E-SENEGASAPONINS A AND B, *Z*-SENEGASAPONINS A AND B, *Z*-SENEGINS II AND III, NEW TYPE INHIBITORS OF ETHANOL ABSORPTION IN RATS FROM SENEGAE RADIX, THE ROOTS OF *POLYGALA SENEGA* L. VAR *LATIFOLIA* TORREY ET GRAY

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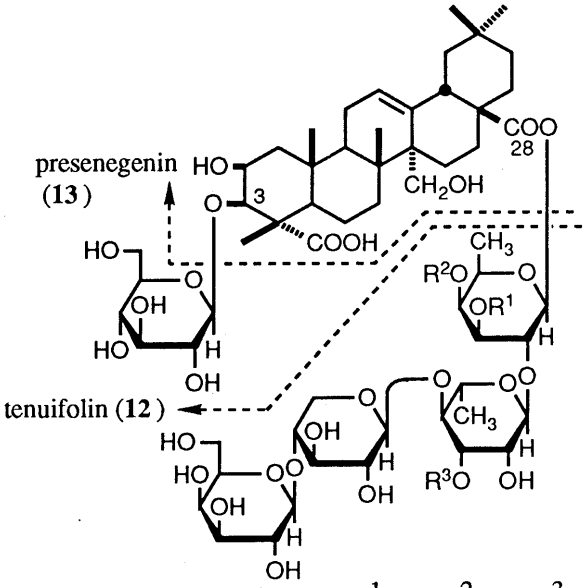
New inhibitors of ethanol absorption, *E*-senegasaponins a and b, *Z*-senegasaponins a and b, *Z*-senegins II and III, were isolated from Senegae Radix, the roots of *Polygala senega* L. var *latifolia* TORREY et GRAY, together with senegins II and III. Their chemical structures have been elucidated on the basis of chemical and physicochemical evidence, and the geometrical isomeric structures of methoxycinnamoyl moiety in each saponin were found to show tautomer-like behavior. The inhibitory effects of senegasaponins, senegins, and their related compounds have been examined, and some structure-activity relationships have been found.

KEY WORDS senegasaponin a ; senegasaponin b ; *Z*-senegin ; Senegae Radix ; *Polygala senega* var *latifolia* ; ethanol absorption inhibitor

Senegae Radix, the root of *Polygala senega* L. and *P. senega* L. var *latifolia* TORREY et GRAY (Polygalaceae), has been used clinically as an expectorant. The oligoglycosidic constituents, which are the principal and bioactive ingredients of this natural medicine, have been investigated extensively, and three bisdesmoside type saponins having *E*-methoxycinnamoyl group, senegins II (**6**), III (**9**) and IV, have been characterized.[1)]



	R^1	R^2	R^3
E-senegasaponin a (**1**) :	H	*E*-MC	Api
Z-senegasaponin a (**2**) :	H	*Z*-MC	Api
desacylsenegasaponin a (**3**) :	H	H	Api
E-senegasaponin b (**4**) :	H	*E*-MC	H
Z-senegasaponin b (**5**) :	H	*Z*-MC	H
senegin II (**6**) :	H	*E*-DMC	H
Z-senegin II (**7**) :	H	*Z*-DMC	H
desacylsenegin II (**8**) :	H	H	H
senegin III (**9**) :	Rha	*E*-MC	H
Z-senegin III (**10**) :	Rha	*Z*-MC	H
desacylsenegin III (**11**) :	Rha	H	H

MC : 4-methoxycinnamoyl
DMC : 3,4-dimethoxycinnamoyl
Api : β-D-apiofuranosyl, Rha : α-L-rhamnopyranosyl

In the course of our studies on the bioactive constituents in natural medicines, several saponin constituents were found to exhibit potent inhibitory effects on ethanol absorption. Furthermore, by examination of the structure-activity relationships, it has been found that the active saponins are classified into the following two types of structure : 1) Oleanolic acid 3-*O*-monodesmosides such as elatosides obtained from *Aralia elata* SEEM. (Araliaceae),[2)] 2) Acylated polyhydroxyoleanane triterpene 3-*O*-monodesmosides such as camelliasaponins from *Camellia japonica* L. (Theaceae)[3a)] and escins from *Aesculus hippocastanum* L. (Hippocastanaceae).[3b)] As a continuing part of our screening to find the inhibitor of ethanol absorption in natural medicines, new type inhibitors named *E*-senegasaponins a (**1**) and b (**4**), *Z*-senegasaponins a (**2**) and b (**5**), *Z*-senegins II (**7**) and III (**10**) were isolated from Japanese Senegae Radix, the roots of *P. senega* var *latifolia*, through bioassay-guided separations. This communication deals with the structure elucidation of **1**, **2**, **4**, **5**, **7**, and **10**, and the inhibitory effects on ethanol absorption in rats.

