Brodiosaponins A−F, Six New Polyhydroxylated Steroidal Saponins from the Tubers of *Brodiaea californica*

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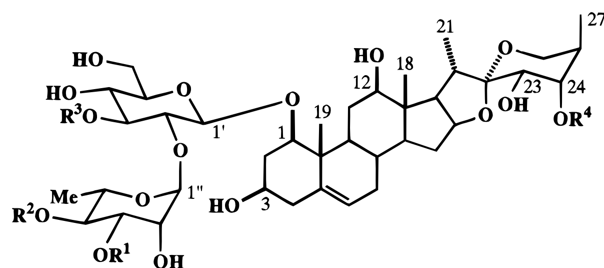
Received December 4, 1996[⊗]

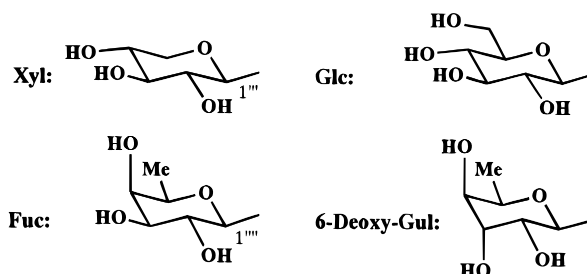
The constituents of the tubers of *Brodiaea californica* have been analyzed as part of a systematic study of plants of the subfamily Allioideae in the Liliaceae. Six new polyhydroxylated steroidal saponins designated as brodiosaponins A−F (**1**−**6**) were isolated, and their structures were elucidated on the basis of spectroscopic analysis, including 2D NMR techniques, and acid- and alkaline-catalyzed hydrolysis. The bisdesmosidic saponins, brodiosaponins D−F (**4**−**6**) contain 6-deoxy-D-gulopyranose, which has been shown for the first time as a saccharide component among the steroidal and triterpene saponins reported to date.

Our previous investigations have revealed that plants belonging to the subfamily Allioideae in the Liliaceae, such as *Agapanthus inapertus*,[1] *Ipheion uniflorum*,[2] *Triteleia lactea*,[3] and *Dichelostemma multiflorum*,[4] as well as certain species of the genus *Allium*,[5−7] contain steroidal saponins, some of which exhibit potent inhibitory activity for cAMP phosphodiesterase. The genera *Ipheion*, *Triteleia*, and *Dichelostemma* are taxonomically closely related and were formerly included in the genus *Brodiaea*.[8] The genus *Brodiaea*, with about 30 species, has a distribution from British Columbia in Canada to Baja California in Mexico. *Brodiaea californica* Lindle. ex Lem., native to northern California in the United States, grows in grasslands at lower elevations on well-drained soils and is the largest and tallest of the *Brodiaea* species that can be commercially purchased as a garden plant in Japan.

Phytochemical examination on *B. californica* tubers resulted in the isolation of six new polyhydroxylated steroidal saponins, named brodiosaponins A−F (**1**−**6**). This paper reports the structural assignment of the new saponins on the basis of the spectroscopic analysis, including 2D NMR techniques, and acid- and alkaline-catalyzed hydrolysis.



	R¹	R²	R³	R⁴
1	H	H	Xyl	H
2	H	H	Xyl	Fuc
3	H	Ac	Xyl	Fuc
4	Glc	H	H	6-Deoxy-Gul
5	H	H	Xyl	6-Deoxy-Gul
6	H	Ac	Xyl	6-Deoxy-Gul



Results and Discussion

The concentrated *n*-BuOH-soluble fraction of the methanolic extract of *B. californica* tubers (fresh wt of 3.0 kg) was repeatedly subjected to Si gel, octadecylsilanized (ODS) Si gel, and Diaion HP-20 column chromatography, as well as preparative reversed-phase HPLC to yield brodiosaponins A (**1**) (24.0 mg), B (**2**) (44.6 mg), C (**3**) (26.1 mg), D (**4**) (125 mg), E (**5**) (304 mg), and F (**6**) (167 mg).

Brodiosaponin A (**1**) ($C_{44}H_{70}O_{20}$, negative-ion FABMS *m/z* 917 $[M - H]^-$) was obtained as an amorphous solid, $[\alpha]_D$ −54.0° (MeOH). The ^{1}H-NMR spectrum in pyridine-d_5 of **1** showed signals for two tertiary methyl groups at δ 1.38 and 1.30 (each 3H, s); three secondary methyl groups at δ 1.68 (3H, d, J = 6.1 Hz), 1.48 (3H, d, J = 6.9 Hz), and 1.09 (3H, d, J = 7.3 Hz); three anomeric protons at δ 6.31 (1H, br s), 4.88 (1H, d, J = 7.5 Hz), and 4.74 (1H, d, J = 7.2 Hz); and an olefinic proton at δ 5.55 (1H, br d, J = 5.6 Hz). The signal at δ 1.68 was due to the methyl group of 6-deoxyhexopyranose. These ^{1}H-NMR data and a quaternary ^{13}C-NMR signal at δ 113.4 suggested **1** to be a spirostanol triglycoside.[9] Acid hydrolysis of **1** with 1 M HCl in dioxane−H_2O (1:1) yielded D-glucose, D-xylose, and L-rhamnose as carbohydrate moieties. The ^{13}C-NMR spectrum of **1** showed a total of 44 resonance lines, 17 of which were attributed to three monosaccharide units. This implied a $C_{27}H_{42}O_7$ molecular formula for the aglycon moiety, which suggested the aglycon to be a spirostanol with five oxygen atoms on the skeleton. Analysis of the ^{1}H−^{1}H COSY spectrum combined with the HOHAHA data, together with one-bond ^{1}H−^{13}C connectivities through the HMQC spectrum, which were measured in a mixed solvent of pyridine-d_5−MeOH-d_4 (11:1) to remove exchangeable protons and minimize signal overlap, allowed assignment of all the ^{1}H- and

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⊗ Abstract published in *Advance ACS Abstracts,* June 1, 1997.

