Six New Presenegenin Glycosides, Reiniosides A—F, from *Polygala reinii* Root

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Received August 29, 1994; accepted November 9, 1994

Six new oleanane-type triterpene saponins, called reiniosides A—F, were isolated from the roots of *Polygala reinii* FR. *et* SAV. and their structures were elucidated by spectroscopic and chemical means.

Key words *Polygala reinii*; reinioside; Polygalaceae; oleanane-triterpene saponin; presenegenin

We earlier reported[1] the isolation and structural elucidation of acylated oligosaccharides from the roots of *Polygala reinii* FR. *et* SAV. We continued the investigation on the constituents of this plant and isolated six new oleanane-type triterpene saponins, called reiniosides A—F (**1**—**6**), from the polar and lipophilic fraction.

A water extract of the roots was passed through a porous polymer gel Diaion HP-20 column and the adsorbed material was successively eluted with 50%, 70% methanolic water and methanol. The methanolic eluate was subjected to preparative HPLC using octadecyl silica (ODS) gel column and gave six saponins.

Reinioside A (**1**) showed its $[M+Na]^+$ ion peak at *m/z* 865 in the FAB-MS and the elemental analysis data was consistent with $C_{42}H_{66}O_{17}$. The ^{1}H-NMR spectrum suggested the presence of five singlet methyl proton (δ 0.87, 1.01, 1.04, 1.50, 1.98), a pair of oxymethylene proton [δ 3.74 (br d, $J=12$ Hz), 4.00 (d, $J=12$ Hz)], a trisubstituted olefinic proton [δ 5.86 (t-like)] in the aglycone moiety and two anomeric proton signals [δ 5.09 (d, $J=8$ Hz), 5.21 (d, $J=8$ Hz)]. The ^{13}C-NMR spectrum suggested the presence of two carboxylic groups (δ 180.1 and 180.3). From these NMR data, **1** was assumed to be a triterpenic glycoside. On acid hydrolysis, **1** afforded **1a** as an aglycone and D-glucose as a sugar moiety. The ^{1}H-NMR spectrum of **1a** revealed five singlet methyl at δ 1.00, 1.03, 1.06, 1.50, 1.97 and two carbinyl proton signals at δ 4.59 (m), 4.71 (d, $J=3.5$ Hz). Compound **1a** afforded a dimethyl ester (**1b**) by treatment with diazomethane. Senegins, which were isolated from *Polygala senega* and had presenegenin (**1c**) as a genuine aglycone, afforded senegenic acid by treatment with acid.[2] We therefore assumed that **1a** was senegenic acid and reinioside A was a presenegenin diglucoside. By comparing the ^{13}C-NMR spectral data of the aglycone moiety of **1** and senegin III,[3] the genuine aglycone of **1** was determined to be presenegenin (olean-12-en-2β,3β,27-trihydroxy-23,28-dioic acid) (**1c**). To determine the binding sites of two glucoses, we undertook a differential nuclear Overhauser effect (NOE) and a heteronuclear multiple bond coherence (HMBC) spectrum after assignment of all proton signals due to a sugar moiety by a ^{1}H–^{1}H COSY spectrum. The NOE was observed at the proton signal at δ 4.59 (d, $J=2$ Hz) due to the H-3 of the aglycone on irradiation at the proton signal at δ 5.09 and at the proton signal at δ 4.01 due to the H-2 of inner glucose on irradiation at the proton signal at δ 5.21, respectively. In the HMBC spectrum, $^3J_{HCOC}$ were observed between the anomeric proton signal at δ 5.09 and the carbon signal at δ 85.4 due to the C-3 of the aglycone, and between the anomeric proton signal at δ 5.21 and the carbon signal at δ 83.7 due to the C-2 of the inner glucose. The anomeric configuration of both of two glucosyl moieties was determined as β from the J values of their proton signals. Consequently, the structure of reinioside A was elucidated as presenegenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Reinioside B (**2**) showed its $[M+Na]^+$ ion peak at *m/z* 1332 in the FAB-MS and the elemental analysis data was consistent with $C_{61}H_{96}O_{30}$. The ^{1}H-NMR spectrum suggested the presence of six singlet methyl proton (δ 0.78, 0.94, 1.10, 1.53, 1.97, 1.99), a pair of oxymethylene proton [δ 3.77 (br d, $J=12$ Hz), 4.00 (overlapped)], a trisubstituted olefinic proton [δ 5.81 (t-like)] and five anomeric proton signals [δ 5.05 (d, $J=7$ Hz), 5.07 (d, $J=8$ Hz), 5.18 (d, $J=8$ Hz), 6.09 (d, $J=8$ Hz), 6.29 (br s)]. The ^{13}C-NMR spectrum suggested the presence of a carboxylic (δ 180.3), two ester carbonyl (δ 171.0, 176.6) and five anomeric carbon signals (δ 94.5, 101.7, 102.8, 106.0, 107.3). The ester carbonyl carbon signal at δ 171.0 was assigned to an acetyl carbon by observation of a ^{1}H–^{13}C long-range correlation with a methyl proton signal at δ 1.97. On acid hydrolysis, compound **2** afforded senegenic acid (**1a**) as an artifact aglycone and D-glucose, D-fucose, L-rhamnose and D-xylose in the ratio 2:1:1:1. On alkaline hydrolysis, compound **2** afforded deacetyl compound (**2a**) as an amorphous powder, which showed its $[M+Na]^+$ ion peak at *m/z* 1290. From these results, we assumed that **2** was a bisdesmoside of presenegenin (**1c**) having five monosaccharide and one acetyl group. We undertook a differential NOE and HMBC spectrum to decide the binding sites of each sugar and acetyl group after assignment of all proton signals due to a sugar moiety by a detailed proton spin decoupling experiment. The NOE and the ^{1}H–^{13}C long-range correlation were observed as shown in Tables III and IV, confirming that the sugar chain at C-3 was β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl and that at C-28 was β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(4-*O*-acetyl)-β-D-fucopyranosyl group.

