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Structure and Bioactivity of Steroidal Saponins Isolated from the Roots of *Chamaelirium luteum* (False Unicorn)

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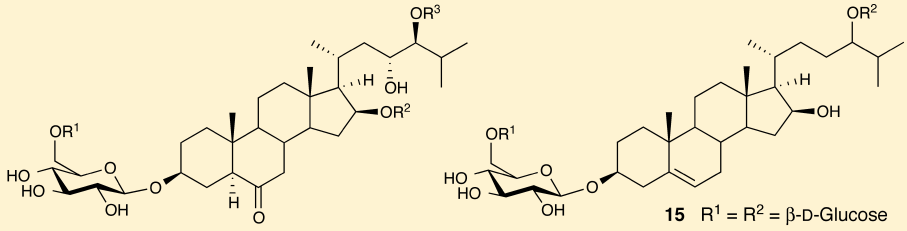
Supporting Information



15 $R^1 = R^2 =$ β-D-Glucose

6-dehydrochamaeliroside A $R^1 =$ β-D-Glucose $R^2 = R^3 = H$
6-dehydrochamaeliroside B $R^1 =$ β-D-Glucose $R^2 = H$ $R^3 =$ β-D-Fucose
6-dehydrochamaeliroside E $R^1 = R^2 =$ β-D-Glucose $R^3 = H$

ABSTRACT: Phytochemical investigation of *Chamaelirium luteum* ("false unicorn") resulted in the isolation of 15 steroidal glycosides. Twelve of these (**1**, **2**, **4**−**9**, **11**−**13**, and **15**) are apparently unique to this species, and eight of these (**6**−**9**, **11**−**13**, and **15**) are previously unreported compounds; one (**15**) possesses a new steroidal aglycone. In addition, the absolute configuration of (23*R*,24*S*)-chiograsterol A (**10**) was defined, and its full spectroscopic characterization is reported for the first time. The structures and configurations of the saponins were determined using a combination of multistage mass spectrometry (MS^n), 1D and 2D NMR experiments, and chemical degradation. The antiproliferative activity of nine compounds obtained in the present work, and eight related compounds generated in previous work, was compared in six human tumor cell lines, with aglycones **3** and **10** and related derivatives **16**, **17**, **19**, and **20** all displaying significant antiproliferative activity.

Chamaelirium luteum (L.) A. Gray (Melanthiaceae), commonly known as "false unicorn" or "devil's bit", is native to North America. The underground parts of *C. luteum* (roots and rhizome) have been used in traditional medicine mainly for the treatment of female reproductive health disorders[1] and are now widely used in many commercial botanical supplements; the annual trade volume of *C. luteum* was recently estimated at 9800−14 300 kg in the United States alone.[2] *C. luteum* is listed as endangered or threatened in several states by the United States Department of Agriculture (USDA),[3] with wildcrafting (the harvest of plant material from the wild) a significant contributor to the decline of wild populations.[4]

Phytochemical characterization of *C. luteum* has been surprisingly limited given the wide use of this herb in traditional medicine and commercial botanical supplements and its inclusion in a clinical trial[5] and an animal model study.[6] Reports from the nineteenth century indicated the presence of steroidal saponins in *C. luteum*, with the isolation of the "bitter principle" of *C. luteum* (named chamaelirin) by Greene in 1878.[7] Subsequently, he characterized its decomposition products as glucose and a substance referred to as chamaeliritin.[8] In 1942, the presence of steroidal saponins in *C. luteum* was confirmed by Cataline and Francke[9] and by Marker et al.[10] The latter isolated small amounts of the steroidal aglycone diosgenin from both "helonias root" and *C. carolinianum* Willd. (now synonymous with *C. luteum*). We recently identified the major phytochemical constituents of *C. luteum* as the steroidal saponins chamaelirosides A and B (**1** and **2**, respectively).[11] These possess an unusual cholestane-derived steroidal skeleton, (23*R*,24*S*)-chiograsterol B (**3**),[11] that is constitutionally identical to an aglycone reported only once previously from *Chionographis japonica* (Willd.) Maxim. but with undefined configuration at C-23 and C-24.[12] A third major constituent, heloside A (**4**), possessing an unusual cholesterol-derived aglycone (helogenin, **5**) was also isolated from *C. luteum* along with the related minor saponin heloside B, which differs only by lacking the 26-*O*-glucosyl residue present in **4**.[13]

