Silenorubicosides A−D, Triterpenoid Saponins from *Silene rubicunda*

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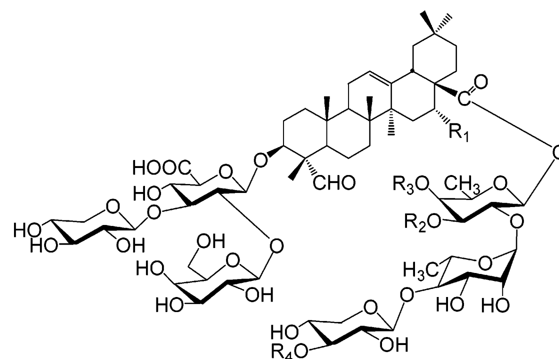
Four novel triterpenoid saponins, silenorubicosides A−D (**1**−**4**), together with four known saponins were isolated from the roots of *Silene rubicunda*. Their structures were established on the basis of spectroscopic analysis, including extensive 1D and 2D NMR (DQF-COSY, TOCSY, HETCOR, HMBC, and phase-sensitive NOESY) studies and chemical degradation.

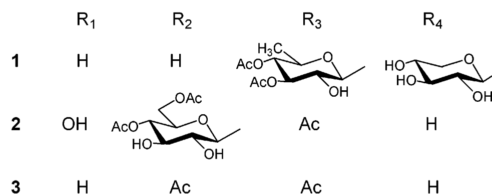
Silene is a genus of Caryophyllaceae consisting of 400 species. *Silene rubicunda* Franch. is an annual herb that is distributed in the southwestern region of the People's Republic of China. *S. rubicunda* is called "Jiuzhishen", and its roots are used as a folk medicine for the treatment of dropsy, cough, amenorrhea, and hernia.[1] The roots of *S.* rubicunda are rich in saponins, and four triterpenoid saponins named rubicunosides A−D have been isolated.[2] The medicinal importance and our continuing interest in the chemistry of triterpenoid saponins prompted us to initiate a chemical investigation of this plant. In this report, we describe the isolation and structure elucidation of four new triterpenoid saponins, named silenorubicosides A−D (**1**−**4**), along with four known saponins from the roots of *S.* rubicunda.

Results and Discussion

A methanolic extract of the roots of *S. rubicunda* was suspended in H_2O and then partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction, on chromatographic purification over Dianion-HP-20, followed by repeated HPLC purification, afforded eight triterpenoid saponins, silenorubicosides A−D (**1**−**4**) and four known saponins. The known saponins were identified as 3-*O*-{*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyrannosyl}-28-*O*-{*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(l→2)-[3,4-di-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranosyl}quillaic acid (pachystegioside A),[3] 3-*O*-{*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyrannosyl}-28-*O*-{*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(l→2)-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→3)]-4-*O*-acetyl-*β*-D-fucopyranosyl}quillaic acid,[4] 3-*O*-{*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl}-28-*O*-[*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-*β*-D-fucopyranosyl]gypsogenin (glanduloside C),[5] and 3-*O*-{*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(l→3)]-*β*-D-glucuronopyranosyl}-28-*O*-[α-L-rhamnopyranosyl-(1→2)-4-*O*-(*E*)-*p*-methoxycinnamoyl-*β*-D-fucopyranosyl]quilllaic acid (sinocrassuloside X),[6] by detailed NMR analyses and comparison of the data in the references. Although known in other genera, this is the first report of the isolation of these known compounds from *Silene*.

Silenorubicoside A (**1**) was obtained as an amorphous solid, $[\alpha]^{22}_D$ −6.3° (MeOH). The negative-ion high-resolution (HR) FABMS of **1** showed an accurate $[M - H]^-$ ion peak at *m/z* 1725.7411, in accordance with an empirical molecular formula of $C_{79}H_{122}O_{41}$, which was supported by the ^{13}C NMR spectrum and various DEPT data. The ^{13}C NMR spectrum showed 79 carbon signals, of which 30 were assigned to the aglycone, four to the two acetyl groups, and 45 to the sugar moieties (Tables 1 and 2). Acid hydrolysis of **1** with 1 M HCl afforded gypsogenin[7] and monosaccharides L-rhamnose, D-fucose, D-galactose, D-xylose, D-quinovose, and D-glucuronic acid (1:1:1:3:1:1) by GC-MS analysis