S0163-3864(96)00749-5 CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognosy

Table 1. ^{1}H- and ^{13}C-NMR Data of the Aglycon Moiety of **1**[a]

position	^{1}H	J (Hz)	^{13}C
1	3.75 dd	11.9, 4.1	85.0
2ax	2.29 q-like	11.9	38.0
2eq	2.55 ddd	11.9, 4.1, 4.1	
3	3.68 m		68.1
4ax	2.57 dd	11.9, 11.9	43.6
4eq	2.50 dd	11.9, 4.3	
5			139.6
6	5.56 br d	5.6	125.1
7a	1.53		31.8
7b	1.89 br dd	11.7, 4.0	
8	1.55		32.2
9	1.72 ddd	11.9, 11.9, 3.5	49.9
10			42.8
11ax	1.81 ddd	11.9, 11.9, 11.9	33.8
11eq	3.21 ddd	11.9, 3.5, 3.5	
12	3.73 dd	11.9, 3.5	79.5
13			46.5
14	1.24		55.6
15α	1.98		31.9
15β	1.58		
16	4.57 ddd	8.7, 7.1, 7.1	83.1
17	2.14 dd	8.7, 7.1	61.2
18	1.22 s		11.4
19	1.34 s		15.0
20	3.05 dq	7.1, 7.1	38.1
21	1.39 d	7.1	13.7
22			113.4
23	3.96 d	3.0	64.7
24	4.07 dd	3.0, 3.0	73.9
25	2.04		38.0
26ax	4.38 dd	11.3, 2.1	59.7
26eq	3.32 br d	11.3	
27	1.08 d	7.4	15.1

[a] Spectra were measured in pyridine-d_5−MeOH-d_4 (11:1). Assignments were established by interpretation of the ^{1}H−^{1}H COSY, HOHAHA, and HMQC spectra.

Table 2. ^{1}H- and ^{13}C-NMR Data of the Saccharide Moieties of **1** and **4**[a]

	1			**4**		
position	^{1}H	J (Hz)	^{13}C	^{1}H	J (Hz)	^{13}C
1′	4.70 d	7.4	100.5	4.72 d	7.2	101.1
2′	3.98 dd	8.8, 7.4	76.2	4.07		75.8
3′	3.94 dd	8.8, 8.8	88.4	4.06		79.4
4′	3.64 dd	8.8, 8.8	69.5	3.84 dd	9.3, 9.3	71.7
5′	3.66 ddd	8.8, 6.3, 1.4	77.5	3.70		77.8
6′	4.32 dd	11.3, 1.4	63.0	4.35 dd	11.3, 2.0	63.1
	4.09 dd	11.3, 6.3		4.21		
1″	6.17 d	1.4	101.6	6.30 d	1.0	101.2
2″	4.61 dd	3.3, 1.4	72.2	4.84 dd	3.1, 1.0	71.8
3″	4.41 dd	9.6, 3.3	72.1	4.74 dd	9.4, 3.1	82.4
4″	4.14 dd	9.6, 9.6	74.0	4.40 dd	9.4, 9.4	73.2
5″	4.67 dq	9.6, 6.2	69.5	4.81 dq	9.4, 6.2	69.2
6″	1.60 d	6.2	19.0	1.57 d	6.2	18.6
1‴	4.82 d	7.7	105.1	5.52 d	7.9	106.3
2‴	3.83 dd	8.8, 7.7	74.6	4.01 dd	8.7, 7.9	76.0
3‴	3.94 dd	8.8, 8.8	78.2	4.15 dd	8.7, 8.7	78.1
4‴	4.01 ddd	10.5, 8.8, 5.3	70.5	4.11 dd	8.7, 8.7	71.6
5‴	4.18 dd	10.5, 5.3	67.1	4.02		78.1
	3.60 dd	10.5, 10.5				
6‴				4.39 dd	10.8, 1.7	62.5
				4.22 dd	10.8, 5.4	
1⁗				5.70 d	8.3	103.9
2⁗				4.50 dd	8.3, 3.2	70.5
3⁗				4.69 dd	3.2, 3.2	73.3
4⁗				4.03 br d	3.2	73.4
5⁗				4.53 br q	6.6	70.1
6⁗				1.50 d	6.6	17.0

[a] Spectra were measured in pyridine-d_5−MeOH-d_4 (11:1). Assignments were established by interpretation of the ^{1}H−^{1}H COSY, HOHAHA, and HMQC spectra.

