Triterpenoid Saponins from *Bellis bernardii*[†,‡]

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Nine saponins were isolated from the deacylated saponin mixture obtained from the whole plants of *Bellis bernardii*. Their structures have been elucidated from NMR and MS data and by chemical derivatization. Three were glycosides of the recently reported bellisonic acid (2β,3β,-23-trihydroxy-16-oxoolean-12-en-28-oic acid), with the major compounds being related glycosides of polygalacic acid (2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid). In addition, a saponin of bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid) was obtained and is the first acyl glycoside of this aglycon with a free hydroxyl group at C-3.

The genus *Bellis* L. includes about 10 species, most of which are native to the Mediterranean region. While three of these, *Bellis perennis* L., the common daisy, *Bellis annua* L., and *Bellis sylvestris* Cyr. are frequently found, the others are endemic to particular geographic regions. One of the latter, *Bellis bernardii* Boiss. et Reuter, grows on Corsica in moist meadows at an altitude of about 1800 m. It reaches a height of only 1−6 cm and is one of the smallest species of the genus.

In our previous paper,[1] we have described a new aglycon of the oleanane series, bellisonic acid, which is the first naturally occurring olean-12-en-28-oic acid with a keto function at position C-16. The present paper gives the full experimental details of the isolation of this compound and describes the isolation and structure elucidation of two additional glycosides of this acid. Furthermore, we have identified five glycosides of polyglacic acid, which is the most commonly found aglycon of the saponins of the genus *Bellis*,[2−5] and one glycoside of bayogenin.

Results and Discussion

Bernardiosides A (**1**), B_1 (**2**), B_2 (**3**), B_3 (**4**), B_4 (**5**), C_1 (**6**), C_2 (**7**), C_4 (**8**), and D (**9**) (Chart 1) were isolated from the mild alkaline hydrolysate of the saponin mixture obtained from the whole plants of *B. bernardii* as described in Experimental Section. A comprehensive multistage procedure was used for structure elucidation. Sugar components were determined by means of GC identification of the pertrimethylsilylated derivatives obtained by methanolysis of the corresponding saponin. ESI-MS and MS/MS of the $[M + Na]^+$ ions supplied information on the molecular weights, the composition of acylglycosidic sugar chains, and the composition of the remaining *O*-glycosidic prosapogenin moiety. Comparison of ^{1}H- and ^{13}C-NMR data with the literature data afforded basic information about the structure of each aglycon and the composition of the carbohydrate moiety, while known compounds were unambiguously identified. When a new compound became evident, 2D NMR experiments (COSY, HMQC, HMBC) and/or GC−MS analysis of the partially methylated alditol acetates was performed. Finally, the absolute configurations of the monosaccharide constituents were determined. Preparation and analysis of the L-cysteine methyl ester derivatives, according to Hara et al.,[6] showed that in all compounds glucose, xylose, and fucose were present as the D-enantiomers and rhamnose as the L-enantiomer.

Hence, by sugar component analysis glucose was identified in **1**, glucose and rhamnose in **3**, glucose, fucose, rhamnose, and xylose in **2**, **6**, **7**, and **9**, and fucose, rhamnose, and xylose in **4** and **8** in the appropriate ratios. A comparison of ^{1}H- and ^{13}C-NMR data with literature data indicated that compounds **1**, **4**, **6**, **8**, and **9** are glycosides of polygalacic acid and compounds **2**, **5**, and **7** are glycosides of bellisonic acid. Overall, the structural analyses afforded four classes of compounds, namely **1** in the first class, **4**, **6**, **8** and **9** in the second, **2**, **5**, and **7** in the third, and **3** in the fourth as detailed below.

In the ^{1}H-NMR spectrum of **1** only one anomeric proton signal (δ 4.47, d, J = 7.7 Hz) was observed showing that **1** contained one β-D-glucose moiety. The ESI-MS afforded molecular ions at m/z 667 $[M + H]^+$, 689 $[M + Na]^+$, and 705 $[M + H]^+$. MS/MS investigation of the sodium adduct gave one intense daughter ion at m/z 645 $[M - CO_2 + Na]^+$ indicating that **1** possesses an unsubstituted carboxylic acid group. This was confirmed from the ^{1}H- and ^{13}C-NMR data and assigned on the basis of the 1D and 2D COSY, HMQC, and HMBC spectra. Even though carbons C-16−C-18, C-22, and C-28 of polygalacic could not be unambiguously assigned, the small downfield shift of C-13 to δ 145.5 was indicative of the presence of a free carboxylic acid group[7] and was supported by the ^{1}H-NMR data. The characteristic shift of H-18 found in acylglycosides of polygalacic acid was not observed and H-19$_A$ was shifted downfield to δ 2.28 (t, J = 13.3 Hz). Cross-peaks in the HMBC spectrum between C-3 of the aglycon (δ 83.7) and H-1 of glucose (δ 4.47) and between H-3 of

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† For a preliminary communication, see Schöpke et al.[1]

‡ Dedicated to Prof. Dr. Drs. h. c. H. Oelschläger on the occasion of his 75th birthday.