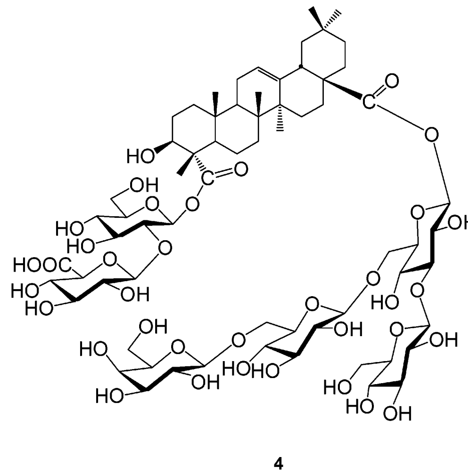


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Table 1. ^{13}C NMR Data (δ) for Aglycone Moieties of **1**−**4** in Pyridine-d_5

position	**1**	**2**	**3**	**4**
1	38.1	38.2	38.1	39.0
2	25.4	25.3	25.4	27.8
3	85.0	84.3	84.8	75.0
4	55.1	55.1	55.1	55.5
5	48.9	48.6	48.8	54.3
6	20.7	20.6	20.7	23.2
7	32.7	32.8	32.5	33.8
8	40.2	40.3	40.2	40.3
9	46.8	46.9	47.8	48.4
10	36.3	36.3	36.3	37.2
11	23.2	23.7	23.4	23.9
12	122.4	122.0	122.5	122.7
13	144.0	144.6	144.0	144.8
14	42.3	42.1	42.2	41.7
15	28.6	36.2	28.4	28.1
16	23.7	73.9	23.7	21.9
17	47.1	49.4	47.2	46.8
18	42.1	41.7	41.9	41.2
19	46.3	47.5	46.3	46.3
20	30.7	30.7	30.7	30.7
21	33.9	36.0	34.0	32.7
22	32.3	31.9	32.3	32.4
23	211.0	209.8	210.4	178.3
24	11.2	11.1	11.3	11.3
25	15.7	15.8	15.7	16.2
26	17.4	17.5	17.4	17.3
27	25.8	27.0	25.9	26.4
28	175.4	176.0	176.3	176.2
29	33.1	33.1	33.1	33.1
30	23.7	24.5	23.7	23.8

following conversion to the trimethylsilyl thiazolidine derivatives.[8] The chemical shifts of δ 85.0 (C-3) and 175.4 (C-28) revealed that **1** was a bisdesmosidic glycoside. The ^1H and ^{13}C NMR spectra showed eight anomeric proton signals at δ 6.29 (br s), 5.95 (d, J = 8.3 Hz), 5.57 (d, J = 7.5 Hz), 5.33 (d, J = 7.5 Hz), 5.17 (d, J = 7.6 Hz), 5.05 (d, J = 7.0 Hz), 5.01 (d, J = 7.1 Hz), and 4.86 (d, J = 7.3 Hz) and the corresponding carbon signals at δ 101.4, 94.3, 104.0, 105.1, 105.7, 105.8, 107.0, and 104.1, respectively. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were assigned using DQFCOSY with the aid of TOCSY and NOESY experiments, while the carbons were assigned by HETCOR and further confirmed by HMBC experiments. On comparison of the ^{13}C NMR data between **1** and pachystegioside A,[3] the superimposable pattern indicated the same sugar sequence. The linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 of galactose (δ 5.57) with C-2 of glucuronic acid (δ 78.2), H-1 of xylose I (δ 5.33) with C-3 of glucuronic acid (δ 86.2), H-1 of glucuronic acid (δ 4.86) with C-3 of the aglycone (δ 85.0). Similarly, the sugar chain at C-28 was established from the following HMBC correlations: H-1 of xylose III (δ 5.17) with C-3 of xylose II (δ 87.1), H-1 of xylose II (δ 5.01) with C-4 of rhamnose (δ 85.3), H-1 of rhamnose (δ 6.29) with C-2 of fucose (δ 74.3), H-1 of quinovose (δ 5.05) with C-4 of fucose (δ 83.8), H-1 of fucose (δ 5.95) with C-28 of the aglycone (δ 175.4). The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment. Attachment of the two acetyl groups to C-3 and C-4 of quinovose was confirmed from HMBC correlations between H-3 (δ 5.62) and H-4 (δ 5.06) of quinovose with the carbonyl carbons of the two acetyl groups (δ 170.4 and 170.1), respectively. From the above evidence, the structure of silenorubicoside A (**1**) was elucidated as 3-*O*-{β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl}-28-*O*-{β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(l→2)-[3,4-di-*O*-acetyl-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranosyl}gypsogenin.