^{13}C-NMR signals arising from the aglycon moiety, as shown in Table 1. The connectivities of the main fragments and the two angular methyls through quaternary carbons were established by interpretation of the HMBC spectrum optimized for an $^{n}J_{C,H}$ parameter of 8 Hz. The quaternary carbon signal observed at δ 42.8 showed $^{2}J_{C,H}$ and $^{3}J_{C,H}$ correlation peaks with the proton signals at δ 2.50 (dd, J = 11.9, 4.3 Hz, H-4eq), 5.56 (br d, J = 5.6 Hz, H-6), and 1.34 (3H, s, Me-19), and was assigned to C-10. Another quaternary carbon at δ 46.5 was assignable to C-13, which was correlated to the proton signals at δ 1.98 (m, H-15α), 4.57 (ddd, J = 8.7, 7.1, 7.1 Hz, H-16), 2.14 (dd, J = 8.7, 7.1 Hz, H-17), 3.05 (dq, J = 7.1, 7.1 Hz, H-20), and 1.22 (3H, s, Me-18). Additional important correlations observed are depicted in Figure 1, leading to the construction of a spirost-5-ene structure with oxygen atoms at C-1, C-3, C-12, C-23, and C-24. The NOE correlations in the phase-sensitive NOESY spectrum, H-8/Me-18 and Me-19, H-14/H-9, H-16 and H-17, H-16/H-17, and Me-18/H-20, provided evidence for the usual B/C *trans*, C/D

Table 3. ^{13}C-NMR Spectral Data for Compounds **1**−**6**[a]

carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	85.0	85.0	85.1	85.8	85.0	85.1
2	38.2	38.3	38.3	39.0	38.2	38.3
3	68.1	68.1	67.9	68.0	68.1	67.8
4	43.7	43.8	43.9	43.8	43.8	43.9
5	139.4	139.6	139.4	139.6	139.5	139.3
6	124.9	124.8	125.0	124.8	124.9	125.1
7	31.7	31.7	31.7	31.7	31.7	31.7
8	32.1	32.1	32.2	32.0	32.1	32.2
9	49.9	50.0	50.0	50.3	49.9	49.9
10	42.8	42.8	42.8	42.8	42.8	42.8
11	34.0	33.9	34.0	33.5	34.0	34.0
12	79.6	79.6	79.6	79.4	79.6	79.5
13	46.5	46.6	46.6	46.6	46.6	46.6
14	55.6	55.6	55.6	55.7	55.6	55.6
15	31.8	32.0	32.0	31.9	32.0	32.0
16	83.0	82.3	82.8	82.8	82.8	82.8
17	61.2	61.4	61.4	61.3	61.4	61.4
18	11.5	11.5	11.5	11.5	11.5	11.5
19	15.0	15.1	15.1	15.0	15.1	15.1
20	38.1	38.6	38.7	38.6	38.6	38.6
21	13.8	13.9	13.9	13.9	14.0	14.0
22	113.4	112.6	112.6	112.7	112.7	112.7
23	64.8	65.5	65.5	65.5	65.5	65.5
24	74.0	82.3	82.2	82.2	82.2	82.2
25	37.9	36.9	36.9	36.9	37.0	37.0
26	59.7	60.4	60.4	60.4	60.4	60.5
27	15.6	15.4	15.4	15.4	15.4	15.4
1′	100.5	100.5	100.5	101.1	100.5	100.5
2′	76.1	76.1	75.1	75.4	76.1	75.1
3′	88.3	88.4	88.6	79.9	88.4	88.6
4′	69.6	69.7	69.7	71.7	69.7	69.6
5′	77.6	77.6	77.6	77.7	77.6	77.7
6′	63.1	63.1	63.1	63.2	63.1	63.1
1″	101.6	101.6	100.8	101.2	101.6	100.8
2″	72.4	72.5	72.2	71.9	72.5	72.2
3″	72.4	72.4	69.9	82.6	72.4	69.9
4″	74.2	74.2	76.5	73.2	74.2	76.5
5″	69.5	69.5	66.6	69.2	69.5	66.6
6″	19.1	19.1	18.6	18.6	19.1	18.6
1‴	105.2	105.2	105.4	106.4	105.2	105.4
2‴	74.7	74.7	74.6	76.2	74.7	74.6
3‴	78.4	78.4	78.6	78.2	78.4	78.6
4‴	70.6	70.6	70.6	71.7	70.6	70.6
5‴	67.2	67.2	67.2	78.3	67.2	67.3
6‴				62.6		
1⁗		106.5	106.5	104.0	104.0	104.0
2⁗		73.2	73.2	70.6	70.6	70.6
3⁗		75.2	75.2	73.5	73.5	73.5
4⁗		72.9	72.9	73.5	73.5	73.5
5⁗		71.6	71.6	70.0	70.1	70.1
6⁗		17.3	17.3	17.0	17.0	17.0
Ac			170.8			170.8
			21.1			21.1

[a] Spectra were measured in pyridine-d_5.

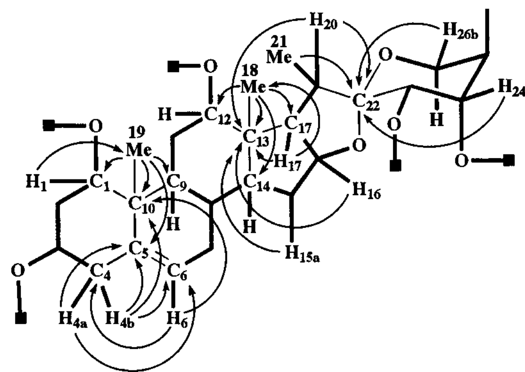

Figure 1. HMBC correlations of the aglycon moiety of **1**.