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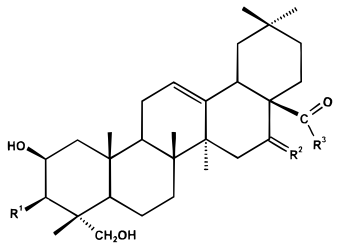
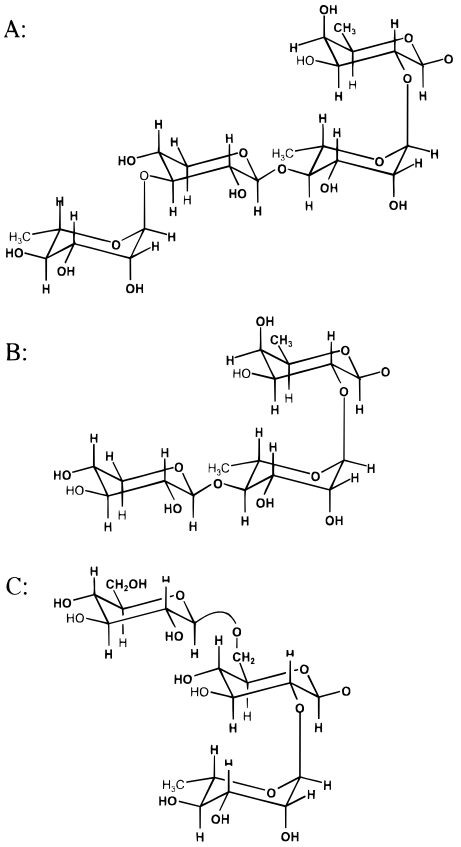
Chart 1



Compound	R^1	R^2	R^3
1	ß-D-Glc	α-OH, ß-H	OH
2	ß-D-Glc	O	B
3	OH	H2	C
4	α-L-Rha	α-OH, ß-H	B
5	α-L-Rha	O	A
6	ß-D-Glc	α-OH, ß-H	B
7	ß-D-Glc	O	A
8	α-L-Rha	α-OH, ß-H	A
9	ß-D-Glc	α-OH, ß-H	A



the aglycon (δ 3.65) and C-1 of glucose (δ 105.5) confirmed that glucose is attached to the aglycon at C-3. Taken together, the NMR and MS data indicated that bernardioside A (**1**) is 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,-16α,23-tetrahydroxyolean-12-en-28-oic acid.

In compounds **4**, **6**, **8**, and **9**, C-28 appeared at δ 177.3 in the ^{13}C-NMR and H-18 at δ 2.98 (dd, J = ca. 5 and 15 Hz), indicating that the carboxyl group was glycosylated. This was confirmed by ESI MS/MS investigation of the sodium adducts of the molecular ions that afforded intense daughter ions corresponding to the sugar chain bound acyl glycosidically. Finally, careful comparison with literature ^{13}C-NMR data established that **4** was identical with besysaponin C_{12},[5] **8** with bellissaponin BS1,[2] and **9** with bellissaponin BS2.[2]

For **6** the ESI-MS afforded molecular ions at m/z 1091 $[M + H]^+$, 1113 $[M + Na]^+$, and 1129 $[M + K]^+$. In the ^{1}H-NMR spectrum there were signals of four anomeric protons and two secondary methyl groups that belonged to four sugar units, three of which were deoxyhexoses. GC−MS analysis of the partially methylated alditol acetates confirmed the presence of terminal xylose and glucose. Additionally, a 1,2-linked fucose and 1,4-linked rhamnose were detected. Comparison of the ^{13}C-NMR data (Table 1) of the carbohydrate moiety with those of compound **5** confirmed that both saponins consisted of identical sugar units. Hence, compound **6** was assigned as 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside.