The MeOH extract of Senegae Radix, cultivated and processed in Hyogo Prefecture, was found to exhibit an inhibitory effect on ethanol absorption; it was partitioned into a AcOEt-water mixture, and the water-soluble portion was further extracted with 1-BuOH. The 1-BuOH-soluble portion with potent inhibitory activity was purified by repeated ordinary SiO_2 column and HPLC (YMC-Pack, D-ODS-5, MeOH-1% AcOH) to afford senegasaponins a and b and senegins II, III and IV (*E* and *Z*-type mixtures). Finally each *E* and *Z*- mixture was subjected to HPLC separation (YMC-Pack-Ph, MeOH-1% AcOH) to give *E*-senegasaponins a (**1**, 0.14% from the natural medicine) and b (**4**, 0.18%), *Z*-senegasaponins a (**2**, 0.16%) and

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Table I. ^{13}C NMR Data for **1**, **2**, **4**, **5**, **7** and **10** (68MHz, d_5-Pyridine, δc)

	1	2	4	5	7	10		1	2	4	5	7	10
C-1	44.3	44.3	44.2	44.3	44.2	44.3	Rha-1	102.3	102.3	101.9	101.9	101.9	102.0
C-2	70.3	70.3	70.3	70.3	70.3	70.3	2	71.8	71.6	71.8	71.7	71.8	71.6
C-3	85.9	85.9	85.9	85.9	85.9	85.9	3	82.4	82.4	72.5	72.5	72.5	72.5
C-4	52.9	52.9	52.8	52.9	52.9	52.9	4	78.8	78.8	85.2	85.2	85.1	84.8
C-5	52.6	52.6	52.5	52.5	52.5	52.5	5	68.4	68.4	68.4	68.4	68.4	68.6
C-6	21.5	21.3	21.6	21.5	21.5	21.4	6	19.1	19.0	18.7	18.7	18.7	18.7
C-7	33.8	33.8	33.5	33.5	33.5	33.6	Xyl-1	104.8	104.8	107.0	107.0	106.9	106.8
C-8	41.1	41.1	41.1	41.1	41.1	41.1	2	75.0	75.1	75.1	75.1	75.1	75.1
C-9	49.3	49.3	49.3	49.3	49.3	49.3	3	77.4	77.4	77.3	77.3	77.3	77.3
C-10	37.1	37.0	37.0	37.0	37.0	37.0	4	78.6	78.6	78.3	78.3	78.4	78.4
C-11	23.6	23.6	23.6	23.6	23.6	23.8	5	64.7	64.7	65.0	65.0	65.0	64.4
C-12	127.8	127.9	127.8	127.9	127.8	127.7	Gal-1	104.5	104.5	104.5	104.5	104.5	104.5
C-13	139.0	139.0	138.9	138.9	139.0	138.9	2	71.8	71.8	71.8	71.8	71.8	71.8
C-14	47.0	47.0	47.0	47.0	47.0	47.1	3	75.0	75.1	75.7	75.7	75.7	75.5
C-15	24.5	24.5	24.5	24.5	24.5	24.5	4	70.1	70.1	70.1	70.1	70.1	70.1
C-16	24.1	24.1	24.0	24.0	24.0	24.0	5	76.6	76.6	76.7	76.7	76.7	76.6
C-17	47.9	47.9	48.0	48.0	48.0	48.0	6	62.3	62.3	62.2	62.2	62.2	62.2
C-18	42.0	42.0	42.0	42.0	42.0	42.0	Rha-1						105.1
C-19	45.4	45.4	45.3	45.4	45.4	45.5	2						72.3
C-20	30.8	30.8	30.8	30.8	30.8	30.8	3						73.6
C-21	33.8	33.8	33.8	33.9	33.9	33.9	4						72.6
C-22	32.4	32.4	32.4	32.4	32.4	32.3	5						70.9
C-23	180.9	180.9	180.7	180.8	180.8	180.7	6						18.6
C-24	14.3	14.3	14.2	14.2	14.2	14.2	Api-1	111.8	111.7				
C-25	17.5	17.5	17.5	17.5	17.5	17.5	2	77.5	77.5				
C-26	18.9	18.9	18.7	18.7	18.7	18.9	3	79.6	79.6				
C-27	64.4	64.4	64.4	64.4	64.4	64.3	4	74.5	74.5				
C-28	176.7	176.7	176.8	176.8	176.8	176.7	5	64.6	64.5				
C-29	33.0	33.0	33.0	33.0	33.0	33.1	Cinnamoyl						
C-30	24.1	24.1	24.0	24.0	24.0	24.0	1	167.7	166.8	167.6	166.7	166.8	166.3
Glu-1	105.4	105.4	105.4	105.5	105.4	105.4	2	116.2	117.1	116.1	117.1	116.9	116.4
2	75.0	75.2	75.2	75.2	75.2	75.2	3	145.2	143.9	145.2	144.0	144.6	144.8
3	78.4	78.4	78.3	78.4	78.4	78.4	1'	127.5	127.9	127.4	127.9	128.2	127.7
4	71.6	71.6	71.5	71.6	71.6	71.6	2'	130.4	133.2	130.4	133.2	114.8	133.3
5	78.4	78.4	78.3	78.4	78.4	78.4	3'	114.8	113.9	114.7	113.9	150.5	114.1
6	62.7	62.7	62.7	62.7	62.7	62.7	4'	161.9	161.0	161.9	161.1	151.2	161.1
Fuc-1	94.5	94.5	94.6	94.6	94.6	94.8	5'	114.8	113.9	114.7	113.9	111.4	114.1
2	75.9	75.9	74.6	74.6	74.6	74.9	6'	130.4	133.2	130.4	133.2	125.8	133.3
3	74.2	73.9	74.4	74.2	74.1	80.9	3'-OMe					55.8	
4	74.7	74.5	74.8	74.7	74.8	73.4	4'-OMe	55.3	55.2	55.0	55.2	55.7	55.2
5	70.8	70.6	70.9	70.7	70.7	70.8							
6	16.6	16.6	16.6	16.6	16.6	16.9							