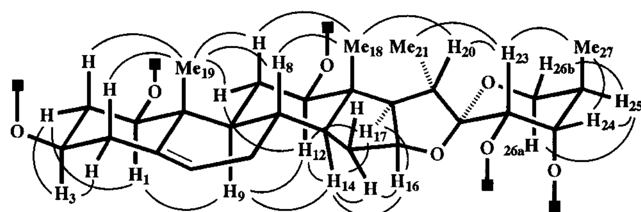

Figure 2. NOE correlations of the aglycon moiety of **1**.

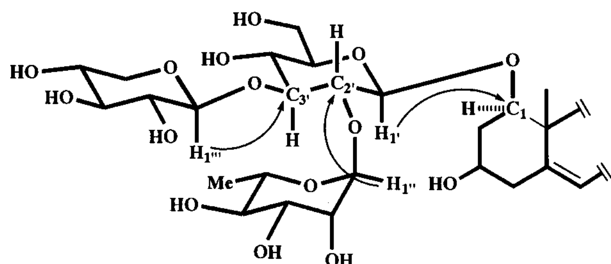

Figure 3. HMBC correlations of the saccharide portion of **1**.

trans, and D/E *cis* ring fusions (Figure 2). The 1β, 3β, and 12β orientations were shown by the spin coupling constants of the H-1, H-3, and H-12 protons: H-1, δ 3.75 (dd, J = 11.9, 4.1 Hz); H-3, δ 3.68 (m, $W_{1/2}$ = 24.5 Hz), and H-12, δ 3.73 (dd, J = 11.9, 3.5 Hz), and supported by the NOEs, H-9/H-1 and H-12, and H-12/H-14 and H-17. The NOEs from H-23 to H-20, Me-21, and Me-27 and from H-24 to H-25 and Me-27 and small coupling constants between H-23 and H-24 (J = 3.0 Hz) and between H-25 and H_2-26 ($J_{H\text{-}25,H\text{-}26a}$ = 2.1 Hz, $J_{H\text{-}25,H\text{-}26b}$ = 0.5 Hz >), allowed us to assign the 22α, 23*S*, 24*S*, and 25*R* configurations. Thus, the structure of the aglycon of **1** was revealed.

Detailed inspection of the ^{1}H–^{1}H COSY and HOHAHA spectra allowed the sequential assignment of the proton resonances for each monosaccharide to be made, starting from the easily distinguished anomeric protons. The HMQC spectrum correlated all the proton signals with the corresponding carbons. The presence of terminal α-L-rhamnopyranosyl and β-D-xylopyranosyl units, and a 2,3-branched β-D-glucopyranosyl unit was presumed by comparison of the ^{13}C-NMR assignment for each monosaccharide with those of authentic methyl glycosides.[9,10] In the HMBC spectrum, the anomeric proton signals at δ 6.17 (d, J = 1.4 Hz), 4.82 (d, J = 7.7 Hz), and 4.70 (d, J = 7.4 Hz) assignable to rhamnose, xylose, and glucose were correlated to the three-bond coupled carbon signals at δ 76.2 (C-2 of glucose), 88.4 (C-3 of glucose), and 85.0 (C-1 of aglycon), respectively (Figure 3). The structure of this triglycoside unit, a 2,3-branched glucose bearing rhamnose at C-2 and xylose at C-3, as well as its linkage to C-1 of the aglycon, were thus apparent. Analysis of all of these data above was consistent with the structure of **1** as (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside].

The ^{1}H-NMR spectrum of brodiosaponin B (**2**) ($C_{50}H_{80}O_{24}$) showed four anomeric proton signals at δ 6.29 (1H, br s), 5.19 (1H, d, J = 7.9 Hz), 4.87 (1H, d, J = 7.7 Hz), and 4.73 (1H, d, J = 7.4 Hz), as well as four steroid methyls at δ 1.43 (3H, d, J = 6.9 Hz), 1.40 (3H, s), 1.25 (3H, s), and 1.08 (3H, d, J = 7.4 Hz), and an olefinic proton at δ 5.57 (1H, br d, J = 5.4 Hz). Acid hydrolysis of **2** with 1 M HCl gave D-glucose, D-xylose, L-rhamnose, and D-fucose. On comparison of the whole ^{13}C-NMR spectrum of **2** with that of **1**, a set of additional six signals corresponding to a terminal β-D-fucopyranosyl moiety appeared at δ 106.5 (CH), 73.2 (CH), 75.2 (CH), 72.9 (CH), 71.6 (CH), and 17.3 (Me), and the signal due to C-24 of the aglycon was displaced downfield by 8.3 ppm by *O*-glycosylation and observed at δ 82.3, confirming that the C-24 hydroxyl group was the glycosylated position to which the additional D-fucose was linked. Partial hydrolysis of **2** with 0.2 M HCl gave **1** as one of the hydrolysates. The structure of **2** was shown to be (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl]-24-*O*-β-D-fucopyranoside.