The ^{13}C-NMR spectra of compounds **2**, **5**, and **7** showed carbonyl signals at δ 210.1 indicative of the presence of a keto function. MS/MS of the sodium adducts of the molecular ions gave intense daughter ions that corresponded to sugar chains linked by an acyl-glycosidic bond. Additionally, $[M - 44]^+$ ions were obtained, although no further carboxyl group was present. As shown in our previous paper, the loss of CO_2 from the Na adduct is characteristic of bellisonic acid arising from skeletal rearrangement of the molecule, corresponding to transfer of the tetrasaccharide from the carboxyl at C-28 to the neighboring ketone (enol) group at C-16 with subsequent elimination of CO_2.[1]

The structure of compound **5** was elucidated previously by MS (ESI, MS/MS), 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, HMQC, HMBC) NMR, GC−MS of the partially methylated alditol acetates, and GC analysis of the trimethylsilylated monosaccharides and L-cysteine methyl ester derivatives as 3-*O*-α-L-rhamnopyranosyl-2*β*,3*β*,-23-trihydroxy-16-oxoolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside as reported in a previous paper.[1] The structures of **2** and **7** were determined straightforwardly from the MS and NMR data by comparison with **6** and **9**, showing that **2** is 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,23-trihydroxy-16-oxoolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside and **7** 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,23-trihydroxy-16-oxoolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranoside.

ESI-MS of **3** gave a molecular ion at m/z 981 $[M + Na]^+$. Considering the constitution of the carbohydrate components, the aglycon must have a molecular weight of 488 Da. On the basis of the 1D (^{1}H, ^{13}C, DEPT) and

Table 1. ^{13}C NMR Chemical Shifts of Compounds **1**, **2**, and **4**−**9** in Methanol-d_4

		1	**2**	**4**	**5**	**6**	**7**	**8**	**9**
aglycon	C-1	44.7	44.4	45.2	45.0	44.5	44.3	45.1	44.5
	C-2	71.2	71.9	72.0	71.7	71.9	71.9	72.0	72.0
	C-3	83.9	83.7	82.6	82.3	84.4	83.9	82.4	84.2
	C-4	43.1	43.2	43.4	43.4	43.1	43.2	43.5	43.2
	C-5	48.2	47.9	48.1	47.9	48.0	47.9	48.4	48.2
	C-6	18.0	18.5	18.8	18.8	18.7	18.7	19.0	19.0
	C-7	33.8	33.3	33.8	33.3	33.8	33.3	33.7	33.7
	C-8	40.8	41.3	40.9	41.4	40.9	41.3	40.9	40.9
	C-9	48.5	47.9	48.5	47.9	48.4	47.9	48.4	48.2
	C-10	37.5	37.5	37.8	37.7	37.5	37.5	37.8	37.6
	C-11	24.6	24.8	24.7	24.7	24.6	24.7	24.6	24.6
	C-12	123.2	126.2	123.6	126.2	123.6	126.2	123.6	123.6
	C-13	145.5	141.2	144.8	141.2	144.8	141.2	144.7	144.7
	C-14	43.0	[b]	43.0	49.2	43.1	[b]	43.0	43.0
	C-15	36.7	46.9	36.5	46.9	36.5	46.9	36.3	36.5
	C-16	[a]	210.2	74.7	210.1	74.7	210.1	74.8	74.8
	C-17	[a]	60.4	50.1	60.4	50.1	60.4	50.2	50.2
	C-18	[a]	48.2	42.4	48.3	42.4	48.2	42.2	42.4
	C-19	47.9	47.7	48.0	47.4	48.0	47.7	48.0	48.0
	C-20	31.4	33.4	31.3	31.4	31.3	31.4	31.3	31.3
	C-21	36.7	35.6	36.5	35.6	36.5	35.6	36.5	36.6
	C-22	[a]	27.5	32.0	27.5	32.0	27.5	31.9	32.0
	C-23	65.6	65.5	65.7	65.5	65.7	65.9	65.8	66.2
	C-24	14.7	14.7	14.7	14.8	14.7	14.8	14.9	14.9
	C-25	17.6	17.6	18.0	17.5	17.6	17.6	18.0	17.9
	C-26	18.0	18.0	17.8	18.0	17.8	18.0	17.9	17.8
	C-27	27.5	27.8	27.3	27.8	27.3	27.8	27.2	27.2
	C-28	[a]	173.4	177.3	173.4	177.3	173.4	177.3	177.3
	C-29	33.6	33.3	33.4	33.3	33.4	33.3	33.3	33.3
	C-30	25.4	23.8	24.9	23.8	24.9	23.8	24.9	24.9
Rha 1	C-1				102.6		102.6	102.5	102.6
	C-2				72.2[c]		72.3[c]	72.3	72.3
	C-3				72.8		72.8	72.3	72.3
	C-4				74.0		74.0	74.0	74.1
	C-5				70.0		70.0	70.0	70.0
	C-6				18.4		18.4	18.4	18.4
Xyl	C-1		107.2	107.2	107.2	107.1	107.2	107.2	107.2
	C-2		76.2	76.1	76.4	76.1	76.4	76.4	76.5
	C-3		78.3	78.2	84.5[d]	78.2	84.6	84.4	84.4
	C-4		71.2	71.1	69.9	71.1	69.9	68.8	68.8
	C-5		67.2	67.3	67.2	67.3	67.2	67.2	67.2
Rha 1,4	C-1		101.3	101.1	101.4	101.1	101.4	101.3	101.4
	C-2		72.3[c]	72.3	71.9	72.3	72.3[c]	72.3	72.3
	C-3		72.8[c]	72.4	72.2[c]	72.7	72.4[c]	72.3	72.4
	C-4		84.4	84.4	84.5[d]	84.4	84.6	84.6	84.6
	C-5		69.0	68.8	69.0	68.8	69.0	69.9	69.9
	C-6		18.0	18.3	18.0	18.3	18.0	17.9	17.9
Fuc 1,2	C-1		95.7	95.2	95.7	95.2	95.7	95.1	95.1
	C-2		74.6	74.2	75.0	74.7	75.0	74.6	74.6
	C-3		76.4	76.7	76.4	76.7	76.5	76.7	76.8
	C-4		73.5	73.6	73.4	73.6	73.4	73.6	73.6
	C-5		72.2	72.3	72.2	72.3	72.3	72.1	72.3
	C-6		16.6	16.5	16.6	16.5	16.6	16.5	16.5
Rha 1[e]	C-1			104.2	104.2			104.1	
	C-2			72.7	72.2[c]			72.6	
	C-3			72.4	72.8[c]			72.3	
	C-4			74.1	74.0			74.0	
	C-5			70.4	70.4			70.4	
	C-6			17.8	17.9			17.9	
Glc 1[e]	C-1	105.5	105.5			105.4	105.5		105.5
	C-2	75.4	75.4			75.4	75.4		75.4
	C-3	77.7	77.8			77.7	77.8		77.8
	C-4	71.1	71.2			71.2	71.2		71.2
	C-5	78.2	78.3			78.2	78.2		78.2
	C-6	62.3	62.4			62.3	62.3		62.4

