Silenosides A−C, Triterpenoid Saponins from *Silene vulgaris*

Michal Glensk,[†] Victor Wray,[‡] Manfred Nimtz ,[‡] and Thomas Schöpke*[,§]

Department of Pharmacognosy, Wroclaw University of Medicine, pl. Nankiera 1, Pl-50-140 Wroclaw, Poland, Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany, and Ernst-Moritz-Arndt-Universität Greifswald, Institut für Pharmazie, Jahnstrasse 17, D-17487 Greifswald, Germany

Received November 11, 1998

Three new triterpenoid saponins named silenosides A−C (**1**−**3**) were obtained from the roots of *Silene vulgaris*. Their structures were elucidated by spectral and chemical methods as *β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-3*β*-hydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→3)-*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside; 3-*O*-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl-3*β*,16α-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→4)-[*β*-D-glucopyranosyl(1→2)]-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside; and 3-*O*-α-L-arabinopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-3*β*,16α-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→4)-[*β*-D-glucopyranosyl(1→2)]-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside, respectively.

Saponins are regarded as a common phytochemical feature of plants belonging to the plant family Caryophyllaceae.[1] As yet, however, the number of species possessing saponins whose structures have been elucidated is limited.

Silene vulgaris (Moench) Garcke (syn. *Silene inflata* Sm.) is a perennial herb that reaches a height of up to 90 cm. It is native to Europe, where it is widespread, and found especially on soil with a high lime content or on sandy ground. In popular medicine, this plant is used to treat anemia. Moreover, the young shoots can be used as a vegetable similar to spinach. Its rhizomes contain saponins, alkaloids, and tannins.[2,3] The present paper reports the isolation and structure elucidation of three major saponins obtained from the roots of *S. vulgaris*, silenosides A−C (**1**−**3**).

Results and Discussion

A multistage procedure was used for the structure elucidation of compounds **1**−**3**. The sugar components were quantified by means of GC of the pertrimethylsilylated derivatives obtained by methanolysis of the corresponding saponin. Their absolute configuration was determined by conversion to the respective trimethylsilyl ether of the methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate, which was analyzed according to Hara et al.[4] ESIMS and MS−MS of the $[M - H]^-$ ions supplied information on the molecular weights, the composition of acyl glycosidic sugar chains, and the composition of the remaining *O*-glycosidic prosapogenin moiety. 2D NMR experiments [^{1}H−^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence spectroscopy (HMQC), ^{1}H-detected multiple bond ^{13}C multiple quantum coherence spectroscopy (HMBC)], in combination with GC−MS analysis of the partially methylated alditol acetates obtained from the monosaccharide constituents of the saponins (methylation analysis), was then used to determine the exact linkage arrangements of all monosaccharide residues and the identity of each aglycon in **1**−**3**.

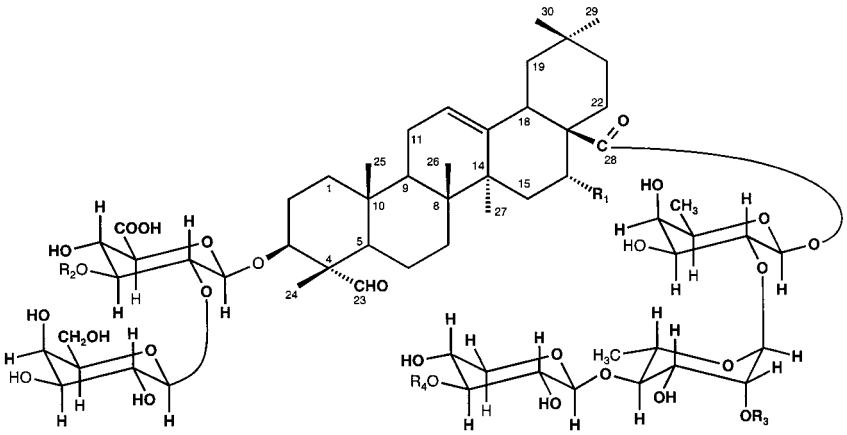
Sugar component analysis of **1**−**3** afforded glucuronic acid, galactose, fucose, rhamnose, and xylose as sugar components of all compounds, while **2** gave, in addition, glucose; and **3**, glucose and arabinose. For the determination of their absolute configuration, as shown in the Experimental Section, L-arabinose and D-xylose (t_R 8.76 min), D-arabinose and L-xylose (t_R 9.24 min), and D-galactose and D-glucuronic acid (t_R 12.31 min) could not be separated under the conditions of chromatography used. However, because the chromatograms did not show peaks of D-arabinose and L-xylose (t_R 9.24 min) and no additional peaks were evident, it was concluded that in **1**−**3** glucuronic acid, glucose, galactose, xylose, and fucose were present as the D-enantiomers; and rhamnose and arabinose as the L-enantiomers.