Silenorubicoside B (**2**) was isolated as an amorphous solid with the molecular formula $C_{76}H_{116}O_{40}$, as determined from data of the negative-ion HRFABMS (*m/z* 1667.6940 [M − H]$^-$), ^{13}C NMR (76 carbon signals), and various DEPT spectra. In contrast to compound **1**, the 1D MNR spectra showed different signal patterns due to both the aglycone and the sugar units. In the ^1H NMR spectrum of **2**, in addition to six methyl proton singlets (1.74, 1.44, 1.06, 0.98, 0.95, 0.82), a broad triplet-like olefinic proton at δ 5.38, and an aldehyde proton at δ 9.98, it also showed a broad singlet for the hydroxymethylene proton at δ 5.21 characteristic of H-16β of quillaic acid. Detailed NMR analysis established the aglycone to be quillaic acid.[3] Acid hydrolysis of **2** yielded quillaic acid, and L-rhamnose, D-fucose, D-galactose, D-xylose, D-glucose, and D-glucuronic acid (1:1:1:2:1:1) as component sugars. The ^1H and ^{13}C NMR data showed seven anomeric proton signals at δ 6.44 (br s), 6.01 (d, J = 8.3 Hz), 5.56 (d, J = 7.5 Hz), 5.33 (d, J = 7.8 Hz), 5.22 (d, J = 7.8 Hz), 5.02 (d, J = 7.6 Hz), and 4.91 (d, J = 7.5 Hz) and the corresponding anomeric carbon signals at δ 101.0, 94.6, 104.3, 105.0, 106.3, 105.2, and 104.0. The ^1H and ^{13}C NMR chemical shift assignments were accomplished by a combination of DQF-COSY, TOCSY, DEPT, HETCOR, HMBC, and phase-sensitive NOESY experiments. From its ^1H and ^{13}C NMR data (Tables 1 and 2), it was evident that the sugar structure at C-3 was the same as that in **1**. The linkage of the remaining four sugars at C-28 was determined from the following HMBC correlations: H-1 of xylose (δ 5.22) with C-4 of rhamnose (δ 82.6), H-1 of rhamnose (δ 6.44) with C-2 of fucose (δ 71.8), H-1 of glucose (δ 5.02) with C-3 of fucose (δ 82.9), H-1 of fucose (δ 6.01) with C-28 of the aglycone (δ 176.0). Attachment of three acetyl groups to C-4 of fucose and to C-4 and C-6 of glucose was confirmed by HMBC correlation between H-4 of fucose (δ 5.77) and H-4 (δ 5.47) and H-6 (δ 4.51 and 4.57) of glucose with the carbonyl carbons of the acetyl groups (δ 170.2, 170.1, and 170.7), respectively. On the basis of the foregoing evidence, the chemical structure of silenorubicoside B (**2**) was concluded to be 3-*O*-{β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl}-28-*O*-{β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(l→2)-[4,6-di-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-4-*O*-acetyl-β-D-fucopyranosyl}quillaic acid.

Silenorubicoside C (**3**), an amorphous solid, had a molecular formula of $C_{68}H_{104}O_{33}$, determined from its positive-ion HRFABMS. The overall structure assignment was accomplished using the same protocol as in **1**. Its ^1H and ^{13}C NMR data revealed that **3** was a bisdesmosidic glycoside with the same aglycone (gypsogenin) as that in **1** and that it contained six sugars and two acetyl groups. Acid hydrolysis afforded gypsogenin and L-rhamnose, D-fucose, D-galactose, D-xylose, and D-glucuronic acid (1:1:1:2:1). The linkage positions were established using HMBC and NOESY correlations. As in compounds **1** and **2**, **3** also contained the same trisaccharide unit at C-3. The remaining three sugars at C-28 were of a linear structure with xylose linked to C-4 (δ 85.0) of rhamnose, and rhamnose to C-2 (δ 72.6) of fucose. Attachment of the two acetyl groups to C-3 and C-4 of fucose was determined from HMBC correlations between H-3 (δ 5.56) and H-4 (δ 5.57) of fucose with the carbonyl carbons of the two acetyl groups (δ 170.7 and 170.1), respectively. On the basis of all the foregoing evidence, silenonbicoside C (**3**) was elucidated as 3-*O*-{β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl}-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-