The spectral features of brodiosaponin C (**3**) ($C_{52}H_{82}O_{25}$) were quite similar to those of **2**. The presence of an acetyl group in the molecule was shown by the IR (ν_{max} 1725 cm^{-1}), ^{1}H-NMR [δ 2.03 (3H, s)], and ^{13}C-NMR [δ 170.8 (C=O) and 21.1 (Me)] spectral data. When **3** was treated with 3% sodium methoxide in MeOH, it was hydrolyzed to yield **2**. Therefore, **3** was found to be a monoacetate of **2**. In the ^{13}C-NMR spectrum of **3**, the signal due to the rhamnose C-4 carbon was shifted to lower field by 2.3 ppm, whereas the signals due to C-3 and C-5 occurred at higher shifts by 2.5 and 2.9 ppm, respectively, as compared with those of **2**. Furthermore, the downfield-shifted proton signal at δ 5.77 (dd, J = 9.7, 9.7 Hz) was assigned to the rhamnose H-4 proton. Thus, the acetyl moiety was revealed to be linked to the rhamnose C-4 hydroxyl position, and the structure of **3** was formulated as (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-[*O*-(4-*O*-acetyl)-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl]-24-*O*-β-D-fucopyranoside.

Brodiosaponin D (**4**) ($C_{51}H_{82}O_{25}$) was shown by its ^{1}H- and ^{13}C-NMR spectra to have the same polyhydroxylated sapogenol structure as compounds **1**–**3**. The ^{1}H-NMR spectrum of **4** displayed four anomeric proton signals at δ 6.41 (1H, br s), 5.77 (1H, d, J = 8.2 Hz), 5.64 (1H, d, J = 7.8 Hz), and 4.75 (1H, d, J = 7.3 Hz). Acid hydrolysis of **4** with 1 M HCl followed by HPLC analysis allowed the identification of D-glucose and L-rhamnose in a ratio of 2:1, but a monosaccharide could not be identified through the HPLC analysis. The triglycoside structure composed of two β-D-glucose units and an α-L-rhamnose unit, and its linkage position to the aglycon was established as *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranose attached to C-1, by the observation of the ^{1}H–^{13}C long-range correlation from each anomeric proton across

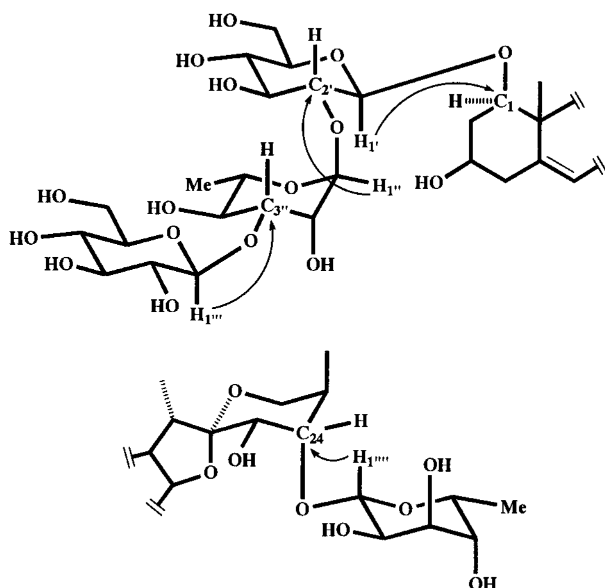

Figure 4. HMBC correlations of the saccharide portions of **4**.

the glycosidic bond to the carbon of other substituted monosaccharide or the aglycon in the HMBC spectrum as shown in Figure 4.

Detailed inspection of the ^{1}H−^{1}H COSY spectrum in conjunction with the HOHAHA data of **4** resulted in the identification of the spin-coupling system of the unidentified monosaccharide, suggesting that it was 6-deoxy-β-gulopyranose. Compound **4** was subjected to acid hydrolysis with 2.5 M HCl in dioxane−MeOH (1:1), followed by purification by column chromatography on Si gel and preparative HPLC gave methyl 6-deoxy-β-D-gulopyranoside, identified by its specific rotation, the ^{1}H- and ^{13}C-NMR spectra, and comparison with published data.[11] This unusual monosaccharide was directly linked to the C-24 hydroxyl group of the aglycon with the formation of a β-glycoside linkage, the evidence for which was obtained from a $^3J_{C,H}$ correlation peak between δ 5.70 (d, J = 8.3 Hz) and δ 82.3 (C-24) in the HMBC spectrum. Therefore, the structure of **4** was formulated as (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-[*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-24-*O*-6-deoxy-β-D-gulopyranoside.

The ^{1}H- and ^{13}C-NMR data of brodiosaponin E (**5**) ($C_{50}H_{80}O_{24}$) showed that it was identical to **1** in terms of the structures of the aglycon and the triglycoside attached to C-1 of the aglycon, but differed only in the monosaccharide structure attached to C-24. In the ^{13}C-NMR spectrum, the six signals appearing at δ 104.0, 70.6, 73.5 × 2, 70.1, and 17.0 could be assigned to C-1 through C-6 of a 6-deoxy-β-D-gulopyranosyl moiety. Total acid hydrolysis of **5** with 1 M HCl gave D-glucose, D-xylose, L-rhamnose, and 6-deoxy-D-gulopyranose, and partial acid hydrolysis with 0.2 M HCl gave **1** as one of the hydrolysates. The structure of **5** was thus characterized as (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl]-24-*O*-6-deoxy-β-D-gulopyranoside.