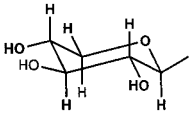
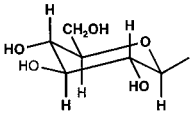
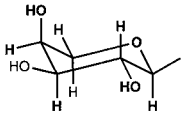
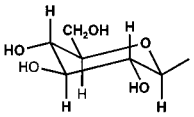
Negative-ion ESIMS of **1** gave a molecular ion at *m*/*z* 1363 $[M - H]^-$. An ESIMS−MS investigation of the molecular ion afforded intense daughter ions at *m*/*z* 807 [M − 132(= pentose) − 132−146(= deoxyhexose) − 146 − $H]^-$ and at *m*/*z* 469 [M − 132 − 132 − 146 − 146 − 162(= hexose) − 176(= hexuronic acid) − $H]^-$. These data suggested that **1** consisted of an aglycon with a molecular weight of 470 bound glycosidically through an acyl unit to a sugar chain consisting of four carbohydrate units (two pentoses and two deoxyhexoses), having a second sugar chain consisting of a hexose and a hexuronic acid bound *O*-glycosidically. Further structural information on the attached oligosaccharides was obtained by methylation analysis, including the additional reduction of the hexuronic acid residue with $NaBD_4$, followed by GC−MS analysis of the resulting partially methylated alditol acetates. On the basis of their retention times and fragmentation patterns, the presence of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylglucitol-6-D_2, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol, 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylxylitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol, and 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylfucitol was determined. These data indicated the presence of a 2-substituted glucuronic acid and fucose, a 4-substituted rhamnose, a 3-substituted xylose, and a terminal galactose and xylose unit. Consequently, a linear arrangement of the acyl glycosidically linked tetrasaccharide was present in **1**.

* To whom correspondence should be addressed. Tel.: +49-3834-864867. Fax: +49-3834-864802. E-mail: thomas@schoepke.de.
[†] Wroclaw University of Medicine.
[‡] Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig
[§] Institut für Pharmazie, Greifswald, Germany.

10.1021/np980505r CCC: $18.00
Published on Web 05/06/1999

Chart 1



	R_1	R_2	R_3	R_4
1	H	H	H	
2	OH	H		H
3	OH			H

The structure of the aglycon of **1** was determined by various NMR techniques. The ^{1}H and ^{13}C NMR spectra of **1** showed signals for six tertiary methyl groups, one olefinic group, and a carboxyl and aldehyde group each. The ^{1}H and ^{13}C NMR data, assigned on the basis of the 1D and 2D COSY, HMQC, and HMBC spectra, indicated 3β-hydroxy-23-oxo-olean-12-en-28-oic acid (gypsogenin) to be the aglycon of **1**.