Reinioside C (**3**) showed its $[M+Na]^+$ ion peak at *m/z* 1374 in the FAB-MS and the elemental analysis data was consistent with $C_{63}H_{98}O_{31}$. The ^{1}H-NMR spectrum was very similar to that of **2**, except for the presence of one more acetyl methyl signal [δ 2.04 (6H, s)]. On alkaline

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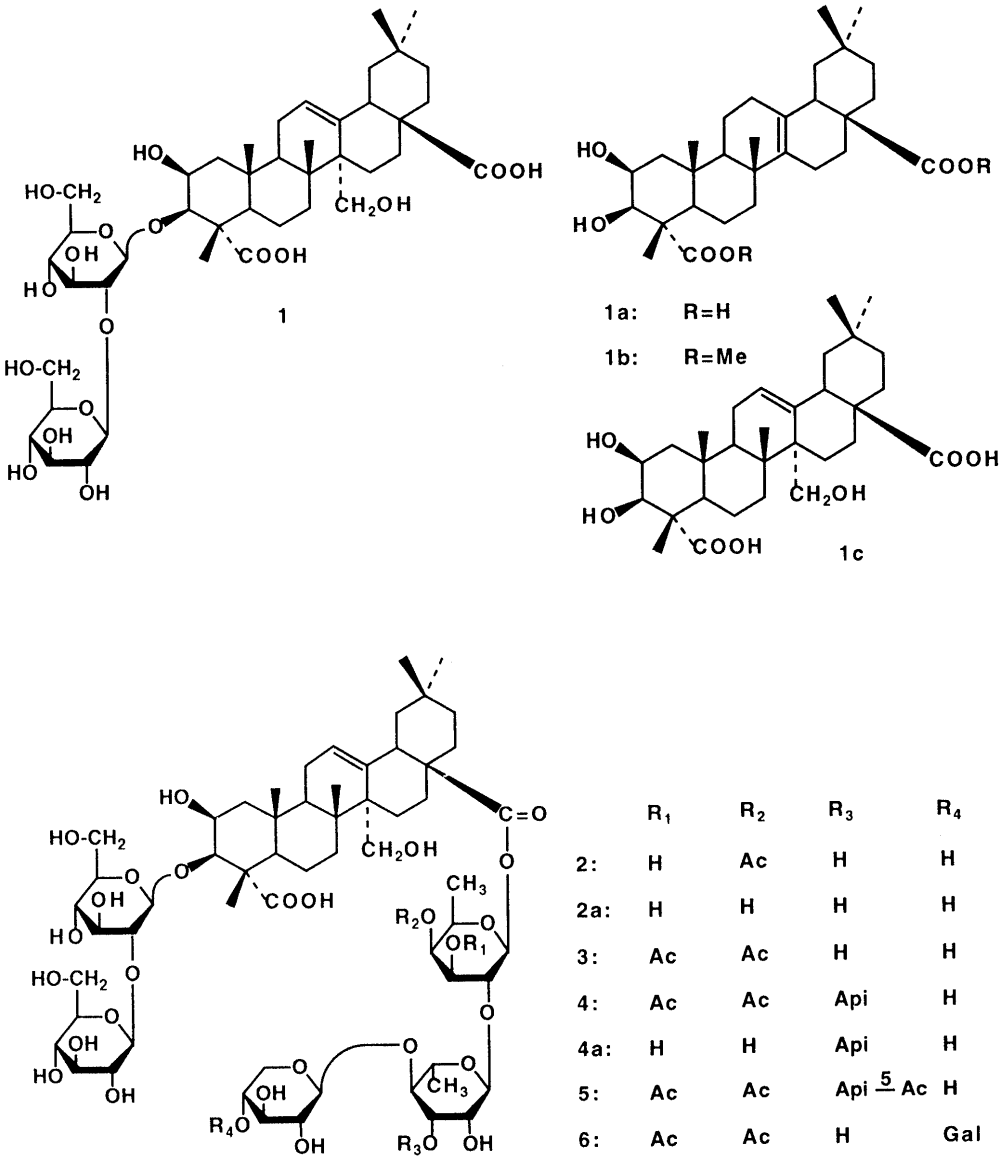

Chart 1

hydrolysis, compound **3** afforded **2a** as a deacetyl compound. In the ^{1}H-NMR spectrum of **3**, the H-3 and H-4 signals of the fucose moiety were shifted to downfield at δ 5.58 (Δ +1.40 ppm) and 5.59 (Δ +1.64 ppm), respectively, compared to those of **2a**. The structure of **3** was therefore determined to be 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-diacetyl)-β-D-fucopyranosyl presenegenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Reiniosides D (**4**), $C_{68}H_{106}O_{35}$ and E (**5**), $C_{70}H_{108}O_{36}$ showed a $[M+Na]^+$ ion peak at *m/z* 1506 and 1548, respectively, in the FAB-MS. Both compounds gave D-glucose, D-fucose, L-rhamnose, D-xylose and D-apiose in the ratio 2 : 1 : 1 : 1 : 1 on acid hydrolysis, while both gave **4a** on alkaline hydrolysis. In the ^{1}H-NMR spectrum, **4** revealed two acetyl signals at δ 2.06 and 2.13, and **5** revealed three acetyl signals at δ 1.88, 2.07 and 2.12. A detailed proton spin decoupling experiment starting from the irradiation at each anomeric proton signal and difference NOE experiment irradiating at each of these signals enabled us to assign the sugar proton signals as shown in Table I. The positions of acetoxyl groups were thus C-3 and C-4 of the fucose moiety in **4** and C-3 and C-4 of the fucose moiety and C-5 of the apiose moiety in **5**. From the NOE and HMBC spectral data (Tables III and IV), the sugar sequences were β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl at C-3 and β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-(3,4-diacetyl)-β-D-fucopyranosyl at C-28 in **4** and β-D-xylopyranosyl-(1→4)-[5-acetyl-β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-(3,4-diacetyl)-β-D-fucopyranosyl at C-28 in **5**.