[a] Unambiguous assignment not possible. [b] Signal under solvent signal. [c,d] Assignments may be interchanged. [e] Sugar bound to the aglycon.

2D (COSY, HMQC, HMBC) NMR experiments, **3** was found to possess a 2,3,23-trihydroxyolean-12-en-28-oic acid unit as aglycon. C-3 did not show a downfield glycosylation shift, while cross-peaks in the HMBC spectrum between H-1 of glc A (δ 5.46) and C-28 of the aglycon (δ 178.1) confirmed that the carbohydrate moiety is bound to the carboxyl group. As all glycosides of bayogenin obtained to date are 3-*O*-glycosides it seemed possible that the hydroxyl at C-3 may have changed orientation, thus hindering 3-*O*-glycosylation. However, H-2−H-3, H-2−H-1_A and H-2−H1_B coupling constants of approximately 3.5 Hz and the absence of a ^4J coupling between H-3 and H-1_{eq} clearly indicated the equatorial orientation of the hydroxyl at C-3 and axial orientation of the hydroxyl at C-2. Hence, bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid) was established as the aglycon of compound **3**. GC−MS analysis of the partially methylated alditol acetates gave a terminal glucose (glc^B), a terminal rhamnose, and a 1,2,6-linked glucose (glc^A), indicating that **3** is a monodesmosidic saponin. Cross-peaks in the HMBC spectrum between H-6$_{A/B}$ of glc A (δ 4.16/3.80) and C-1 of glc B (δ 104.7) and between H-1 of rhamnose (δ 5.39) and C-2 of glc A (δ 77.2) showed that glc B is attached to C-6 and rhamnose to C-2 of glc A. The H-1−H-2 coupling constants of 7.2 and 7.8 Hz demonstrated the β-glycosidic linkage of the glucose units and a H-1−C-1 coupling constant of ca. 170 Hz the α-glycosidic linkage of rhamnose. Consequently, compound **3** was determined as 2β,3β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside.