The IR, ^{1}H-, and ^{13}C-NMR spectra of brodiosaponin F (**6**) ($C_{52}H_{82}O_{25}$) showed the presence of an acetyl group in the molecule [IR: ν_{max} 1725 cm^{-1}; ^{1}H NMR: δ 2.03 (3H, s); ^{13}C NMR: δ 170.8 (C=O) and 21.1 (Me)]. Alkaline hydrolysis of **6** gave **5**. The acylation shifts of an acetyl group linkage were recognized at C-4 (+ 2.3 ppm) of the rhamnose and its neighboring carbons [C-3 (− 2.5 ppm) and C-5 (− 2.9 ppm)] by comparison of the ^{13}C-NMR spectrum of **6** with that of **5**, and at the H-4 proton [δ 5.78 (dd, J = 9.7, 9.7 Hz)] by comparison of the ^{1}H-NMR spectrum with that of **3**. The structure of **6** was shown to be (23*S*,24*S*,25*R*)-spirost-5-ene-1β,3β,12β,23,24-pentol 1-*O*-[*O*-(4-*O*-acetyl)-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl]-24-*O*-6-deoxy-β-D-gulopyranoside.

Brodiosaponins A−F (**1**−**6**) are new polyhydroxylated spirostanol saponins, and brodiosaponins D−F (**4**−**6**) are especially interesting structurally because they have been found to contain 6-deoxy-D-gulopyranose for the first time as a saccharide component among the steroidal and triterpene saponins reported up to the present.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrophotometer, and MS, on a VG AutoSpec E instrument. Elemental analysis was carried out using an Elementar Vario EL elemental analyzer. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^{1}H-NMR), and 2D NMR, on a Bruker AM-500 (500 MHz for ^{1}H-NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. Si gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-Kasei), and ODS Si gel (Nacalai Tesque) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a Tosoh HPLC system composed of a CCPM pump; a CCP controller PX-8010, a UV-8000, or an RI-8010 detector; and Rheodyne injection port with a 2-mL sample loop for preparative HPLC and a 20-μL sample loop for analytical HPLC. A CAPCELL PAK C_{18} column (Shiseido, 10 mm i.d. × 250 mm, ODS, 5 μm) was used for preparative HPLC, and a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm) was employed for analytical HPLC.

Plant Material. The tubers of *B. californica* were purchased from Heiwaen, Japan, in October 1994. The plant specimen is on file in our laboratory (TOYAKU-94−8) and was authenticated by Prof. Jin Murata of Makino Herbarium, Tokyo Metropolitan University.

Extraction and Isolation. The plant material (fresh wt, 3.0 kg) was extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate was partitioned between H_2O and *n*-BuOH. Column chromatography of the *n*-BuOH-soluble phase on Si gel and elution with a gradient mixture of $CHCl_3$−MeOH system (6:1; 4:1; 2:1), and finally with MeOH, gave three fractions (I−III). Fraction II was passed through a Diaion HP-20 column eluting with increased amounts of MeOH in H_2O. The MeOH eluate portion was further fractionated by subjecting it to ODS Si gel column chromatography eluting with MeOH−H_2O (4:1), to produce three fractions (IIa−IIc). Each fraction was chromatographed

on ODS Si gel eluting with H_2O−MeCN (IIa, 4:1; IIb, 3:1; IIc, 7:3) and finally purified by preparative HPLC with H_2O−MeCN (IIa, 4:1; IIb, 3:1; IIc, 7:3). Compounds **2** (44.6 mg) and **5** (304 mg) were isolated from fraction IIa, compounds **3** (26.1 mg) and **6** (167 mg) from IIb, and compound **1** (24.0 mg) from IIc. Fraction III was passed through a Diaion HP-20 column, and the MeOH eluate portion was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (20:10:1), followed by purification by preparative HPLC with H_2O−MeCN (4:1) to yield compound **4** (125 mg).

Brodiosaponin A (1): amorphous solid; $[\alpha]^{28}_D$ −54.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3425 (OH), 2930 (CH), 1040, 980 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.31 (1H, br s, H-1″), 5.55 (1H, br d, J = 5.6 Hz, H-6), 4.88 (1H, d, J = 7.5 Hz, H-1‴), 4.74 (1H, d, J = 7.2 Hz, H-1′), 4.44 (1H, dd, J = 11.2, 2.0 Hz, H-26a), 3.35 (1H, br d, J = 11.2 Hz, H-26b), 1.68 (3H, d, J = 6.1 Hz, Me-6″), 1.48 (3H, d, J = 6.9 Hz, Me-21), 1.38 (3H, s, Me-19), 1.30 (3H, s, Me-18), 1.09 (3H, d, J = 7.3 Hz, Me-27); negative-ion FABMS *m/z* 917 [M − H]$^-$; *anal.* C 55.19%, H 8.03%, calcd for $C_{44}H_{70}O_{20}\cdot 2\ H_2O$, C 55.34%, H 7.81%.