Reinioside F (**6**), $C_{69}H_{108}O_{36}$ showed its $[M+Na]^+$ ion peak at *m/z* 1536 in the FAB-MS. The ^{1}H-NMR spectrum suggested the presence of two acetyl methyl proton at δ 2.04 (6H, s) and six anomeric proton signals at δ 4.94 (d, J=8 Hz), 4.98 (d, J=7.5 Hz), 5.08 (d, J=8 Hz), 5.22 (d, J=7.5 Hz), 5.67 (d, J=1.5 Hz) and 6.15 (d, J=8 Hz) in addition to the signals due to the aglycone moiety. On acid hydrolysis, **6** gave D-glucose, D-fucose, L-rhamnose, D-xylose and D-galactose in the ratio 2 : 1 : 1 : 1 : 1. The position of two acetoxyl groups were C-3 and C-4 of the

TABLE I. ^{1}H-NMR Spectral Data of Reiniosides A(**1**)—F(**6**) in C_5D_5N

Proton No.	1	2	2a	3	4	4a	5	6
Aglycone moiety								
2	4.69 (m)	4.70 (m)	4.80 (m)	4.71 (m)	4.71 (m)	b)	4.71 (m)	4.69 (m)
3	4.59 (d, 2)	4.59 (d, 2)	4.60 a)	4.60 a)	4.61 (d, 3)	b)	4.62 (d, 2)	4.59 (d, 3)
12	5.86 (t-like)	5.81 (t-like)	5.79 (t-like)	5.81 (t-like)	5.79 (t-like)	5.77 (t-like)	5.79 (t-like)	5.80 (t-like)
24	1.98 (s)	1.99 (s)	1.97 (s)	1.98 (s)	2.02 (s)	2.02 (s)	2.02 (s)	1.98 (s)
25	1.50 (s)	1.53 (s)	1.54 (s)	1.55 (s)	1.58 (s)	1.56 (s)	1.58 (s)	1.54 (s)
26	1.04 (s)	1.10 (s)	1.02 (s)	1.09 (s)	1.08 (s)	1.11 (s)	1.08 (s)	1.09 (s)
27	3.74 (br d, 12)	3.77 (br d, 12)	b)	3.77 (br d, 12)	3.78 (br d, 12)	b)	3.77 (br d, 12)	3.78 (br d, 12)
	4.00 (d, 12)	4.00 a)	b)	4.02 a)	4.02 a)	b)	3.99 a)	4.01 (d, 12)
29	0.87 (s)	0.78 (s)	0.78 (s)	0.79 (s)	0.80 (s)	0.78 (s)	0.80 (s)	0.79 (s)
30	1.01 (s)	0.94 (s)	0.93 (s)	0.94 (s)	1.00 (s)	0.95 (s)	1.00 (s)	0.95 (s)
Sugar moiety at C-3								
Inner Glc								
1	5.09 (d, 8)	5.07 (d, 8)	5.08 (d, 7.5)	5.09 (d, 8)	5.09 (d, 7.5)	5.10 (d, 8)	5.10 (d, 8)	5.08 (d, 8)
2	4.01 a)	4.00 a)	b)	4.02 a)	4.02 a)	b)	4.02 (br t, 8)	4.05 (br t, 8.5)
3	4.19 (br t, 9)	4.18 (br t, 9.5)	b)	4.19 (br t, 9.5)	4.20 (br t, 9)	b)	4.20 a)	4.20 (br t, 9)
4	4.09 (br t, 9)	4.09 (br t, 9.5)	b)	4.08 (br t, 8.5)	4.13 a)	b)	4.11 (br t, 9.5)	4.08 (br t, 8.5)
5	3.86 (m)	3.85 a)	b)	3.85 (m)	3.85 (m)	b)	3.86 (m)	3.85 (m)
6	4.23 (dd, 11.5, 5)	4.22 (dd, 11.5, 6)	b)	4.23 (dd, 12, 5.5)	4.24 (dd, 11, 5)	b)	4.24 (dd, 12.5, 3.5)	4.23 (dd, 12, 5)
	4.42 (dd, 11.5, 1.5)	4.42 (dd, 11.5, 1.5)	b)	4.42 (dd, 12, 1.5)	4.42 a)	b)	4.43 (dd, 12.5, 5)	4.42 a)
Terminal Glc								
1	5.21 (d, 8)	5.18 (d, 8)	5.19 (d, 7.5)	5.20 (d, 8)	5.21 (d, 7.5)	5.20 (d, 8)	5.21 (d, 8)	5.22 (d, 7.5)
2	3.96 (br t, 8.5)	3.95 (br t, 8)	b)	3.96 (br t, 8)	3.97 (br t, 8)	b)	3.97 (br t, 8)	3.98 (dd, 8.5, 7.5)