As discussed in our previous paper, bellisonic acid is a new sapogenin, as well as the first genuine naturally occurring derivative of 16-oxoolean-12-en-28-oic acid.[1] Hence, all of the glycosides of bellisonic acid described here are novel triterpenoid saponins. The observation of a number of saponins of 2β,3β,23-trihydroxy-16-oxoolean-12-en-28-oic acid in considerable quantities seems to be a unique chemical feature of *B. bernardii* as glycosides of this aglycon have so far not been found in the intensively investigated *B. perennis*[1,3,4] and *B. sylvestris*.[5] On the other hand, the presence of the more common bellissaponins BS1 [= bernardioside C_4 (**8**)] and BS2 [bernardioside D (**9**)] as the major deacylsaponins clearly confirms that, from a chemical point of view, *B. bernardii* belongs to the genus *Bellis*.

In addition to the novel bellisonic acid derivatives **2**, **5**, and **7**, another new triterpenoid saponin, **3**, has been identified. Moreover, it also represents a new class of compounds as it is the first glycoside of bayogenin with a carbohydrate substituent at C-28 and no sugar moiety at C-3 of the aglycon.

Experimental Section

General Experimental Procedures. 1D and 2D NMR spectra were recorded in CD_3OD at 300 K on a Bruker AM 600 NMR spectrometer (^1H, 600.14 MHz; ^{13}C, 150.91 MHz) as described previously.[3] Mass spectra were obtained on a Finnigan TSQ 700 equipped with a Finnigan electrospray source (ESI MS and MS/MS) and a Kratos MS 50 FS connected to a Carlo Erba Mega Series gas chromatograph (GC−MS). $[\alpha]_D$ values were measured on a Perkin-Elmer 241-C polarimeter and infrared spectra on a Mattson Genesis FTIR. TLC was carried out on silica gel 60 plates or foils (Merck), column chromatography on Sephadex LH-20 (Pharmacia) and silica gel 60, 0.063-0.2 mm (Merck), and HPLC on a Hitachi/Merck D-6000 equipped with a L-4000 UV detector.

Plant Material. Whole plants of *B. bernardii* were collected during Aug 1991 on Corsica (Pozzi) at an altitude of about 1800 m. The material was dried at

Table 2. Mass Spectral and Methylation Analysis Data of Saponins Isolated from *B. bernardii*

compd	molecular ion $[M + Na]^+$ (m/z)	fragment ion of the acyl-linked saccharide (m/z)	difference (parent−daughter)[a] (m/z)	per-*O*-acetylated derivatives
2	1111	447 $dhex_2pent - H_2O + Na$	664 hex-bellisonic acid	2,3,4,6-tetra-*O*-methylglucitol, 2,3,4-tri-*O*-methylxylitol, 3,4-di-*O*-methylfucitol, 2,3-di-*O*-methylrhamnitol
3	981	493 $hex_2dhex - H_2O + Na$	488 bayogenin	2,3,4,6-tetra-*O*-methylglucitol, 2,3,4-tri-*O*-methylrhamnitol, 3,4-di-*O*-methylglucitol
4	1097	447 $dhex_2pent - H_2O + Na$	650 dhex-polygalacic acid	2,3,4-tri-*O*-methylrhamnitol, 2,3,4-tri-*O*-methylxylitol, 3,4-di-*O*-methylfucitol, 2,3-di-*O*-methylrhamnitol
5	1241	593 $dhex_3pent - H_2O + Na$	648 dhex-bellisonic acid	2,3,4-tri-*O*-methylrhamnitol (2 mol), 2,4-di-*O*-methylxylitol, 3,4-di-*O*-methylfucitol, 2,3-di-*O*-methylrhamnitol
6	1113	447 $dhex_2pent - H_2O + Na$	666 hex-polygalacic acid	2,3,4,6-tetra-*O*-methylglucitol, 2,3,4-tri-*O*-methylxylitol, 3,4-di-*O*-methylfucitol, 2,3-di-*O*-methylrhamnitol
7	1257	593 $dhex_3pent - H_2O + Na$	664 hex-bellisonic acid	2,3,4,6-tetra-*O*-methylglucitol, 2,3,4-tri-*O*-methylrhamnitol, 2,4-di-*O*-methylxylitol, 3,4-di-*O*-methylfucitol, 2,3-di-*O*-methylrhamnitol

[a] Molecular mass corresponding to aglycon and substituents at C-3. [b] Derivatives detected in approximately equimolar amounts, if not stated otherwise.