Steroidal saponins are common in medicinal herbs, and most steroidal saponins isolated from terrestrial plants fall into two

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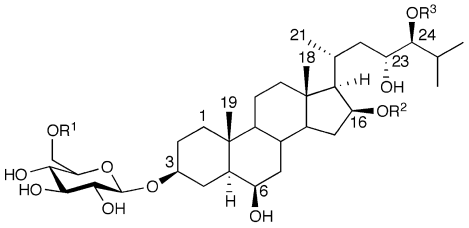
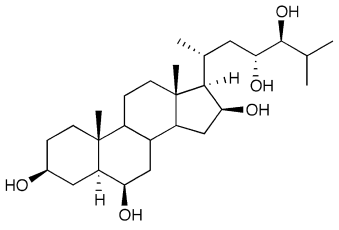
Chart 1



1 R^1 = β-D-Glc R^2 = R^3 = H
2 R^1 = β-D-Glc R^2 = H R^3 = β-D-Fuc
6 R^1 = β-D-Glc R^2 = H R^3 = 4-*O*-acetyl-β-D-Fuc
7 R^1 = β-D-Glc R^2 = H R^3 = α-L-Ara
8 R^1 = R^2 = β-D-Glc R^3 = H



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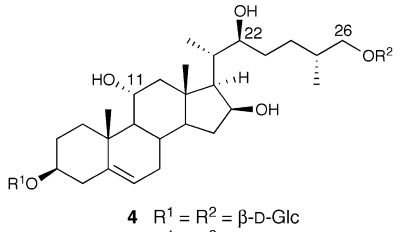
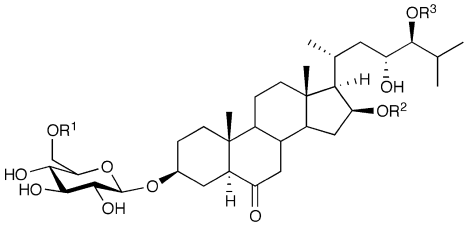
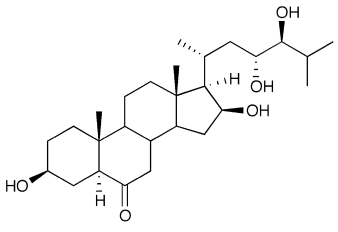


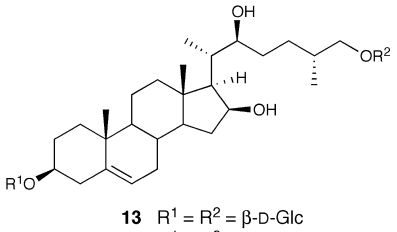
4 R^1 = R^2 = β-D-Glc
5 R^1 = R^2 = H



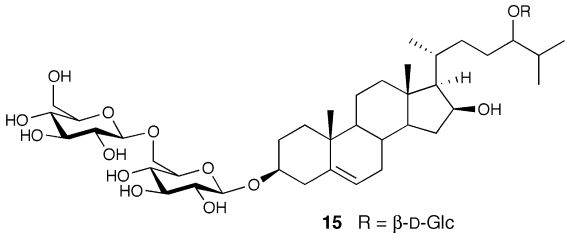
9 R^1 = β-D-Glc R^2 = R^3 = H
11 R^1 = β-D-Glc R^2 = H R^3 = β-D-Fuc
12 R^1 = R^2 = β-D-Glc R^3 = H



10



13 R^1 = R^2 = β-D-Glc
14 R^1 = R^2 = H



15 R = β-D-Glc

structural subclasses, namely, spirostanol and furostanol saponins. Saponins are thought to be responsible for the therapeutic effect proposed for some medicinal plants, as they exhibit cytotoxic, anti-inflammatory, antibacterial, antifungal, and hemolytic activities.[14] The major steroidal saponins of *C. luteum*[11,13] represent a further structural subclass of steroidal saponins: the open-chain steroidal saponins, which lack the additional ring(s) derived from the C-17 side chain that are characteristic of spirostanol and furostanol saponins. While open-chain steroidal saponins are abundant in marine organisms,[15−18] they are less common in terrestrial plants.[19]