Acid Hydrolysis of 1. A solution of **1** (3 mg) in 1 M HCl (dioxane−H_2O, 1:1, 3 mL) was heated at 100 °C for 3 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column and fractionated using a Sep-Pak C_{18} cartridge (Waters), eluting with H_2O−MeOH (4:1, 5 mL) followed by MeOH (5 mL), to give a sugar fraction (0.9 mg) and an aglycon fraction (1.2 mg). TLC analysis of the aglycon fraction showed that it contained several unidentified artifactual sapogenols. The sugar fraction (0.9 mg) was dissolved in H_2O (1 mL), to which (−)-α-methylbenzylamine (5 mg) and Na[BH_3CN] (8 mg) in EtOH (1 mL) was added. After being set aside at 40 °C for 4 h followed by addition of AcOH (0.2 mL) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O−MeCN (4:1; 1:1; 1:9, each 5 mL) mixtures as solvents. The H_2O−MeOH (1:9) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[12,13] which was then analyzed by HPLC under the following conditions: solvent, MeCN−H_2O (2:3); flow rate, 0.8 mL min^{-1}; detection, UV 230 nm. The derivatives of D-glucose, D-xylose, and L-rhamnose were detected; t_R (min): 18.11 (derivative of D-xylose); 23.44 (derivative of D-glucose); 26.83 (derivative of L-rhamnose).

Brodiosaponin B (2): amorphous solid; $[\alpha]^{28}_D$ −38.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3430 (OH), 2930 (CH), 1040, 980 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.29 (1H, br s, H-1″), 5.57 (1H, br d, J = 5.4 Hz, H-6), 5.19 (1H, d, J = 7.9 Hz, H-1⁗), 4.87 (1H, d, J = 7.7 Hz, H-1‴), 4.73 (1H, d, J = 7.4 Hz, H-1′), 1.66 (3H, d, J = 6.1 Hz, Me-6″), 1.53 (3H, d, J = 6.4 Hz, Me-6⁗), 1.43 (3H, d, J = 6.9 Hz, Me-21), 1.40 (3H, s, Me-19), 1.25 (3H, s, Me-18), 1.08 (3H, d, J = 7.4 Hz, Me-27); negative-ion FABMS *m/z* 1063 [M − H]$^-$; *anal.* C 54.24%, H 7.86%, calcd for $C_{50}H_{80}O_{24}\cdot 2\ H_2O$, C 54.54%, H 7.69%.

Acid Hydrolysis of 2. Compound **2** (3 mg) was subjected to acid hydrolysis using the same procedures as described for **1** to give a sugar fraction (1.2 mg). The monosaccharide constituents in the fraction were converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives, which were then analyzed by HPLC. The derivatives of D-glucose, D-xylose, D-fucose, and L-rhamnose were detected; t_R (min): 17.92 (derivative of D-xylose); 20.04 (derivative of D-fucose); 23.34 (derivative of D-glucose); 26.31 (derivative of L-rhamnose).

Partial Hydrolysis of 2. A solution of **2** (11.2 mg) in 0.2 M HCl (dioxane−H_2O, 1:1, 4 mL) was heated at 90 °C for 30 min under an Ar atmosphere. The reaction mixture was neutralized by passage through an Amberlite IRA-93ZU column and then chromatographed over Si gel, eluting with $CHCl_3$−MeOH−H_2O (20:10:1), to give **1** (1.4 mg) as one of the hydrolysates.

Brodiosaponin C (3): amorphous solid; $[\alpha]^{28}_D$ −48.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400 (OH), 2925 (CH), 1725 (C=O, ester), 1035, 980 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.43 (1H, br s, H-1″), 5.77 (1H, dd, J = 9.7, 9.7 Hz, H-4″), 5.62 (1H, br d, J = 5.4 Hz, H-6), 5.21 (1H, d, J = 7.9 Hz, H-1⁗), 4.84 (1H, d, J = 7.7 Hz, H-1‴), 4.74 (1H, d, J = 7.3 Hz, H-1′), 2.03 (3H, s, Ac), 1.53 (3H, d, J = 6.4 Hz, Me-6⁗), 1.43 (3H, d, J = 6.9 Hz, Me-21), 1.41 (3H, d, J = 6.3 Hz, Me-6″), 1.38 (3H, s, Me-19), 1.27 (3H, s, Me-18), 1.09 (3H, d, J = 7.4 Hz, Me-27); negative-ion FABMS *m/z* 1105 [M − H]$^-$.

Alkaline Hydrolysis of 3. Compound **3** (5 mg) was treated with 3% NaOMe in MeOH (3 mL) at room temperature for 30 min. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo) column and then chromatographed over Si gel, eluting with $CHCl_3$−MeOH−H_2O (20:10:1), to yield **2** (4.2 mg).

Brodiosaponin D (4): amorphous solid; $[\alpha]^{28}_D$ −38.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400 (OH), 2930 (CH), 1055, 980 cm^{-1}; ^1H-NMR (C_5D_5N) δ 6.41 (1H, br s, H-1″), 5.77 (1H, d, J = 8.2 Hz, H-1⁗), 5.64 (1H, d, J = 7.8 Hz, H-1‴), 5.54 (1H, br d, J = 5.6 Hz, H-6), 4.75 (1H, d, J = 7.3 Hz, H-1′), 1.58 (3H, d, J = 6.1 Hz, Me-6″), 1.53 (3H, d, J = 6.6 Hz, Me-6⁗), 1.42 (3H, s, Me-19), 1.41 (3H, d, J = 6.9 Hz, Me-21), 1.23 (3H, s, Me-18), 1.07 (3H, d, J = 7.3 Hz, Me-27); negative-ion FABMS *m/z* 1093 [M − H]$^-$; *anal.* C 52.90%, H 7.86%, calcd for $C_{51}H_{82}O_{25}\cdot 7/2\ H_2O$, C 52.89%, H 7.74%.

