (25 °C), respectively. The ratio of components [*ca.* 1.0 : 1.1 (**5** : **6**), 1.0 : 1.0 (**10** : **11**), 3.3 : 1.0 (**13** : **14**)] in the reaction solution was characterized by HPLC (the same conditions as described above for characterization of the geometrical ratio in the isomerization reaction of **1** and **2**).

ii) Powdered **5**, **10**, and **13** (5 mg) were each left standing for 24 h under photo-irradiation with four 20 W fluorescent lamps at room temperature (26 °C). The ratio of the components [*ca.* 1.3 : 1.1 (**5** : **6**), 1.0 : 1.4 (**10** : **11**), 1.0 : 3.6 (**13** : **14**)] was characterized by HPLC as described above.

Bioassay for the Hypoglycemic Activity in Rats Male Wistar rats (Kiwa Laboratory Animals Ltd., Wakayama) 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 100 mg/kg. 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 analysis of variance (ANOVA) followed by Dunnett's test and results were expressed as the mean ± S.E. (Table 3).

Bioassay for Inhibitory Effect on Ethanol Absorption Male Wistar rats (Kiwa Laboratory Animals Ltd.) weighing 170—180 g were starved for 20—24 h but given water *ad libitum*. The tested samples were dissolved in water (5 ml/kg), and they were orally administered to the rats at various doses. 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 of differences was estimated by the same method as in the case of hypoglycemic activity and results were expressed as the mean ± S.E. (Table 4).

Acknowledgement The authors are grateful to the Ministry of Education, Science, Sports and Culture of Japan for financial support (Grant No. 07557292).

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