The NMR data of the carbohydrate moieties in the molecule of **1** confirmed and extended the results obtained by mass spectrometric techniques. ^{1}H and ^{13}C NMR spectra showed six signals for anomeric protons (δ 5.26, 5.21, 4.41, 4.40, 4.25, and 4.13) and anomeric carbons (δ 104.9, 104.7, 104.2, 101.0, 99.5, and 93.3). The presence of two methyl doublets (δ 1.14, J = 6.1 Hz and δ 1.05, J = 6.1 Hz) confirmed that **1** consisted of six carbohydrate moieties, of which two were deoxyhexoses. The ring protons of the monosaccharide residues were assigned starting from the anomeric protons by means of the COSY, HMQC, and HMBC spectra, as summarized in Table 1. Cross peaks in the HMBC spectrum between H-1 of galactose (δ 4.25) and C-2 of glucuronic acid (δ 81.5), between H-1 of glucuronic acid (δ 4.13) and C-3 of the aglycon (δ 81.3), and reverse cross peaks between H-3 of the aglycon (δ 3.77) and C-1 of glucuronic acid (δ 101.0) and between H-2 of glucuronic acid (δ 3.17) and C-1 of galactose (δ 104.7) showed that a galactopyranosyl(1→2)-glucuronopyranoside sugar chain was attached to the aglycon at C-3. The sequence of the second sugar chain was also obtained from the HMBC spectrum. Thus, the appearance of cross peaks between H-1 of xylose^B (δ 4.41) and C-3 of xylose^A (δ 85.6), H-1 of xylose^A (δ 4.40) and C-4 of rhamnose (δ 82.8), H-1 of rhamnose (δ 5.21) and C-2 of fucose (δ 72.2), and H-1 of fucose (δ 5.26) and C-28 of the aglycon (δ 175.5) indicated that a xylopyranosyl(1→3)-xylopyranosyl(1→4)-rhamnopyranosyl(1→2)-fucopyranoside unit was attached to the aglycon at position C-28. This finding was also confirmed by the appearance of reverse cross peaks between H-2 of fucose (δ 3.61) and C-1 of rhamnose (δ 99.5), between H-4 of rhamnose (δ 3.38) and C-1 of xylose^A (δ 104.9), and between H-3 of xylose^A (δ 3.32) and C-1 of xylose^B (δ 104.2). H-1, H-2 vicinal coupling constants between 7 and 8 Hz for glucuronic acid, galactose, xylose, and fucose indicated that these sugars occurred in **1** as the β-anomers in $^4C_1$ configurations. Although the observed small H-1, H-2 coupling constant of rhamnose allowed either an equatorial or axial orientation of H-1, the H-1, C-1 coupling constant of 171.8 Hz indicated an equatorial orientation of the hydrogen and thus the presence of the α-anomer.[5] Although the H-4,H-5 coupling constant could not be determined due to overlap of signals, the common $^1C_4$ configuration is assumed. Hence, **1** was β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl-3β-hydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (silenoside A).

Negative-ion ESIMS−MS of compound **2** yielded a molecular ion at m/z 1409 [M − H]$^-$ and an intense fragment ion at m/z 823 after CID, resulting from the loss of an acyl glycosidically linked tetrasaccharide chain [M − 146 − 146 − 162 − 132 − H]$^-$. Another fragment ion at m/z 485 was generated by the further elimination of the *O*-glycosidically linked disaccharide [hex−hexA], indicating the molecular weight of the aglycon to be 486 and suggesting the presence of an additional hydroxyl group compared to **1**. Methylation analysis confirmed the presence of the same *O*-linked disaccharide as in **1**, while the acyl-linked tetrasaccharide

Table 1. NMR Data of Compounds **1**−**3** (in DMSO-d_6 for Compound **1**, in a 1:1 Mixture of CD_3OD and D_2O for **2** and **3**)