3	4.15 (br t, 8.5)	4.14 (br t, 8.5)	b)	4.15 (br t, 8.5)	4.10 (br t, 9)	b)	4.15 (br t, 8)	4.17 (br t, 8.5)
4	4.09 (br t, 9)	4.07 (br t, 8.5)	b)	4.08 (br t, 8.5)	b)	b)	b)	b)
5	4.03 a)	4.00 a)	b)	4.02 a)	4.02 a)	b)	4.03 a)	4.07 a)
6	4.35 (dd, 11.5, 4.5)	4.33 (dd, 11, 2)	b)	4.34 a)	4.34 (dd, 11.5, 4.5)	b)	4.35 (dd, 12, 5)	4.35 a)
	4.60 (dd, 11.5, 1.5)	4.58 a)	b)	4.60 a)	4.59 (br d, 11.5)	b)	4.60 (br d, 12)	4.62 (br d, 11)
Sugar moiety at C-28								
Fuc								
1		6.09 (d, 8)	6.06 (d, 8)	6.16 (d, 8)	6.15 (d, 8)	6.07 (d, 8)	6.15 (d, 8)	6.15 (d, 8)
2		4.56 (br t, 8)	4.64 (br t, 8)	4.57 a)	4.52 (dd, 9.5, 8)	b)	4.53 (dd, 9.5, 8)	4.55 a)
3		4.37 (dd, 9.5, 3)	4.18 a)	5.58 a)	5.56 (dd, 9.5, 3)	b)	5.55 (dd, 9.5, 3)	5.57 a)
4		5.52 (br d, 3)	3.95 a)	5.59 (br s)	5.60 (d, 3)	b)	5.60 (d, 3)	5.58 a)
5		4.03 a)	3.90 a)	4.12 a)	4.12 a)	b)	4.12 a)	4.13 a)
6		1.26 (d, 6.5)	1.48 (d, 6)	1.20 (d, 6.5)	1.22 (d, 6.5)	1.55 (d, 6)	1.22 (d, 6.5)	1.20 (d, 6.5)
Ac at C-3				2.04 (s)	2.13 (s)		2.12 (s)	2.04 (s)
Ac at C-4		1.97 (s)		2.04 (s)	2.06 (s)		2.07 (s)	2.04 (s)
Rha								
1		6.29 (br s)	6.39 (br s)	5.69 (br s)	5.66 (br s)	6.25 (br s)	5.64 (br s)	5.67 (d, 1.5)
2		4.78 (br s)	b)	4.54 (br s)	4.68 (dd, 3, 1.5)	b)	4.65 (br s)	4.53 (br s)
3		4.66 (dd, 9.5, 3)	b)	4.48 (dd, 9.5, 2.5)	4.39 (dd, 9.5, 3)	b)	4.37 (dd, 9.5, 3)	4.48 (dd, 9, 3)
4		4.32 (br t, 9.5)	4.30 a)	4.29 (br t, 9.5)	4.46 (br t, 9.5)	b)	4.45 (br t, 9.5)	4.22 a)
5		4.51 (m)	b)	4.34 a)	4.28 (m)	b)	4.26 (m)	4.32 a)
6		1.78 (d, 6)	1.68 (d, 8)	1.74 (d, 6)	1.69 (d, 6)	1.69 (d, 6)	1.68 (d, 6)	1.69 (d, 6)
Xyl								
1		5.05 (d, 7)	5.04 (d, 7)	5.06 (d, 6.5)	5.31 (d, 7.5)	5.32 (d, 8)	5.30 (d, 8)	4.98 (d, 7.5)
2		4.01 a)	b)	4.02 a)	3.95 (br t, 8)	b)	3.96 (br t, 8)	3.95 (dd, 8.5, 7.5)
3		4.01 a)	b)	4.02 a)	4.13 (br t, 8.5)	b)	4.09 (br t, 8.5)	4.05 (br t, 8.5)
4		4.21 a)	b)	4.20 a)	4.20 a)	b)	4.15 a)	4.22 a)
5		3.50 (br t, 11)	b)	3.51 (br t, 11)	3.45 (br t, 10)	b)	3.52 (br t, 11)	3.45 (br t, 11)
		4.15 a)	b)	4.16 a)	4.19 (d, 10)	b)	4.20 a)	4.27 a)
Gal								
1							4.94 (d, 8)	
2							4.45 a)	
3							4.15 a)	
4							b)	
5							4.22 a)	
6							b)	
							b)	
Api								
1					6.00 (d, 4.5)	6.05 (d, 4)	5.98 (d, 4)	
2					4.70 (d, 4.5)	b)	4.48 (d, 4)	
4					4.19 a)	b)	4.16 (d, 9.5)	
					4.55 (d, 10)	b)	4.29 (d, 9.5)	
5					4.02 a)	b)	4.42 a)	
					4.02 a)	b)	4.55 (d, 11.5)	
Ac							1.88 (s)	

Recorded at 500 MHz at 35°C; the figures in parentheses are coupling constants (*J*) in Hz. *a*) Overlapping with other signals. *b*) No assignment.