b (**5**, 0.10%), *Z*-senegins II (**7**, 0.25%) and III (**10**, 0.07%) together with senegin II (*E* form, **6**)[1] and III (*E* form, **9**).[1]

E-Senegasaponin a (**1**), colorless fine crystals, mp 228-231°C, $[\alpha]_D$ -12.9° (MeOH), $C_{74}H_{110}O_{35}$, IR (KBr) : 3432, 1750, 1717, 1707, 1637 cm^{-1}, FAB-MS : m/z 1557 $(M-H)^-$, liberated methyl *E*-4-methoxycinnamate and desacylsenegasaponin a (**3**) upon sodium methoxide treatment (1% NaOMe-MeOH, r.t., 15min). Methanolysis of **3** with 9% HCl-MeOH furnished methyl D-glucoside, methyl D-fucoside, methyl L-rhamnoside, methyl D-apioside, methyl D-xyloside, and methyl D-galactoside. The ^1H NMR (d_5-pyridine, J in Hz) and ^{13}C NMR data (Table I) were assigned by COSY (^1H-^1H, ^1H-^{13}C), HMBC, and HOHAHA (^1H-^1H, ^1H-^{13}C). The ^1H NMR data of **1** showed signals ascribable to six anomeric protons [δ 5.10 (d, J=7.6, Glu-1-H), 6.21 (d, J=8.3, Fuc-1-H), 6.31 (br s, Rha-1-H), 6.08 (d-like, Api-1-H), 5.31 (d, J=7.6, Xyl-1-H), 4.96 (d, J=7.9, Gal-1-H) and *E*-4-methoxycinnamoyl group [δ 6.51 (d, J=15.8, 2-H), 7.94 (d, J=15.8, 3-H), 7.00, 7.44 (both d, J=8.9, aromatic Hx4)]. HMBC correlations were observed between the following carbons and protons in the oligosaccharide moieties of **1** and **3** : 3-C & Glu-1-H, 28-C & Fuc-1-H, Fuc-2-C & Rha-1-H, Rha-3-C & Api-1-H, Rha-4-C & Xyl-1-H, Xyl-4-C & Gal-1-H. Based on these findings together with comparisons of ^{13}C NMR data for **1**, **3**, **6**, and **9** and observation of the HMBC correlation between Fuc-4-H [δ 5.73 (d-like)] and *E*-4-methoxycinnamoyl-1-C in **1**, the structure of *E*-senegasaponin a has been determined as **1**.