	carbon	**1**	**2**	**3**	proton(s)	**1**	**2**	**3**
aglycon	C-1	37.6	38.8	38.8	H-1A,B	1.55, 0.98	1.76, 1.13	1.77, 1.14
	C-2	24.1	25.2	25.3	H-2A,B	1.92, 1.60	2.04, 1.84	2.04, 1.84
	C-3	81.2	85.1	85.9	H-3	3.76	3.98	3.98
	C-4	53.9	56.4	56.4				
	C-5	47.2	48.6	48.6	H-5	1.27	*a*	1.38
	C-6	19.7	21.2	21.2	H-6A,B	1.44	*a*	1.53, 1.50
	C-7	28.7	33.1	33.1	H-7A,B	1.91, 1.73	*a*	1.53, 1.33
	C-8	39.3	40.7	40.7				
	C-9	46.8	47.5	47.5	H-9	1.57	1.74	1.75
	C-10	35.5	36.6	36.6				
	C-11	22.9	24.1	24.1	H-11A,B	1.80	1.96	1.96
	C-12	121.6	123.5	123.5	H-12	5.16	5.38	5.39
	C-13	143.1	143.8	143.8				
	C-14	41.3	42.3	42.3				
	C-15	27.2	36.0	36.0	H-15A,B	1.83,1.03	1.66, 1.51	1.67, 1.49
	C-16	22.4	74.2	74.4	H-16A,B	1.98,1.49	4.52	4.52
	C-17	45.9	49.9	49.6				
	C-18	41.2	41.8	41.8	H-18	2.73	2.97, dd, J = 14.2/*ca*. 3 Hz	2.97, dd, J = 13.5/3.0 Hz
	C-19	45.5	47.5	47.5	H-19A	1.65	2.24, t, J = 13.5 Hz	2.24, t, J = 13.5 Hz
					H-19B	1.05	1.12	1.12, dd, J = 13.5/*ca*. 3 Hz
	C-20	30.3	30.9	30.9				
	C-21	33.1	36.0	36.0	H-21A,B	[a]	1.80,1.25	1.81,1.26
	C-22	29.0	31.2	31.2	H-22A,B	[a]	1.96,1.80	1.96,1.81
	C-23	209.6	212.4	212.4	H-23	9.45, s	9.49, s	9.48, s
	C-24	10.2	10.5	10.7	H_3-24	1.05, s	1.19, s	1.21, s
	C-25	15.4	16.3	16.3	H_3-25	0.89, s	1.03, s	1.03, s
	C-26	16.7	17.5	17.6	H_3-26	0.67, s	0.76, s	0.76, s
	C-27	25.5	24.0	27.0	H_3-27	1.09, s	1.38, s	1.38, s
	C-28	175.5	177.6	177.5				
	C-29	32.8	33.1	33.1	H_3-29	0.86, s	0.92, s	0.92, s
	C-30	23.5	24.6	24.7	H_3-30	0.87, s	0.98, s	0.98, s
3-*O*-sugar chain								
GlcA	C-1	101.0	103.2	103.7	H-1	4.13, d, J = 7.3 Hz	4.44, d, J = 7.8 Hz	4.48, d J = 7.6 Hz
	C-2	81.5	79.7	77.8	H-2	3.17	3.58	3.74
	C-3	76.4	77.4	85.1	H-3	3.32	*a*	3.80
	C-4	71.8	73.0	71.6	H-4	3.15	*a*	3.59
	C-5	73.6	77.2	77.7	H-5	3.24	*a*	3.68
	C-6	173.1	176.4	176.2				
Gal	C-1	104.7	104.1	103.2	H-1	4.25, d, J = 7.4 Hz	4.63, d, J = 6.5 Hz	4.83 (overlapped)
	C-2	72.5	72.8	72.8	H-2	3.31	3.52	3.52
	C-3	72.9	74.1	74.6	H-3	3.29	*a*	3.59
	C-4	67.6	70.6	70.4	H-4	3.67	*a*	3.88
	C-5	75.1	76.4	76.4	H-5	3.33	*a*	3.60
	C-6	59.7	62.1	62.1	H-6A,B	3.57, 3.49	*a*	3.87, 3.74
Ara	C-1			104.0	H-1			4.65, d, J = 7.2 Hz
	C-2			72.5	H-2			3.62
	C-3			74.4	H-3			3.58
	C-4			69.6	H-4			3.93
	C-5			67.4	H-5A,B			3.97, 3.67
28-*O*-sugar chain								
Fuc	C-1	93.3	94.5	95.5	H-1	5.26, d, J = 7.8 Hz	5.33, d, J = 7.7 Hz	5.33, d, J = 7.6 Hz
	C-2	72.2	75.8	75.8	H-2	3.61	3.78	3.79
	C-3	74.5	75.8	75.9	H-3	3.59	*a*	3.78
	C-4	71.4	73.8	73.8	H-4	3.41	*a*	3.64
	C-5	70.7	72.6	72.6	H-5	3.59	3.78	3.77
	C-6	16.7	16.1	16.3	H_3-6	1.05, d, J = 6.1 Hz	1.24, d, J = 6.3 Hz	1.25, d, J = 6.2 Hz
Rha	C-1	99.5	100.1	100.1	H-1	5.21, br s	5.75, br s	5.74, br s
	C-2	69.7	81.7	81.7	H-2	3.74	4.10	4.11
	C-3	70.4	71.5	71.5	H-3	3.64	3.95	3.95
	C-4	82.8	82.8	82.9	H-4	3.38	3.66	3.65
	C-5	66.7	68.8	68.8	H-5	3.62	3.80	3.78
	C-6	17.7	18.2	18.2	H_3-6	1.14, d, J = 6.1 Hz	1.35, d, J = 6.2 Hz	1.35, d, J = 6.0 Hz
Glc	C-1		105.8	105.8	H-1		4.55, d, J = 7.8 Hz	4.55, d, J = 7.8 Hz
	C-2		74.6	74.7	H-2		3.36	3.36
	C-3		76.9	76.9	H-3		3.46	3.44
	C-4		70.8	70.8	H-4		3.35	3.40
	C-5		77.2	77.2	H-5		3.48	3.42
	C-6		62.1	62.0	H-6A,B		3.95, 3.74	3.93, 3.72
Xyl^A	C-1	104.9	106.1	106.1	H-1	4.40, d, J = 7.5 Hz	4.60, d, J = 7.6 Hz	4.60, d, J = 7.8 Hz
	C-2	73.9	75.5	75.5	H-2	3.15	3.27	3.27
	C-3	85.6	74.6	74.7	H-3	3.36	3.45	3.44
	C-4	67.6	77.3	77.2	H-4	3.36	3.57	3.58
	C-5	65.5[b]	66.5	66.5	H-5A,B	3.74, 3.09	3.90, 3.29	3.91, 3.28
Xyl^B	C-1	104.2			H-1	4.41, d, J = 7.5 Hz		
	C-2	73.6			H-2	3.09		
	C-3	76.2			H-3	3.17		
	C-4	69.4			H-4	3.32		
	C-5	65.7[b]			H-5A,B	3.74, 3.09		