50−60 °C. A voucher specimen is deposited at the herbarium of the Department of Pharmacy, Humboldt-University, herbal no. Scho-3.

Extraction and Isolation. An aliquot (200 g) of the dried plant material was refluxed twice for 1 h with 2000 mL of 80% MeOH. The solvent was removed under reduced pressure, and the residue was diluted with H_2O to 750 mL. The extract was defatted twice with $CHCl_3$ and extracted four times with *n*-BuOH. The dried *n*-BuOH extract was dissolved in MeOH and dropped into an excess of Et_2O to give 10.5 g of a yellow-brown, powdery crude glycoside mixture.

A 3 g aliquot of the crude glycoside mixture was subjected to a Sephadex LH-20 column (MeOH) and gave 2.3 g of a saponin-containing fraction. This fraction was hydrolyzed with 2000 mL of 1% KOH for 2 h at room temperature. After neutralization with HCl, the deacylated saponins were extracted three times with *n*-BuOH (500 mL each). The resulting mixture of deacylated saponins (2 g) was separated by column chromatography on silica gel ($CHCl_3$−MeOH−H_2O 7:3:1, lower layer) to give saponin fractions A (69 mg), B (150 mg), C (502 mg), and D (265 mg). While fraction D consisted of a single compound (bernardioside D [**9**]), fractions A−C had to be purified further by HPLC. HPLC of fraction A on LiChrosorb RP-18 (250 mm × 25 mm i.d., particle size 7 μm, solvent MeOH−H_2O 65:35, 8 mL min^{-1}, detection 206 nm) afforded 25 mg of bernardioside A (**1**). From fraction B (solvent MeOH−H_2O 65:35), 14 mg of bernardioside B_1 (**2**), 13 mg of bernardioside B_2 (**3**), 40 mg of bernardioside B_3 (**4**), and 16 mg of bernardioside B_4 (**5**) were obtained. HPLC of fraction C (solvent MeOH−H_2O 65:35) yielded 91 mg of bernardioside C_1 (**6**), 15 mg of bernardioside C_2 (**7**), and 225 mg of bernardioside C_4 (**8**).

Identification of the Component Monosaccharides. The determination was performed according to Kusumoto et al.[8] using 1 mg of each compound. GLC conditions: column J&W Scientific DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm), oven temperature: 170 °C for 10 min, then increasing by 2° min^{-1}, 250 °C injection port and detector temperature, carrier gas He (0.4 mL/s). Retention times: xylose 11.73 min, rhamnose 8.88 and 9.29 min, fucose 10.01 and 10.71 min, glucose 19.13 and 19.49 min.

Determination of the Absolute Configuration of the Sugars. The determination was performed according to Hara *et al.*[6] using about 1 mg of each compound. GLC conditions: column J&W Scientific DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm), 250 °C oven temperature, 280 °C injection port and detector temperature, carrier gas He (22.3 L/h). Retention times: D-xylose 9.11 min (L-xylose 9.72 min), L-rhamnose 9.90 min, D-fucose 10.44 min (L-fucose 11.25 min), D-glucose 12.15 min (L-glucose 12.83 min).

Preparation and Analysis of the Partially Methylated Alditol Acetates.[9,10] One hundred μg each were dissolved in 150 mL of DMSO and methylated according to Hakamori.[11] Hydrolysis was performed using trifluoroacetic acid, reduction using $NaBH_4$, and acetylation using acetic acid anhydride. GLC conditions: column J&W Scientific DB-1 (30 m × 0.32 mm i.d., film thickness 0.1 μm), temperature program: 3 min 80 °C, 10° min^{-1} to 150 °C, 6° min^{-1} to 230 °C, 15° min^{-1} to 300 °C. The respective partially methylated alditol acetates were identified by comparison with standard compounds, their characteristic EI MS fragments, and their retention times.