Table II. Inhibitory Effects of Senegasaponins, Senegins, and Their Related Compounds from Senegae Radix on Ethanol Absorption (100mg/kg, *p.o.*)

	n	Ethanol concentration in blood (mg/ml) 1h	2h	3h
E, *Z*-Senegasaponins a (**1**, **2**)	4	0.06±0.02*	0.07±0.03*	0.03±0.01
Desacylsenegasaponin a (**3**)	4	0.22±0.08*	0.22±0.04	0.03±0.01
E, *Z*-Senegasaponins b (**4**, **5**)	5	0.07±0.01**	0.02±0.01*	0.03±0.01
E, *Z*-Senegins II (**6**, **7**)	5	0.09±0.06*	0.09±0.04	0.04±0.01
Desacylsenegin II (**8**)	4	0.26±0.12	0.10±0.05	0.05±0.01
Tenuifolin (**12**)	4	0.49±0.01	0.11±0.02	0.01±0.00
Presenegenin (**13**)	4	0.51±0.01	0.15±0.02	0.01±0.00
Control	10	0.54±0.01	0.21±0.01	0.01±0.00

*p<0.05, **p<0.01

Alkaline treatment of *Z*-senegasaponin a (**2**)[4] with 1% NaOMe-MeOH furnished methyl *Z*-4-methoxycinnamate and **3**. Comparisons of ^{1}H and ^{13}C NMR data for **2** with those for **1** and **3** have corroborated the structure of *Z*-senegasaponin a (**2**) having *Z*-4-methoxycinnamoyl group [δ 6.00 (d, J=12.9, 2-H), 6.85 (d, J=12.9, 3-H)]. In addition, it was found that, by standing for 24h at room temperature in aq. MeOH solution, **2** was changed to **1** to yield the mixture of *E* and *Z*-senegasaponin a (*ca* 1:1.3).[5] The structures of *E* and *Z*-senegasaponins b (**4**, **5**),[6] *Z*-senegins II (**7**)[7] and III (**10**)[8] were elucidated in the same way. By the alkaline treatment, **4**, **5**, and **7** yielded desacylsenegin II (**8**) and organic acid methyl ester (**4** : methyl *E*-4-methoxycinnamate, **5** : methyl *Z*-4-methoxycinnamate, **7** : methyl *Z*-3, 4-dimethoxycinnamate), while **10** furnished desacylsenegin III (**11**) and methyl *Z*-4-methoxycinnamate. Detailed examinations of ^{1}H and ^{13}C NMR experiments as described for **1** led us to formulate the structures of *E*- and *Z*-senegasaponins b (**4**, **5**), *Z*-senegins II (**7**) and III (**10**). Treatment of *Z*-type saponins (**5**, **7**, **10**) with aq. MeOH as described for **2** yielded the *E* and *Z*-mixture (1:2.5, 1.1:1, 1:1)[5], while *E*-type saponins (**4**, **6**, **9**) also gave the *E* and *Z*-mixture (4.5:1, 2:1, 1.6:1)[5], respectively.

Inhibitory effects of senegasaponins a and b, and senegin II (the *E* and *Z*-mixture) and their related compounds such as desacyl derivatives and a prosapogenol on ethanol absorption are summarized in Table II. Senegasaponins a and b and senegin II were found to exhibit a potent inhibitory effect on ethanol absorption. On the other hand, desacylsenegasaponin a (**3**) and desacylsenegin II (**8**) showed weaker inhibitory activity than senegasaponins a and b and senegin II. Furthermore, tenuifolin (**12**) and presenegenin (**13**) eliminated the activity completely, indicating that the 28-*O*-glycoside moiety with methoxycinnamoyl group was essential to the inhibition of ethanol absorption. We are currently working on the further characterization of structure-activity relationships for acylated bisdesmoside saponins.