[a] Assignment not possible. [b] Assignments interchangeable.

contained a 2-substituted fucose residue, a 2,4-disubstituted rhamnose residue, as well as terminal glucose and xylose units.

The NMR data of **2** showed the presence of additional hydroxymethylene and hydroxymethine groups, attributable to the substitution of a hexose for a pentose moiety and the presence of an additional oxygen in the aglycon to give quillaic acid, rather than gypsogenin found in **1**. Analysis of the long-range $^{13}C-^{1}H$ NMR correlations confirmed that **2** had the same disaccharide linked to C-3 of the aglycon as in **1**, as the expected sequence correlations were observed. In contrast, the same 2D NMR spectrum indicated that the acyl-glycosidically linked sugar was different from that in **1**, as indicated by the methylation analysis. Although all long-range correlations for this moiety could not be unambiguously determined, sufficient were observed to establish the structure. Thus, cross peaks were observed from H-1 of fucose to C-28 of the aglycon, from H-2 of this fucose unit to C-1 of rhamnose, from H-1 of glucose to C-2 of rhamnose, and from H-1 of xylose to C-4 of the same rhamnose unit. A prerequisite for making these assignments was the need to assign the C-2 and C-4 signals of the rhamnose moiety unambiguously. This followed from the observation of a long-range correlation of the latter with the protons of the C-6 methyl group and comparison of the data for C-2 with those in **3,** where the assignment was unambiguously established from a one-bond correlation. Thus, **2** was 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-3*β*,16α-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→4)-[*β*-D-glucopyranosyl(1→2)]-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside (silenoside B).