Bernardioside A (1): white, amorphous powder; mp 233 °C; $[\alpha]^{20}_D$ +19.7° (*c* 0.27, MeOH); TLC R_f 0.88 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 14.9 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH−H_2O 65:35, 1 mL/min); IR 3440 (OH), 2927 (−CH_3, −CH_2−) cm^{-1}; ^{1}H NMR, aglycon δ 0.92, 0.96, 0.98, 0.98, 1.25, 1.38 (6 × CH_3), 1.19 (H-19$_B$), 1.22 (dd, $J \approx$ 14.0, 3.5 Hz, H-1$_B$), 2.01 (H-11$_{A/B}$), 2.12 (dd, $J \approx$ 2.5, 14.0 Hz, H-1$_A$), 2.28 (t, J = 13.3 Hz, H-19$_A$), 3.31 (H-23$_B$), 3.65 (d, J = 3.9 Hz, H-3), 3.68 (H-23$_A$), 4.37 (H-2), 5.36 (H-12), sugar δ 4.47 (d, J = 7.7 Hz, H-1), 3.32 (H-2), 3.36 (H-5), 3.40 (H-3), 3.40 (H-4), 3.73 (dd, J = 11.8, 4.6 Hz, H-6$_B$), 3.85 (dd, J = 11.8 and 2.0 Hz, H-6$_A$); ^{13}C NMR, see Table 1; ESI-MS m/z 667 $[M + H^+]$, 689 $[M + Na^+]$, 705 $[M + K^+]$.

Bernardioside B_1 (2): white, amorphous powder; mp 170 °C; TLC R_f 0.51 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 13.5 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH−H_2O 61:39, 1 mL/min); IR 3440, 2930, 1720 cm^{-1}; ^{1}H NMR, aglycon δ 0.91, 0.97, 0.98, 1.00, 1.24, 1.35 (6 × CH_3), 2.83 (d, $J \approx$ 14 Hz, H-15A), 5.59 (br s, H-12), sugar methyl protons δ 1.28 (d, J = 6.5 Hz), 1.32 (d, J = 6.2 Hz), sugar anomeric protons δ 4.47 (d, J = 7.8 Hz), 4.49 (d, J = 7.7 Hz), 5.40 (br s), 5.45 (d, J = 8.0 Hz); ^{13}C NMR, see Table 1; ESI-MS m/z 1089 $[M + H]^+$, 1111 $[M + Na]^+$, 1127 $[M + K]^+$.

Bernardioside B_2 (3): white, amorphous powder; mp 170 °C (unclear); TLC R_f 0.51 ($CHCl_3$−MeOH−H_2O 7:4:

1), HPLC t_R 15.3 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH−H_2O 61:39, 1 mL/min); IR 3440 (OH), 2924 (−CH_3, −CH_2−), 1733 (−CCOOR) cm^{-1}; ^1H NMR, aglycon δ 2.12 (H-1_A), 1.29 (H-1_B), 4.14 (H-2), 3.65 (H-3), 1.30 (H-5), 1.53 (H-$6_{A,B}$), 1.64 (H-7_A), 1.45 (H-7_B), 1.62 (H-9), 2.08 (H-11_A), 1.99 (H-11_B), 5.33 (H-12), 1.70 (H-15_A), 1.38 (H-15_B), 2.90 (H-18), 1.79 (H-19_A), 1.22 (H-19_B), 1.45 (H-21_A), 1.29 (H-21_B), 1.87 (H-22_A), 1.60 (H-22_B), 3.58 (H-23_A), 3.32 (H-23_B), 0.98 (H_3-24), 1.37 (H_3-25), 0.82 (H_3-26), 1.25 (H_3-27), 0.97 (H_3-29), 1.01 (H_3-30), Glc^A δ 5.46 (H-1), 3.65 (H-2), 3.62 (H-3), 3.52 (H-4), 3.55 (H-5), 4.16 (H-6_A), 3.80 (H-6_B), Glc^B δ 4.41 (H-1), 3.27 (H-2), 3.41 (H-3), 3.37 (H-4), 3.31 (H-5), 3.82 (H-6_A), 3.75 (H-6_B), Rha δ 5.39 (H-1), 4.00 (H-2), 3.73 (H-3), 3.44 (H-4), 3.81 (H-5), 1.22 (H_3-6); ^{13}C NMR, aglycon δ 45.3 (C-1), 72.1 (C-2), 73.7 (C-3), 42.7 (C-4), 48.3 (C-5), 18.9 (C-6), 33.1 (C-7), 40.8 (C-8), 49.1 (C-9), 37.8 (C-10), 24.7 (C-11), 123.7 (C-12), 144.9 (C-13), 43.3 (C-14), 29.1 (C-15), 24.2 (C-16), 42.8 (C-18), 47.4 (C-19), 31.5 (C-20), 34.9 (C-21), 32.8 (C-22), 67.8 (C-23), 14.1 (C-24), 17.7 (C-25), 18.0 (C-26), 26.3 (C-27) 178.1 (C-28), 33.5 (C-29), 24.3 (C-30), Glc^A δ 95.2 (C-1), 77.4 (C-2), 78.9 (C-3), 71.3 (C-4), 77.6 (C-5), 69.7 (C-6), Glc^B δ 104.7 (C-1), 75.2 (C-2), 78.0 (C-3), 71.4 (C-4), 78.0 (C-5), 62.8 (C-6), Rha δ 101.7 (C-1), 72.2 (C-2), 72.3 (C-3), 73.8 (C-4), 70.3 (C-5), 18.2 (C-6); ESI-MS *m/z* 981 [M + Na^+], 997 [M + K^+].