Table 2. ^{13}C and ^{1}H NMR Data for Sugar Moieties of **1**−**3** in Pyridine-d_5[a]

	1		**2**		**3**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-*O*-sugar						
Glc A						
1	104.1	4.86 d (7.3)	104.0	4.91 d (7.5)	104.1	4.89 d (7.6)
2	78.2	4.29	78.7	4.37	78.6	4.35 dd (9.2, 7.6)
3	86.2	4.23	86.0	4.27 t (8.9)	86.1	4.24
4	71.4	4.45	71.3	4.46	71.3	4.45 t (9.2)
5	77.3	4.50	77.3	4.51	77.4	4.52
6	172.0		171.8		171.8	
Gal						
1	104.0	5.57 d (7.5)	104.3	5.56 d (7.5)	104.2	5.57 d (7.5)
2	73.6	4.48	73.8	4.47	73.7	4.47
3	75.5	4.12	75.5	4.15	75.5	4.13
4	70.2	4.55	70.3	4.57	70.2	4.57
5	76.5	3.98	76.8	4.00	76.7	4.00
6	61.6	4.39, 4.53	61.7	4.45, 4.52	61.7	4.43, 4.53
Xy l I						
1	105.1	5.33 d (7.5)	105.0	5.33 d (7.8)	105.1	5.33 d (7.6)
2	75.1	3.94	75.3	3.97	75.3	3.96 t (8.3)
3	78.6	4.10	78.6	4.08	78.6	4.10
4	70.8	4.11	70.9	4.10	70.8	4.11
5	67.3	3.66 t (10.1), 4.27	67.4	3.65 t (10.5), 4.24 dd (11.4, 4.9)	67.4	3.65 t (11.0), 4.24
28-*O*-sugar						
Fuc						
1	94.3	5.95 d (8.3)	94.6	6.01 d (8.3)	94.1	6.12 d (8.2)
2	74.3	4.53	71.8	4.66 t (8.5)	72.6	4.58
3	76.6	4.18	82.9	4.37	74.8	5.56
4	83.8	3.97	73.8	5.77 d (3.2)	71.2	5.57
5	71.3	3.94	71.1	4.06	70.8	4.11
6	17.0	1.51 d (6.6)	16.8	1.26 d (6.2)	16.0	1.17 d (6.9)
Fuc-3-$COCH_3$					170.7	
Fuc-3-$COCH_3$					20.6	2.06 s
Fuc-4-$COCH_3$			170.2		170.1	
Fuc-4-$COCH_3$			20.9	1.99 s	20.4	2.05 s
Rham						
1	101.4	6.29 br s	101.0	6.44 br s	102.0	5.70 br s
2	71.6	4.73 br s	72.0	4.75 br s	71.4	4.55 br s
3	72.5	4.58	72.3	4.65 d (8.7)	72.4	4.45
4	85.3	4.27	82.6	4.42	85.0	4.29
5	68.1	4.38	68.6	4.50	68.9	4.35
6	18.5	1.64 d (6.2)	18.6	1.71 d (6.0)	18.6	1.77 d (5.9)
Xy l II						
1	107.0	5.01 d (7.1)	106.3	5.22 d (7.8)	107.5	5.09 d (7.3)
2	75.4	3.96	76.1	4.00	76.1	4.00
3	87.1	4.02	78.6	4.06	78.7	4.05
4	68.8	4.08	70.8	4.11	70.0	4.15
5	67.0	3.49 t (10.8), 4.21	67.4	3.44 t (10.3), 4.18	67.4	3.52 t (10.5), 4.23
Xy l III						
1	105.7	5.17 d (7.6)				
2	75.0	4.04				
3	78.0	4.11				
4	70.7	4.16				
5	67.4	3.66 t (10.1), 4.27				
Qui						
1	105.8	5.05 d (7.0)				
2	73.1	4.01				
3	76.0	5.62 t (9.6)				
4	74.4	5.06 t (9.6)				
5	70.3	3.70				
6	17.7	1.26 d (6.2)				
Qui-3-$COCH_3$	170.4					
Qui-3-$COCH_3$	20.7	1.97 s				
Qui-4-$COCH_3$	170.1					
Qui-4-$COCH_3$	20.6	2.05 s				
Glc						
1			105.2	5.02 d (7.6)		
2			72.6	3.97		
3			75.0	4.11		
4			71.8	5.47 t (9.6)		
5			75.5	3.96		
6			62.8	4.51, 4.57		
Glc-4-$COCH_3$			170.1			
Glc-4-$COCH_3$			20.7	1.90 s		
Glc-6-$COCH_3$			170.7			
Glc-6-$COCH_3$			20.8	2.14 s		