The molecular ion of compound **3** at *m/z* 1541 $[M - H]^-$ obtained by negative ion ESIMS indicated the additional presence of a pentose unit compared to **2**, that was shown to be arabinose from compositional analysis, and this was found to be attached to the *O*-glycosidically bound carbohydrate moiety by MS−MS. This result was confirmed by methylation analysis by the detection of a derivative characteristic of a 2,3-disubstituted (instead of a 2-monosubstituted) glucuronic acid observed for **1** and **2.** These data indicated that the additional pentose residue was attached at O-3 of the glucuronic acid. NMR data showed the presence of quillaic acid as aglycon. The additional presence of an arabinose residue linked at O-3 of the glucuronic acid unit was confirmed unequivocally by the detection of a cross peak between H-1 of arabinose and C-3 of glucuronic acid in the HMBC spectrum. The remaining sugar residues had the same arrangement as in **2**. The H-1,H-2 vicinal coupling constant of 7.2 Hz for arabinose indicated the presence of the α-anomer in the $^4C_1$ configuration. Thus, **3** is 3-*O*-α-L-arabinopyranosyl(1→3)-[*β*-D-galactopyranosyl(1→2)]-*β*-D-glucuronopyranosyl-3*β*,16α-dihydroxy-23-oxoolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→4)-[*β*-D-glucopyranosyl(1→2)]-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside (silenoside C).

Compounds **1**−**3** are novel triterpenoid saponins and have been named silenosides A−C. However, both the aglycon moieties and compositions of the carbohydrate moieties are similar to those of saponins from *Acanthophyllum squarrosum*,[6] *Gypsophila paniculata*,[7,8] *Gypsophila arrostii*,[8] *Gypsophila oldhamiana*,[9] *Agrostemma githago* var. *githago*,[10] and *Silene jenisseensis*.[11,12] The present structural assignments further indicate that glycosides of gypsogenin and quillaic acid, possessing a glucuronic acid unit attached to C-3 and a fucose unit attached to C-28, are common chemical features of plants of the family Caryophyllaceae.

Experimental Section

General Experimental Procedures. Melting points were run on a DSC 25 apparatus (Mettler, Toledo). 1D and 2D NMR spectra of **1** were recorded in DMSO-d_6 and those of **2** and **3** in a mixture of CD_3OD and D_2O (1:1) at 300 K on a Bruker AM 600 NMR spectrometer (^1H, 600.14 MHz, ^{13}C, 150.91 MHz). MS were obtained on a Finnigan TSQ 700 equipped with a Finnigan electrospray source (ESIMS and MS−MS) and on a Finnigan GCQ ion trap mass spectrometer (GC−MS). Analytical HPLC was performed on a Knauer HPLC system model 64 (Knauer, Berlin, Germany). TLC was carried out on Si gel 60 plates or foils (Merck) and on HPTLC precoated plates (Merck) using $CHCl_3$−MeOH−AcOH−H_2O (7:4:1:1) as mobile phase. Column chromatography was performed on Bakerbond octadecyl RP_{18}, 40 μm (J. T. Baker) and Si gel 60, 0.063−0.2 mm (Merck). Anisaldehyde−H_2SO_4 (anisaldehyde 0.5 mL, H_2SO_4 5.0 mL, HOAc 10.0 mL, MeOH 100 mL) was used as the visualization reagent for TLC.

Plant Material. Roots of *S. vulgaris* were collected in September 1995, in the Trzebnica region, Poland, and were air-dried at room temperature. A voucher specimen (voucher number MiGle-01) has been deposited in the Department of Pharmacognosy, Wroclaw University of Medicine, Poland.