Acid Hydrolysis of 4. Compound **4** (3 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (1.3 mg). The monosaccharide constituents in the fraction were converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives, which were then analyzed by HPLC. The derivatives of D-glucose and L-rhamnose were detected; t_R (min): 23.34 (derivative of D-glucose); 26.32 (derivative of L-rhamnose).

Identification of 6-Deoxy-D-gulose. Compound **4** (100 mg) was treated with 2.5 M HCl (dioxane−MeOH, 1:1, 5 mL) at 100 °C for 1 h under an Ar atmosphere. The reaction mixture was neutralized by passage through an Amberlite IRA-93ZU column and then chromatographed over Si gel, eluting with a gradient mixture of $CHCl_3$−MeOH−H_2O (9:1; 4:1; 2:1), to give a glycoside fraction. The fraction was further subjected to Si gel column chromatography, eluting with $CHCl_3$−MeOH (9:1) and EtOAc-MeOH (12:1), and to preparative HPLC eluting with H_2O−MeOH (14:5) to furnish methyl

6-deoxy-β-D-gulopyranoside (1.9 mg): $[\alpha]^{28}_{D}$ −94.7° (*c* 0.19, MeOH); ^{1}H NMR (CD_3OD) δ 4.47 (1H, d, J = 8.2 Hz, H-1), 3.55 (1H, dd, J = 8.2, 3.4 Hz, H-2), 3.94 (1H, dd, J = 3.4, 3.4 Hz, H-3), 3.46 (1H, dd, J = 3.4, 1.3 Hz, H-4), 4.00 (1H, qd, J = 6.6, 1.3 Hz, H-5), 1.22 (3H, d, J = 6.6 Hz, Me-6), 3.48 (3H, s, OMe); ^{13}C NMR (CD_3OD) δ 103.4 (C-1), 70.1 (C-2), 73.4 (C-3), 73.6 (C-4), 69.6 (C-5), 16.2 (C-6), 56.9 (OMe).

Brodiosaponin E (5): amorphous solid; $[\alpha]^{28}_{D}$ −82.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3420 (OH), 2930 (CH), 1035, 980 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ 6.30 (1H, br s, H-1″), 5.77 (1H, d, J = 8.2 Hz, H-1⁗), 5.56 (1H, br d, J = 5.7 Hz, H-6), 4.87 (1H, d, J = 7.6 Hz, H-1‴), 4.74 (1H, d, J = 7.4 Hz, H-1′), 1.66 (3H, d, J = 6.1 Hz, Me-6″), 1.53 (3H, d, J = 6.6 Hz, Me-6⁗), 1.44 (3H, d, J = 6.9 Hz, Me-21), 1.39 (3H, s, Me-19), 1.25 (3H, s, Me-18), 1.08 (3H, d, J = 7.4 Hz, Me-27); negative-ion FABMS *m*/*z* 1063 [M − H]$^-$; *anal.* C 54.44%, H 7.93%, calcd for $C_{50}H_{80}O_{24}\cdot 2\ H_2O$, C 54.54%, H 7.69%.

Acid Hydrolysis of 5. Compound **5** (3 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction (1.4 mg). The monosacchraide constituents in the fraction were converted to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives, which were then analyzed by HPLC. The derivatives of D-glucose, D-xylose, 6-deoxy-D-gulose, and L-rhamnose were detected; t_R (min): 17.82 (derivative of D-xylose); 23.24 (derivative of D-glucose); 24.78 (derivative of 6-deoxy-D-gulose); 26.22 (derivative of L-rhamnose).

Brodiosaponin F (6): amorphous solid; $[\alpha]^{28}_{D}$ −56.0° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3400 (OH), 2970 and 2920 (CH), 1725 (C=O, ester), 1035, 985 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.44 (1H, br s, H-1″), 5.79 (1H, d, J = 8.2 Hz, H-1⁗), 5.78 (1H, dd, J = 9.7, 9.7 Hz, H-4″), 5.62 (1H, br d, J = 5.5 Hz, H-6), 4.85 (1H, d, J = 7.6 Hz, H-1‴), 4.75 (1H, d, J = 7.5 Hz, H-1′), 2.03 (3H, s, Ac), 1.53 (3H, d, J = 6.5 Hz, Me-6⁗), 1.44 (3H, d, J = 6.9 Hz, Me-21), 1.41 (3H, d, J = 6.2 Hz, Me-6″), 1.37 (3H, s, Me-19), 1.27 (3H, s, Me-18), 1.08 (3H, d, J = 7.3 Hz, Me-27); negative-ion FABMS *m*/*z* 1105 [M − H]$^-$.

Alkaline Hydrolysis of 6. Compound **6** (5 mg) was subjected to alkaline hydrolysis using the same procedures as described for **3**, and the reaction mixture was chromatographed over Si gel, eluting with $CHCl_3$−MeOH−H_2O (20:10:1), to yield **5** (4.1 mg).

Acknowledgment. We are grateful to Dr. Y. Shida, Mrs. C. Sakuma, and Mr. H. Fukaya of Tokyo University of Pharmacy and Life Science for the measurements of the mass and 2D NMR spectra, and elemental analysis, respectively.

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NP960749G