TABLE II. ^{13}C-NMR Spectral Data of Reiniosides A(**1**)—F(**6**) in C_5D_5N

Carbon No.	**1a**	**1**	**2**	**2a**	**3**	**4**	**4a**	**5**	**6**
Aglycone moiety									
1	44.9	44.0	44.0	44.1	44.1	44.2	44.1	44.1	44.1
2	71.8	70.2	70.1	70.2	70.0	70.0	70.2	70.0	70.0
3	76.0	85.4	85.4	85.5	85.4	85.4	85.4	85.3	85.4
4	54.0	52.6	52.6	52.7	52.6	52.8	52.6	52.7	52.6
5	52.0	52.4	52.4	52.6	52.4	52.5	52.7	52.5	52.5
6	18.5	21.0	21.1	21.2	21.1	21.1	21.1	21.1	21.2
7	39.5	33.4	33.5	33.6	33.5	33.7	33.8	33.8	33.5
8	38.4	40.8	41.0	41.2	41.1	41.1	41.1	41.1	41.1
9	57.3	49.4	49.2	49.7	49.3	49.3	49.4	49.3	49.3
10	37.2	37.0	36.9	37.1	37.0	37.1	37.0	37.0	37.0
11	21.1	23.7	23.5	23.6	23.7	23.8	23.6	23.7	23.8
12	24.0	127.1	127.8	127.9	127.8	127.9	127.9	127.9	127.8
13	130.7	139.7	138.8	138.9	138.8	138.9	138.9	138.8	138.9
14	137.0	48.1	47.9	48.0	47.9	47.9	47.9	47.9	48.0
15	21.9	24.6	24.4	24.6	24.5	24.6	24.5	24.5	24.5
16	31.9	24.1	23.9	24.1	23.9	23.9	23.7	23.9	24.0
17	45.3	46.5	46.9	46.9	47.0	47.0	46.8	47.0	47.0
18	39.9	41.8	41.9	42.0	41.8	41.9	42.0	41.9	41.9
19	41.9	45.5	45.3	45.3	45.3	45.5	45.4	45.4	45.4
20	30.9	31.0	30.7	30.8	30.7	30.8	30.8	30.8	30.8
21	34.6	34.1	33.7	33.9	33.8	33.8	33.8	33.8	33.9
22	32.2	33.2	32.3	32.4	32.3	32.3	32.4	32.3	32.4
23	180.8	180.3	180.3	180.4	180.3	180.5	180.6	180.6	180.4
24	13.4	14.1	14.1	14.2	14.1	14.2	14.2	14.2	14.2
25	18.1	17.3	17.4	17.5	17.4	17.5	17.5	17.5	17.5
26	20.8	18.7	18.7	18.6	18.7	18.9	18.8	18.9	18.7
27		64.5	64.3	64.4	64.4	64.5	64.5	64.5	64.4
28	180.2	180.1	176.6	176.7	176.3	176.4	176.7	176.4	176.4
29	32.7	33.2	33.0	33.1	33.0	33.1	33.1	33.1	33.0
30	25.1	23.9	23.9	24.1	23.9	24.1	24.1	24.1	24.0
Sugar moiety at C-3									
Inner Glc									
1		102.8	102.8	102.9	102.8	102.9	102.8	102.7	102.7
2		83.7	83.7	83.9	83.3	83.8	83.6	83.8	83.6
3		77.9	77.9	78.0	77.9	77.9	78.0	77.9	77.9
4		71.1[a]	71.1[b]	71.1[c]	71.1	71.2	71.2	71.2	71.2
5		78.1	78.0	78.2[d]	78.1	78.1	78.1[e]	78.1	78.2
6		62.6	62.5	62.7	62.5	62.5	62.6	62.6	62.5[g]
Terminal Glc									
1		106.1	106.0	106.2	106.1	106.2	106.2	106.2	106.1
2		76.9	76.8	76.9	76.8	76.9	76.9	76.9	76.9
3		78.3	78.2	78.3[d]	78.2	78.3	78.3	78.3	78.4
4		71.2[a]	70.9[b]	70.9[c]	71.1	71.0	71.1	71.2	71.2
5		78.3	78.2	78.3[d]	78.2	78.3	78.3[e]	78.3	78.4
6		62.6	62.5	62.7	62.5	62.5	62.6	62.6	62.7[g]
Sugar moiety at C-28									
Fuc									
1			94.5	94.8	94.2	94.4	94.7	94.3	94.2
2			74.0	74.2	72.8	74.6	75.5[f]	74.7	72.7
3			74.1	76.7	74.6	74.1	75.7[f]	74.1	74.6
4			74.7	73.3	71.4	71.3	73.1	71.2	71.2
5			70.5	72.5	70.1	70.2	72.3	70.2	70.1
6			16.4	16.9	16.0	16.1	16.9	16.1	16.1
Ac at C-3					20.6	20.7		20.6	20.6
					170.0	170.2		170.1	170.1
Ac at C-4			20.7		20.3	20.4		20.4	20.4
			171.0		170.7	170.8		170.8	170.8
Rha									
1			101.7	101.3	102.0	102.4	101.7	102.4	102.0
2			71.6	71.9	71.0	71.5	71.5	71.6	71.3
3			72.4	72.5	72.3	82.0	82.2	81.9	72.3
4			84.9	85.1	84.5	78.3	78.7	78.1	84.5
5			68.4	68.3	69.0	69.3	68.4	69.2	69.0
6			19.1	18.8	18.6	19.0	19.0	19.0	18.6

TABLE II. (continued)

Carbon No.	1a	1	2	2a	3	4	4a	5	6
Xyl									
1			107.3	107.4	107.2	105.1	105.3	105.2	106.8
2			76.1	76.2	76.0	75.7	76.2	75.6	75.5
3			78.6	78.8	78.7	78.6	78.7	78.5	76.7
4			70.8[b]	71.2	70.8	71.3	71.2	71.2	78.1
5			67.4	67.5	67.5	67.2	67.2	67.2	65.0
Gal									
1									104.5
2									71.8
3									75.0
4									70.2
5									77.4
6									62.3[g]
Api									
1						111.8	111.7	111.3	
2						77.7	77.7	77.6	
3						79.6	79.6	78.1	
4						74.6	74.6	74.5	
5						64.6	64.7	67.0	
Ac								20.7	
								170.8	

Recorded at 125.6 MHz at 35°C. *a—g*) Assignments may be interchanged in each column.