Extraction and Isolation. A portion (500 g) of the roots was extracted with CH_2Cl_2, MeOH, and 50% aqueous MeOH, respectively, at room temperature. The combined MeOH and 50% MeOH extracts were concentrated under reduced pressure and purified by column chromatography on RP_{18}. The column was washed first with H_2O, then with 30% aqueous MeOH, and finally with MeOH to elute 7 g of the saponin mixture. The saponin mixture (6 g) was chromatographed over Si gel with $CHCl_3$−MeOH−H_2O (8:4:1, lower layer), with 20-mL fractions collected and analyzed by TLC. Fractions having similar compositions were combined and finally purified by column chromatography on Si gel with the solvent system CH_2Cl_2−MeOH−H_2O (8.5:4:1, lower layer), to afford **1** (26 mg), **2** (15 mg), and **3** (39 mg).

Silenoside A (1): obtained as a light yellow powder; ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 1363 $[M - H]^-$; MS−MS of *m/z* 1363 (CID) *m/z* 1231 $[M - 132 - H]^-$, 953 $[M - 132 - 132 - 146 - H]^-$, 807 $[M - 132 - 132 - 146 - 146 - H]^-$, 469 $[M - 132 - 132 - 146 - 146 - 176 - 162 - H]^-$; TLC R_f 0.4 ($CHCl_3$−MeOH−H_2O−CH_3COOH, 7:4:1:1); HPLC t_R 52.08 min (LiChrospher 100 RP_{18} 5 μm, 250 × 4 mm i. d., 0.1% aqueous H_3PO_4−MeCN (71:29), 1 mL/min).

Silenoside B (2): obtained as a light yellow powder; mp 240 °C; ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 1409 $[M - H]^-$; MS−MS of *m/z* 1409 (CID) *m/z* 1247 $[M - 162 - H]^-$, 969 $[M - 162 - 132 - 146 - H]^-$, 823 $[M - 162 - 132 - 146 - 146 - H]^-$, 485 $[M - 162 - 132 - 146 - 146 - 132 - 162 - 176 - H]^-$; TLC R_f 0.2 ($CHCl_3$−MeOH−H_2O−CH_3COOH, 7:4:1:1); HPLC t_R 18.04 min (LiChrospher 100 RP_{18} 5 μm, 250 × 4 mm i. d., 0.1% aqueous H_3PO_4−MeCN (71:29), 1 mL/min).

Silenoside C (3): obtained as a light yellow powder; mp 238 °C; ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 1541 $[M - H]^-$; MS−MS of *m/z* 1541 (CID) *m/z* 955 $[M - 162 - 132 - 146 - 146 - H]^-$, 485 $[M - 162 - 132 - 146 - 146 - 132 - 162 - 176 - H]^-$; TLC R_f 0.15 ($CHCl_3$−MeOH−H_2O−CH_3COOH, 7:4:1:1); HPLC t_R 19.33 min (LiChrospher 100 RP_{18} 5 μm, 250 × 4 mm i. d., 0.1% aqueous H_3PO_4−MeCN (71:29), 1 mL/min).

Quantitation of the Component Monosaccharides in 1−3. Monosaccharides were analyzed as the corresponding methyl glycosides after methanolysis and trimethylsilylation by GC−MS.[13] Monosaccharide ratios were determined by electronic integration of all relevant peaks of the total ion current chromatogram. GC conditions and instrument setup were identical to those used for the methylation analysis (see below).

Determination of the Absolute Configuration of the Sugars in 1−3. This was performed according to the method of Hara et al.[4] using about 5 mg of each compound. GLC conditions: column DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm, J&W Scientific Inc., Folsom, CA), oven temperature 250 °C, injection port temperature 260 °C, detector temperature 280 °C, carrier gas He (14 cm/s), injection volume 1 μL, split ratio 1:50.