[a] Coupling constants (*J* in Hz) are in parentheses. Assignments were made on the basis of DQFCOSY, TOCSY, HMQC, and HMBC.

rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-*β*-D-fucopyranosyl]-gypsogenin.

Silenorubicoside D (**4**) was isolated as an amorphous solid, with the molecular formula $C_{66}H_{104}O_{36}$, as determined from data of the positive-ion HRFABMS (*m/z* 1471.6193 [M − H]$^-$), ^{13}C NMR, and various DEPT spectra. The ^{13}C NMR spectrum showed 66 carbon signals, of which 30 were assigned to the aglycone moiety and 36 to the sugar portion. On comparison of the ^{13}C NMR data of the aglycone with those in **1**, the absence of an aldehyde carbon signal and observation of an additional carbonyl carbon signal at δ 178.3 suggested the aglycone of **4** was most likely gypsogenic acid, which was supported by extensive 2D NMR analysis and the result of acid hydrolysis.[9] The chemical shifts of C-23 and C-28 revealed that **4** was a bisdesmosidic glycoside. Six sugar units were indicated, as there were six anomeric protons and carbons displayed in the ^{1}H and ^{13}C NMR spectra. The sugars were determined to be D-galactose, D-glucose, and D-glucuronic acid (1:4:1) by GC-MS analysis after acid hydrolysis. The ^{1}H and ^{13}C NMR chemical shift assignments were accomplished by a combination of DEPT, DQF-COSY, TOCSY, HETCOR, HMBC, and phase-sensitive NOESY experiments. The *β*-anomeric configurations for the glucose and glucuronic acid were determined from their $^3J_{H1,H2}$ coupling constants (7.8−8.2 Hz), while the α-anomeric configuration for the galactose was determined from a direct comparison of the ^{1}H and ^{13}C NMR data with those of the literature data.[10] The tetrasaccharide moiety attached to C-28 was established by the following HMBC correlations: H-1 of galactose (δ 5.46) with C-6 of glucose IV (δ 68.0), H-1 of glucose IV (δ 4.88) with C-6 of glucose II (δ 68.9), H-1 of glucose III (δ 5.70) with C-3 of glucose II (δ 88.0), H-1 of glucose II (δ 5.95) with C-28 of the aglycone (δ 176.2). The linkage of the remaining two sugars attached to C-23 was determined from the following HMBC correlations: H-1 of glucuronic acid (δ 5.66) with C-2 of glucose I (δ 78.4), H-1 of glucose I (δ 6.29) with C-23 of the aglycone (δ 178.3). Thus, the structure of silenorubicoside D (**4**) was determined to be 23-*O*-[*β*-D-glucuronopyranosyl-(1→2)-*β*-D-glucopyranosyl]-28-*O*-{*β*-D-glucopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl}gypsogenic acid. A triterpenoid saponin with α-D-galacopyranose as a component sugar is rare in nature, which has been reported from *Saponaria officinalis* (Caryophyllaceae).[10]