Bernardioside B_3 (4): white, amorphous powder; mp 216 °C; $[\alpha]^{20}_D$ −28.5° (*c* 0.21, MeOH); TLC R_f 0.51 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 22.6 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm i.d., MeOH−H_2O 61:39, 1 mL/min); IR 3440, 2935, 1735 cm^{-1}; ^1H NMR, see ref 5; ^{13}C NMR, see Table 1; ESI-MS *m/z* 1075 [M + H]$^+$, 1097 [M + Na]$^+$, 1113 [M + K]$^+$.

Bernardioside B_4 (5): white, amorphous powder; mp 193 °C; TLC R_f 0.51 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 24.1 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH-H_2O 61:39, 1 mL/min); IR 3440, 2930, 1715, 1635, 1455, 1385, 1240, 1125, 1050, 980, 915, 815, 755 cm^{-1}; ^1H NMR, see ref 1; ^{13}C NMR, see Table 1; HR-LSI MS *m/z* [M + H]$^+$ 1219.6071 Da (calcd 1219.6111 Da); ESI-MS *m/z* 1219 [M + H]$^+$, 1241 [M + Na]$^+$.

Bernardioside C_1 (6): white, amorphous powder; mp 231−237 °C; $[\alpha]^{20}_D$ −13.6° (*c* 0.39, MeOH); TLC R_f 0.46 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 12.6 (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 61:39, 1 mL/min); IR 3440, 2930, 1730 cm^{-1}; ^1H NMR, see ref 12; ^{13}C NMR, see Table 1; ESI-MS *m/z* 1091 [M + H]$^+$, 1113 [M + Na]$^+$, 1129 [M + K]$^+$.

Bernardioside C_2 (7): white, amorphous powder; mp 203−204 °C; TLC R_f 0.46 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 14.1 (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 61:39, 1 mL/min); IR 3440, 2935, 1715 cm^{-1}; ^1H NMR, aglycon δ 0.91, 0.98, 0.98, 1.01, 1.25, 1.36 (6 × CH_3), 2.83 (d, $J \approx 14$ Hz, H-15_A), 2.25 (d, $J \approx 14$ Hz, H-15_B), 5.60 (t, $J \approx 3$ Hz), sugar methyl protons δ 1.28 (d, $J = 5.9$ Hz), 1.30 (d, $J = 6.1$ Hz), 1.32 (d, $J = 6.4$ Hz), sugar anomeric protons δ 4.47 (d, $J = 7.7$ Hz), 4.51 (d, $J = 7.7$ Hz), 5.17 (d, $J = 1.1$ Hz), 5.37 (d, $J = 1.3$ Hz), 5.46 (d, $J = 8.0$ Hz); ^{13}C NMR, see Table 1; ESI-MS *m/z* 1235 [M + H]$^+$, 1257 [M + Na]$^+$.

Bernardioside C_4 (8): white, amorphous powder; mp 224−227 °C; $[\alpha]^{20}_D$ −36.4° (*c* 1.00, MeOH); TLC R_f 0.46 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 23.3 (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 61.39, 1 mL/min); IR 3440, 2930, 1735 cm^{-1}; ^1H NMR, see ref 5; ^{13}C NMR, see Table 1.

Bernardioside D (9): white, amorphous powder; mp 235 °C; $[\alpha]^{20}_D$ −36.4° (*c* 0.97, MeOH); TLC R_f 0.42 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 15.3 (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 60:40, 1 mL/min); IR 3440, 2930, 1750 cm^{-1}; ^1H NMR, see ref 12; ^{13}C NMR, see Table 1.

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