Preparation and Analysis of Partially Methylated Alditol Acetates in 1−3.[14,15] Aliquots of each compound (10 μg) were dissolved in 150 μL of DMSO and methylated according to the method of Hakamori.[16] To detect hexuronic acids, the permethylated sample was reduced with $NaBD_4$ leading to the conversion of acid methyl esters to the respective 6-alcohol derivatives with two deuterium atoms incorporated at C-6. Purification of the permethylated sample, hydrolysis using trifluoracetic acid, reduction using $NaBH_4$, and acetylation using Ac_2O were performed as described.[17] All GC−MS analyses were performed on a Finnigan GCQ ion trap mass spectrometer running in the positive-ion EI mode equipped with a 30-m capillary column. GLC conditions: column DB-5 (30 m × 0.32 μm i.d., film thickness 0.1 μm, J&W Scientific Inc., Folsom, CA), temperature program, 3 min 80 °C, 10 °C per min to 300 °C. The respective partially methylated alditol acetates were identified by comparison with standard compounds, their characteristic EIMS fragments and their retention times: t_R 12:07 min 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-xylitol and 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-arabitol; t_R 13:29 min 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-fucitol; t_R 13:38 min 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol; t_R 13:54 min, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-galactitol; t_R 14:11 min 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-rhamnitol; t_R 15:53 min 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-glucitol-6-D_2; t_R 16:43 min 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl-glucitol-6-D_2.

Acknowledgment. The authors wish to thank Helmar Döhnert (Humboldt University of Berlin, Institute of Pharmacy) for carrying out gas chromatography for determination of the absolute configurations of the carbohydrate moieties of **1**−**3**, and C. Kakoschke, A. Wassmann, and C. Kamp (all GBF, Braunschweig) for technical assistance.

References and Notes

(1) Watson, L.; Dallwitz, M. J. *The Families of Flowering Plants: Descriptions, Illustrations, Identification, and Information Retrieval*; (1992 onward). Version: 15th October 1998. http://biodiversity.uno.edu/delta/angio/index.htm.
(2) Laghetti, G.; Perrino, P.; Hammer, K. *Econ. Bot.* **1994**, *48*, 337−339.
(3) Ballero, M.; Fresu, I. *Fitoterapia* **1993**, *64*, 141−150.
(4) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−506.
(5) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293−297.
(6) Lacaille-Dubois, M. A.; Hanquet, B.; Rustaiyan, A.; Wagner, H. *Phytochemistry* **1993**, *34*, 489−495.
(7) Kim, Y. C.; Higuchi, R.; Komori, T. *Liebigs Ann. Chem.* **1992**, 941−946
(8) Frechet, D.; Christ, B.; Monegier, D. U.; Sorbier, B.; Fischer, H.; Vuilhorgne, M. *Phytochemistry* **1991**, *30*, 927−931.
(9) Liu, Z.; Li, D.; Owen, N. L.; Grant, D. M.; Cates, R. G.; Jia, Z. *J. Nat. Prod.* **1995**, *58*, 1632−1635.
(10) Siepmann, C.; Bader, G.; Hiller, K.; Wray, V.; Domke, T.; Nimtz, M. *Planta Med.* **1998**, *64*, 159−164.
(11) Lacaille-Dubois, M. A.; Hanquet, B.; Cui, Z. H.; Lou, Z. C.; Wagner, H. *Phytochemistry* **1995**, *40*, 509−514.
(12) Lacaille-Dubois, M. A.; Hanquet, B.; Cui, Z. H.; Lou, Z. C.; Wagner, H. *Phytochemistry* **1997**, *45*, 985−990.
(13) Chaplin, M. F. *Anal. Biochem.* **1982**, *123*, 336−341.
(14) Björndal, H.; Hellerqvist, C. G.; Lindberg, B.; Svensson, S. *Angew. Chem.* **1970**, *82*, 643−652.
(15) Jansson, P.-E.; Kenne, L.; Liedgren, H.; Lindberg, B.; Lönngren, J. *Chem. Commun., University Stockholm* **1976**, *8*, 1−75.
(16) Hakomori, S. I. *J. Biochem. (Tokyo)* **1964**, *55*, 205−208.
(17) Nimtz, M.; Mort, A.; Domke, T.; Wray, V.; Zhang, Y.; Qiu, F.; Choplin, D.; Geider, K. *Carbohydr. Res.* **1996**, *287*, 59−76.

NP980505R