Table 3. ^{13}C and ^{1}H NMR Data for Sugar Moieties of **4** in Pyridine-d_5[a]

position	δ_C	δ_H
23-*O*-sugar		
Glc I		
1	93.8	6.29 d (8.2)
2	78.4	4.37
3	78.8	4.29
4	70.6	4.24
5	79.3	3.84
6	61.5	3.88
GlcA		
1	104.6	5.66 d (7.8)
2	74.9	4.03
3	78.6	4.11
4	72.6	4.53
5	78.5	4.25
6	172.3	
28-*O*-sugar		
Glc II		
1	94.5	5.95 d (7.8)
2	72.9	4.03
3	88.0	3.77 t (8.7)
4	68.6	4.37
5	77.3	3.74
6	68.9	4.19 dd (11.9, 4.6), 4.52
Glc III		
1	103.5	5.70 d (7.8)
2	75.5	3.94 dd (9.1, 7.8)
3	77.6	4.38
4	71.7	4.11
5	77.0	4.11
6	62.3	4.25, 4.48
Glc IV		
1	105.2	4.88 d (7.8)
2	74.9	3.86
3	78.3	4.11
4	71.8	4.05
5	76.2	3.86
6	68.0	4.24, 4.47
Gal		
1	100.5	5.46 d (3.7)
2	70.5	4.63 dd (9.7, 3.7)
3	71.6	4.52 dd (9.7, 3.2)
4	71.1	4.58
5	72.5	4.58
6	62.7	4.38

[a] Coupling constants (*J* in Hz) are in parentheses. Assignments were made on the basis of DQFCOSY, TOCSY, HMQC, and HMBC.

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco microscopic apparatus and are uncorrected. IR spectra were determined using a JASCO D-300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The ^{1}H and ^{13}C NMR measurements were recorded using a JEOL ECP-500 NMR spectrometer. Chemical shifts are expressed in δ (ppm) referring to TMS. FABMS and HRFABMS were conducted using a JEOL JMS-700 MStation mass spectrometer. Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (silica gel 60, Merck), and ODS (Chromatorex, 100−200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan) were used for column chromatography. Preparative HPLC was performed using an ODS column (Pegasil ODSII, Senshu Pak, 10 mm i.d. × 250 mm, Senshu Scientific Co., Ltd., Tokyo, Japan, detector: UV 210 nm). GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS instrument.

Plant Material. The roots of *S. rubicunda* were collected in Kunming, Yunnan Province, People's Republic of China, in November 1999, and identified by Prof. W. Fan (Kunming Institute of Traditional Chinese Medicine). A voucher specimen (NP199901) was deposited at the National Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, China.

Extraction and Isolation. The finely cut roots of *S. rubicunda* (8.0 kg) were extracted with MeOH three times under reflux for 1 h. The combined MeOH extracts were concentrated (1495 g), suspended in H_2O, and then partitioned successively with EtOAc (44.6 g) and *n*-BuOH (218 g). The *n*-BuOH-soluble fraction was applied to a column of Diaion HP-20 and eluted with 30, 50, 70, and 100% MeOH. The fractions eluted with 50% MeOH and 70% MeOH were combined and repeatedly chromatographed over silica gel with $CHCl_3$−MeOH−H_2O (65:25:4) and ODS columns to give several saponin fractions. Further HPLC purification (70−75% MeOH in H_2O, UV detector, 210 nm) afforded **1** (25 mg), **2** (28 mg), **3** (25 mg), **4** (42 mg), pachystegioside A[3] (57 mg), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyrannosylquillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(l→2)-[3,4-di-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside[4] (20 mg), glanduloside C[5] (80 mg), and sinocrassuloside X[6] (18 mg), respectively.

Silenorubicoside A (1): amorphous white solid; mp 205−207 °C; $[\alpha]^{22}_D$ −6.3° (*c* 0.83, MeOH); IR (KBr) ν_{max} 3405, 2936, 1750, 1676, 1420, 1377, 1253, 1134, 1042 cm^{-1}; ^{1}H NMR

(pyridine-d_5, 500 MHz) δ 10.00 (1H, s, H-23), 5.37 (1H, t-like, J = 3.2 Hz, H-12), 3.07 (1H, dd, J = 13.3, 3.4 Hz, H-18), 1.44 (3H, s, H_3-24), 1.20 (3H, s, H_3-27), 1.01 (3H, s, H_3-26), 0.97 (3H, s, H_3-30), 0.87 (3H, s, H_3-29), 0.78 (3H, s, H_3-25); other NMR data, see Tables 1 and 2; FABMS (negative) *m/z* 1725 $[M - H]^-$; HRFABMS (negative) *m/z* 1725.7411 $[M - H]^-$ (calcd for $C_{79}H_{121}O_{41}$, 1725.7395).