TABLE III. Data of ^1H $\{^1H\}$ NOE Difference Spectra of Reiniosides A(**1**)—F(**6**)

Compd.	H (Irradiated)	H (Effected)
1	$Glc_{inn.}$-1	3, $Glc_{inn.}$-3, $Glc_{inn.}$-5
	$Glc_{ter.}$-1	$Glc_{inn.}$-2, $Glc_{ter.}$-3, $Glc_{ter.}$-5
2	$Glc_{inn.}$-1	3, $Glc_{inn.}$-3, $Glc_{inn.}$-5
	$Glc_{ter.}$-1	$Glc_{inn.}$-2, $Glc_{ter.}$-3, $Glc_{ter.}$-5
	Fuc-1	Fuc-3, Fuc-5
	Rha-1	Fuc-2, Rha-2
	Xyl-1	Rha-4, Xyl-3, Xyl-5α
3	$Glc_{inn.}$-1	3, $Glc_{inn.}$-3, $Glc_{inn.}$-5
	$Glc_{ter.}$-1	$Glc_{inn.}$-2, $Glc_{ter.}$-3, $Glc_{ter.}$-5
	Fuc-1	Fuc-3, Fuc-5
	Rha-1	Fuc-2, Rha-2
	Xyl-1	Rha-4, Xyl-3, Xyl-5α
4	$Glc_{inn.}$-1	3, $Glc_{inn.}$-3, $Glc_{inn.}$-5
	$Glc_{ter.}$-1	$Glc_{inn.}$-2, $Glc_{ter.}$-3, $Glc_{ter.}$-5
	Fuc-1	Fuc-3, Fuc-5
	Rha-1	Fuc-2, Rha-2
	Xyl-1	Rha-4, Xyl-3, Xyl-5α
	Api-1	Rha-3
5	$Glc_{inn.}$-1	3, $Glc_{inn.}$-3, $Glc_{inn.}$-5
	$Glc_{ter.}$-1	$Glc_{inn.}$-2, $Glc_{ter.}$-3, $Glc_{ter.}$-5
	Fuc-1	Fuc-3, Fuc-5
	Rha-1	Fuc-2, Rha-2
	Xyl-1	Rha-4, Xyl-3, Xyl-5α
	Api-1	Rha-3
6	$Glc_{inn.}$-1	3, $Glc_{inn.}$-3, $Glc_{inn.}$-5
	$Glc_{ter.}$-1	$Glc_{inn.}$-2, $Glc_{ter.}$-3, $Glc_{ter.}$-5
	Fuc-1	Fuc-3, Fuc-5
	Rha-1	Fuc-2, Rha-2
	Xyl-1	Rha-4, Xyl-3, Xyl-5α
	Gal-1	Xyl-4, Gal-5

fucose moiety by comparison of the NMR data with those of compounds **3**—**5**. The proton spin decoupling, NOE experiment and HMBC spectrum (Tables III and IV) enabled us to identify the structure of **6** as 28-*O*-β-D-galactopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-diacetyl)-β-D-fucopyranosyl presenegenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

The structures of these saponins were similar to those of *P. senega* L.,[4] *P. senega* var. *latifolia* TORR. *et* GRAY[5] and *P. tenuifolia* WILLD.,[6] except that the acyl groups were all acetyl groups and the sugar chain at C-3 was diglucoside.

Experimental

General Procedure ^1H- and ^{13}C-NMR spectra were obtained with a JEOL GSX 500 spectrometer and chemical shifts were given in δ ppm with tetramethylsilane as an internal standard. FAB-MS was recorded on a JEOL JMS SX102 spectrometer. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Gas chromatography (GC) was run on a HITACHI G-3000 gas chromatograph. Preparative and analytical HPLC were done on a JASCO model 800 instrument.

Extraction and Isolation *Polygala reinii* FR. *et* SAV. was collected in Shizuoka, Japan, in June, 1993 and the dried roots (350 g) were extracted three times with boiling water (5 l). The extract was passed through a porous polymer gel, Diaion HP-20 (Mitsubishi Chemical Co.) column (9 cm × 28 cm) and the adsorbed material was eluted successively with 50% and 70% MeOH aq. and MeOH. These eluates were concentrated *in vacuo* to give yellowish brown residues (yield, 7.57 g, 6.58 g, 6.09 g, respectively). From the MeOH eluate, six saponins were isolated by preparative HPLC [Develosil Lop-ODS, 5 × 50 cm × 2; MeOH–H_2O (55:45—72:28) linear gradient]: **1** (84 mg), **2** (1.17 g), **3** (1.15 g), **4** (250 mg), **5** (222 mg), **6** (327 mg).

Reinioside A (1) An amorphous powder, $[\alpha]_D^{25}$ +25.2° (c=1.05, MeOH). *Anal.* Calcd for $C_{42}H_{66}O_{17}\cdot 2H_2O$: C, 57.39; H, 8.03. Found: C, 57.44; H, 7.98. FAB-MS *m/z*: 865 ($[M+Na]^+$). ^1H- and ^{13}C-NMR: Tables I and II.