REFERENCES AND NOTES

1) Y. Tsukitani, J. Shoji, *Chem. Pharm. Bull.*, **21**, 1564 (1973) and the literatures cited in the paper.

2) M. Yoshikawa, E. Harada, H. Matsuda, T. Murakami, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **41**, 2069 (1993).

3) a) M. Yoshikawa, E. Harada, T. Murakami, H. Matsuda, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **42**, 742 (1994) ; b) M. Yoshikawa, E. Harada, T. Murakami, H. Matsuda, N. Wariishi, J. Yamahara, N. Murakami, I. Kitagawa, *ibid.*, **42**, 1357 (1994).

4) **2** : colorless fine crystals, mp 237-240°C, $[\alpha]_D$ -22.0° (MeOH), $C_{74}H_{110}O_{35}$, IR (KBr) : 3432, 1750, 1719, 1708, 1639 cm^{-1}, ^{1}H NMR : δ 5.10 (d, J=7.9, Glu-1-H), 6.16 (d, J=7.9, Fuc-1-H), 6.26 (br s, Rha-1-H), 6.08 (d, J=5.0, Api-1-H), 5.30 (d, J=7.9, Xyl-1-H), 4.96 (d, J=7.9, Gal-1-H), 6.00 (d, J=12.9, Cin-2-H), 6.85 (d, J=12.9, Cin-3-H). FAB-MS : m/z 1557 $(M-H)^-$.

5) Each approximate ratio of the geometrical isomers (*E* and *Z*) was characterized by HPLC.

6) a) **4** : colorless fine crystals, mp 251-254°C, $[\alpha]_D$ +7.4° (MeOH), $C_{69}H_{102}O_{31}$, IR (KBr) : 3432, 1750, 1717, 1708, 1636 cm^{-1}, ^{1}H NMR : δ 5.09 (d, J=7.6), 6.21 (d, J=8.3), 6.40 (br s), 5.02 (d-like), 4.99 (d, J=7.9)(Glu, Fuc, Rha, Xyl, Gal-1-H), 6.50 (d, J=15.8, 2-H), 7.92 (d, J=15.8, 3-H). FAB-MS : m/z 1425 $(M-H)^-$.; b) **5** : colorless fine crystals, mp 252-255°C, $[\alpha]_D$ -13.2° (MeOH), $C_{69}H_{102}O_{31}$, IR (KBr) : 3432, 1750, 1717, 1707, 1636 cm^{-1}, ^{1}H NMR : δ 5.10 (d, J=7.6), 6.16 (d, J=8.2), 6.34 (br s), 5.02 (d-like), 4.99 (d, J=8.3)(Glu, Fuc, Rha, Xyl, Gal-1-H), 5.94 (d, J=12.9, 2-H), 6.84 (d, J=12.9, 3-H). FAB-MS : m/z 1425 $(M-H)^-$.

7) **7** : colorless fine crystals, mp 238-242°C, $[\alpha]_D$ -24.0° (MeOH), $C_{70}H_{104}O_{32}$, IR (KBr) : 3432, 1750, 1719, 1707, 1630 cm^{-1}, ^{1}H NMR : δ 5.08 (d, J=7.9), 6.16 (d, J=8.2), 6.33 (br s), 5.01 (d-like), 4.98 (d, J=7.9)(Glu, Fuc, Rha, Xyl, Gal-1-H), 5.97 (d, J=12.5, 2-H), 6.86 (d, J=12.5, 3-H). FAB-MS : m/z 1455 $(M-H)^-$.

8) **10** : colorless fine crystals, mp 243-246°C, $[\alpha]_D$ -17.0° (MeOH), $C_{75}H_{112}O_{35}$, IR (KBr) : 3453, 1750, 1719, 1707, 1637 cm^{-1}, ^{1}H NMR : δ 5.08 (d, J=7.6), 6.09 (d, J=7.6), 5.91 (br s), 4.99 (br s), 4.98 (d, J=7.9), 5.70 (br s)(Glu, Fuc, Rha, Xyl, Gal-1-H), 5.91 (d, J=12.9, 2-H), 6.86 (d, J=12.9, 3-H). FAB-MS : m/z 1571$(M-H)^-$.

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