Silenorubicoside B (2): amorphous white solid; mp 207−209 °C; $[\alpha]^{22}_D$ −7.2° (*c* 0.97, MeOH); IR (KBr) ν_{max} 3404, 2931, 1732, 1636, 1451, 1379, 1244, 1153, 1078, 1043 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 9.98 (1H, s, H-23), 5.56 (1H, t-like, J = 3.2 Hz, H-12), 5.21 (1H, brs, H-16), 3.35 (1H, dd, J = 13.5, 3.6 Hz, H-18), 2.72 (1H, t, J = 13.5 Hz, H-19), 1.74 (3H, s, H_3-27), 1.44 (3H, s, H_3-24), 1.06 (3H, s, H_3-26), 0.98 (3H, s, H_3-30), 0.95 (3H, s, H_3-29), 0.82 (3H, s, H_3-25); other NMR data, see Tables 1 and 2; FABMS (negative) *m/z* 1667 $[M - H]^-$; HRFABMS (negative) *m/z* 1667.6940 $[M - H]^-$ (calcd for $C_{76}H_{115}O_{40}$, 1667.6955).

Silenorubicoside C (3): amorphous white solid; mp 205−207 °C; $[\alpha]^{22}_D$ +7.1° (*c* 0.96, MeOH); IR (KBr) ν_{max} 3411, 2938, 1742, 1676, 1637, 1420, 1377, 1253, 1142, 1076, 1046 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 9.97 (1H, s, H-23), 5.38 (1H, t-like, J = 3.5 Hz, H-12), 3.09 (1H, dd, J = 13.4, 4.0 Hz, H-18), 1.46 (3H, s, H_3-24), 1.22 (3H, s, H_3-27), 1.02 (3H, s, H_3-26), 0.89 (3H, s, H_3-29), 0.88 (3H, s, H_3-30), 0.80 (3H, s, H_3-25); other NMR data, see Tables 1 and 2; FABMS (negative) *m/z* 1447 $[M - H]^-$; HRFABMS (negative) *m/z* 1447.6390 $[M - H]^-$ (calcd for $C_{68}H_{103}O_{33}$, 1447.6384).

Silenorubicoside D (4): amorphous white solid; mp 171−173 °C; $[\alpha]^{22}_D$ +28.0° (*c* 0.93, MeOH); IR (KBr) ν_{max} 3403, 2925, 1739, 1676, 1431, 1348, 1205, 1143, 1071 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.38 (1H, t-like, J = 3.5 Hz, H-12), 4.67 (1H, t, J = 8.3 Hz, H-3), 3.13 (1H, dd, J = 13.2, 4.1 Hz, H-18), 1.81 (3H, s, H_3-24), 1.34 (3H, s, H_3-26), 1.21(3H, s, H_3-25), 1.12 (3H, s, H_3-27), 0.88 (3H, s, H_3-29), 0.85 (3H, s, H_3-30); other NMR data, see Tables 1 and 3; FABMS (negative) *m/z* 1471 $[M - H]^-$; HRFABMS (negative) *m/z* 1471.6193 $[M - H]^-$ (calcd for $C_{66}H_{103}O_{36}$, 1471.6218).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides. A solution of **1** (10 mg) in 1 M HCl (dioxane−H_2O, 1:1, 1 mL) was heated at 100 °C for 2 h. After dioxane was removed, the solution was extracted with EtOAc (l mL × 3). The extract was washed with H_2O, dried over $MgSO_4$, and evaporated to give gypsogenin (2 mg). The H_2O layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column and concentrated to furnish a monosaccharide residue. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 mL) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried in vacuo, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, EQUITY-1 (30 m × 0.25 mm × 0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier, N_2 gas. By the same method, **2**, **3**, and **4** (each 10 mg) afforded the aglycone quillaic acid (2 mg), gypsogenin (2 mg), and gypsogenic acid (2 mg), respectively. In the acid hydrolysate of **1**−**4**, D-glucose, L-rhamnose, D-fucose, D-galactose, D-xylose, D-quinovose, and D-glucuronic acid were confirmed by comparison of the retention times of their derivatives with those of D-glucose, L-glucose L-rhamnose, D-fucose, D-galactose, D-xylose, D-quinovose, and D-glucuronic acid derivatives prepared in a similar way, which showed retention times of 11.02, 11.43, 7.58, 7.82, 11.59, 6.39, 7.37, and 8.73 min, respectively.

References and Notes

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