Reinioside B (2) An amorphous powder, $[\alpha]_D^{25}$ +3.6° (c=0.97, MeOH). *Anal.* Calcd for $C_{61}H_{96}O_{30}\cdot 3H_2O$: C, 53.74; H, 7.54. Found: C, 53.75; H, 7.80. FAB-MS *m/z*: 1332 ($[M+Na]^+$). ^1H- and ^{13}C-NMR: Tables I and II.

Reinioside C (3) An amorphous powder, $[\alpha]_D^{25}$ +10.4° (c=1.40, MeOH). *Anal.* Calcd for $C_{63}H_{98}O_{31}\cdot 7/2H_2O$: C, 53.49; H, 7.48. Found: C, 53.40; H, 7.56. FAB-MS *m/z*: 1374 ($[M+Na]^+$). ^1H- and ^{13}C-NMR: Tables I and II.

Reinioside D (4) An amorphous powder, $[\alpha]_D^{25}$ −5.4° (c=0.93, MeOH). *Anal.* Calcd for $C_{68}H_{106}O_{35}\cdot 7/2H_2O$: C, 52.81; H, 7.36. Found: H, 52.88; H, 7.57. FAB-MS *m/z*: 1506 ($[M+Na]^+$). ^1H- and ^{13}C-NMR:

TABLE IV. ^1H–^{13}C Long-Range Correlations Observed in the HMBC Spectrum of Reiniosides A(**1**)—F(**6**) ($J=8$ Hz)

Compd.	H	C
Aglycone moiety of **1**—**6**		
	24	3, 4, 5, 23
	25	1, 6, 9, 10
	26	7, 8, 9, 14
	27	8, 15
	29	19, 20, 21, 30
	30	19, 20, 21, 29
Sugar moiety of		
1	$Glc_{inn.}$-1	3
	$Glc_{ter.}$-1	$Glc_{inn.}$-2
2	$Glc_{inn.}$-1	3
	$Glc_{ter.}$-1	$Glc_{inn.}$-2
	Fuc-1	28
	Fuc-4	C=O of Ac (δ 171.0)
	Fuc-6	Fuc-4, Fuc-5
	Ac (δ 1.97)	C=O of Ac (δ 171.0)
	Rha-1	Fuc-2, Rha-3, Rha-5
	Rha-6	Rha-4, Rha-5
	Xyl-1	Rha-4
3	$Glc_{inn.}$-1	3
	$Glc_{ter.}$-1	$Glc_{inn.}$-2
	Fuc-1	28
	Fuc-4	C=O of Ac (δ 170.7)
	Fuc-6	Fuc-4, Fuc-5
	Ac (δ 2.04)	C=O of Ac (δ 170.0; 170.7)
	Rha-1	Fuc-2, Rha-3, Rha-5
	Rha-6	Rha-4, Rha-5
4	$Glc_{inn.}$-1	3
	$Glc_{ter.}$-1	$Glc_{inn.}$-2
	Fuc-1	28
	Fuc-3	C=O of Ac (δ 170.2)
	Fuc-4	C=O of Ac (δ 170.8)
	Fuc-6	Fuc-4, Fuc-5
	Ac (δ 2.06)	C=O of Ac (δ 170.8)
	Ac (δ 2.13)	C=O of Ac (δ 170.2)
	Rha-1	Fuc-2, Rha-3, Rha-5
	Rha-6	Rha-4, Rha-5
	Xyl-1	Rha-4
	Api-1	Rha-3
5	$Glc_{inn.}$-1	3
	$Glc_{ter.}$-1	$Glc_{inn.}$-2
	Fuc-1	28
	Fuc-4	C=O of Ac (δ 170.8)
	Fuc-6	Fuc-4, Fuc-5
	Ac (δ 2.07)	C=O of Ac (δ 170.8)
	Ac (δ 2.12)	C=O of Ac (δ 170.1)
	Rha-1	Fuc-2, Rha-3, Rha-5
	Rha-6	Rha-4, Rha-5
	Xyl-1	Rha-4
	Api-1	Rha-3
6	$Glc_{inn.}$-1	3
	$Glc_{ter.}$-1	$Glc_{inn.}$-2
	Fuc-1	28
	Fuc-4	C=O of Ac (δ 170.8)
	Fuc-6	Fuc-4, Fuc-5
	Ac (δ 2.04)	C=O of Ac (δ 170.8)
	Rha-1	Fuc-2, Rha-3, Rha-5
	Rha-6	Rha-4, Rha-5
	Xyl-1	Rha-4
	Gal-1	Xyl-4

Tables I and II.

Reinioside E (5) An amorphous powder, $[\alpha]_D^{25}$ $-4.5°$ ($c=1.34$, MeOH). *Anal.* Calcd for $C_{70}H_{108}O_{36}\cdot 4H_2O$: C, 52.63; H, 7.31. Found: C, 52.72; H, 7.40. FAB-MS *m/z*: 1548 ($[M+Na]^+$). ^1H- and ^{13}C-NMR: Tables I and II.

Reinioside F (6) An amorphous powder, $[\alpha]_D^{25}$ $+5.2°$ ($c=0.87$, MeOH). *Anal.* Calcd for $C_{69}H_{108}O_{36}\cdot 8H_2O$: C, 49.99; H, 7.54. Found: C, 49.84; H, 7.33. FAB-MS *m/z*: 1536 ($[M+Na]^+$). ^1H- and ^{13}C- NMR: Tables I and II.

Acid Hydrolysis of 1—6 Compound **1** (20 mg) was heated in 5% H_2SO_4 (0.5 ml) and dioxane (0.5 ml) at 100 °C for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate 3 times. The ethyl acetate layer was washed with water and concentrated. The residue was subjected to HPLC [YMC ODS-5, 10 mm × 25 cm; MeCN–H_2O (45 : 55) + 0.05% trifluoroacetic acid (TFA)] to give pure **1a** (1.5 mg). ^1H-NMR (pyridine-d_5, 35 °C) δ: 1.00, 1.03, 1.06 (each 3H, s, H_3-26, H_3-29, H_3-30), 1.50 (3H, s, H_3-25), 1.97 (3H, s, H_3-24), 4.59 (1H, m, H-2), 4.71 (1H, d, $J=3.5$ Hz, H-3). ^{13}C-NMR: Table II. **1a** (1 mg) was dissolved in MeOH (0.5 ml) and treated with CH_2N_2–ether solution in the usual manner to give **1b** (1 mg). ^1H-NMR ($CDCl_3$) δ: 0.87, 0.89, 0.93 (each 3H, s, H_3-26, H_3-29, H_3-30), 1.16 (3H, s, H_3-25), 1.35 (3H, s, H_3-24), 3.60, 3.70 (each 3H, s, OMe × 2), 3.98 (1H, d, $J=4$ Hz, H-3), 4.19 (1H, m, H-2). $[\alpha]_D^{25}$ $+6.3°$ ($c=0.08$, $CHCl_3$). From these NMR and $[\alpha]_D$ value, **1a** and **1b** were identified as senegenic acid and dimethyl senegenate.[2] From each saponin an aglycone (senegenic acid, **1a**) and component monosaccharides were identified as follows. Each saponin (2 mg) was heated with 5% H_2SO_4 (0.05 ml) and dioxane (0.05 ml) at 100 °C for 1 h. After dilution with water, the reaction mixture was extracted with ethyl acetate twice and the water layer was passed through an Amberlite IRA-60E column. The water eluate was concentrated and the residue was reacted with D-cysteine[7] (0.05 mg) in water (0.03 ml) and pyridine (0.015 ml) at 60 °C for 1 h with stirring. After evaporation of the solvent, pyridine (0.015 ml), hexamethyl disilazane (0.015 ml) and trimethyl silylchloride (0.015 ml) were added to the residue. The reaction mixture was heated at 60 °C for 30 min, then centrifuged and the supernatant was applied to GC. The ethyl acetate layer was concentrated and subjected to HPLC to reveal a peak due to senegenic acid from every saponin. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R, D-apiose 10.3 min, L-apiose 9.7 min,[8] D-xylose 10.7 min, L-xylose 9.7 min, L-rhamnose 12.2 min, D-rhamnose 12.0 min,[8] D-fucose 13.2 min, L-fucose 12.3 min, D-glucose 17.8 min, L-glucose 17.2 min, D-galactose 19.5 min, L-galactose 18.3 min.[8] D-Glucose was detected from **1** and D-glucose, D-fucose, L-rhamnose and D-xylose were detected from **2** and **3** at the ratio 2 : 1 : 1 : 1. D-Glucose, D-fucose, L-rhamnose, D-xylose and D-apiose were detected from **4** and **5** in the ratio 2 : 1 : 1 : 1 : 1. D-Glucose, D-fucose, L-rhamnose, D-xylose and D-galactose were detected from **6** in the ratio 2 : 1 : 1 : 1 : 1. The ratio of each sugar was determined by its peak area. HPLC conditions: column, YMC R-ODS-7, 4.6 mm × 25 cm; solvent, MeCN–H_2O (1 : 1) + 0.05% TFA; flow rate, 1.0 ml/min. UV nm; 205. t_R, senegenic acid 7.5 min.

Alkaline Hydrolysis of 2—5 **2** (30 mg) was stirred for 1 h in 0.1% NaOH aq. (2 ml) at room temperature. The reaction mixture was acidified with dil. HCl and passed through a porous polymer gel Diaion HP-20 column (1.5 × 10 cm). After washing with water, the adsorbed material was eluted with MeOH. The MeOH eluate was subjected to HPLC [Develosil ODS-10, 2 × 25 cm; MeCN–H_2O (3 : 7) + 0.05% TFA] to give amorphous powder (**2a**, 17 mg). $[\alpha]_D^{25}$ $+7.4°$ ($c=1.88$, MeOH). FAB-MS *m/z*: 1290 ($[M+Na]^+$). ^1H- and ^{13}C-NMR: Tables I and II. Compound **4** (9 mg) was stirred for 4 h in 0.1% NaOH aq. (1 ml) at room temperature. The reaction mixture was treated as above to give amorphous powder (**4a**, 3 mg). $[\alpha]_D^{25}$ $-5.8°$ ($c=0.77$, MeOH). FAB-MS *m/z*: 1422 ($[M+Na]^+$). ^1H- and ^{13}C-NMR: Tables I and II. Compounds **3** and **5** (each 1 mg) were treated in the same manner as described above. Compounds **3** and **5** gave **2a** and **4a**, respectively, which were identified by HPLC. HPLC conditions: column, YMC R-ODS-7, 4.6 mm × 25 cm; solvent, MeCN–H_2O (35 : 65) + 0.05% TFA; flow rate, 1.0 ml/min. t_R, 6.5 min (**2a**) and MeCN–H_2O (1 : 3) + 0.05% TFA; flow rate, 1.0 ml/min. UV nm; 205. t_R, 10.5 min (**4a**).

Acknowledgement We thank the staff of the Central Analytical Laboratory of this university for elemental analyses and measurement of MS.

References and Notes

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