In the present work, we provide a complete profile of steroidal saponins present in *C. luteum* and report the characterization of eight new saponins. In addition, the absolute configuration of (23*R*,24*S*)-chiograsterol A (**10**) is defined and previously unavailable spectroscopic characterization is reported for this compound. The isolated saponins include seven new compounds that are structurally related to chamaelirosides A and B (**1** and **2**)[11] and one new saponin related to heloside A (**4**).[13] Three known saponins derived from the spirostanol pennogenin aglycone were also isolated. We chose to perform a preliminary screen for antiproliferative activity of the isolated saponins and their aglycones against human cancer cell lines, as this is a common bioactivity of many steroidal saponins.[14]

RESULTS AND DISCUSSION

Semipreparative reversed-phase (RP) HPLC of a crude methanolic extract of *C. luteum* underground parts afforded 15 steroidal saponins, approximately 0.6% of the dry weight of the plant material. Four of these we identified previously as chamaelirosides A and B (**1** and **2**),[11] heloside A (**4**),[13] and heloside B.[13] Eight (**6**−**9**, **11**−**13**, and **15**) were determined to be new saponins, and their structures were elucidated using a combination of MS^n studies, 1D and 2D NMR experiments, and chemical degradation. The remaining three compounds were identified as (25*S*)-27-hydroxypennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside,[20,21] pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyanoside,[21,22] and pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside.[21,22]

Compound **6** was an amorphous solid, and positive-ion HRESIMS provided an ion at *m*/*z* 987.5120, which corresponded to a molecular formula of $C_{47}H_{80}O_{20}$. Fragmentation in the negative-ion $ESIMS^n$ of the quasi-molecular ion at *m*/*z* 963 resulted in neutral losses of both 42 and 60 Da, consistent with the presence of an acetyl group. This was followed by the loss of both 146 and 162 Da from the $[M - 42 - H]^-$ precursor ion, indicating the presence of both terminal deoxyhexose and hexose residues in **6**. The $[M - 162 - 42 - H]^-$ precursor ion at *m*/*z* 759 further fragmented to yield neutral losses of 146 and 164 Da. The observed fragmentation pattern was consistent with the presence of one acetyl group, one deoxyhexose, and two hexose residues in **6** along with a C_{27} skeleton bearing five oxygen atoms. This accounted for the eight degrees of unsaturation provided by the molecular formula for **6** and pointed to a saturated cholestane aglycone structure. The ^1H NMR spectrum of **6** (in pyridine-d_5) displayed signals for two methyl groups attached to quaternary carbons at δ_H 1.06 (s, H_3-18) and 1.27 (s, H_3-19), as well as signals for three methyl groups attached to methine carbons [δ_H 1.06 (d, *J* = 6.5 Hz, H_3-26/27), 1.14 (d, *J* = 6.6 Hz, H_3-21), and 1.27 (d, *J* = 6.4 Hz, H_3-26/27)]. An additional methyl

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Table 1. ^{1}H NMR Data for the Aglycone of Compounds 6−9, 11−13, and 15 (750 MHz)

	6[a]	7[b]	8[a]	9[a]	11[a]	12[b]	13[b]	15[b]
1	1.64 m[c]	1.64 m[c]	1.60 m	1.61 m[c]	1.60 m[c]	1.60 m[c]	1.73 m[c]	1.81 m[c]
	1.02 m	1.04 m	1.02 m[c]	1.21 m[c]	1.20 m[c]	1.21 m[c]	0.98 m[c]	1.12 m[c]
2	2.19 m	2.23 m	2.19 m	2.17 m[c]	2.17 m	2.19 m[c]	2.16 m[c]	2.30 m[c]
	1.79 m	1.82 m	1.79 m	1.62 m[c]	1.62 m[c]	1.63 m[c]	1.77 m[c]	1.81 m[c]
3	4.19 m[c]	4.18 m[c]	4.19 m[c]	4.08 m[c]	4.08 m[c]	4.07 m[c]	3.95 m[c]	3.97 m[c]
4	2.28 m[c]	2.32 m[c]	2.28 m	2.38 m[c]	2.40 m	2.37 m	2.74 ddd (13.3, 4.4, 2.0)	2.74 m
	2.10 m[c]	2.14 m[c]	2.09 m	1.73 m	1.73 q (12.2)	1.73 m[c]	2.50 br t (12.2)	2.52 m
5	1.09 m[c]	1.12 m[c]	1.07 m[c]	2.17 m[c]	2.17 m	2.16 br d (12.6)		
6	3.95 m	3.99 m	3.90 m				5.34 d (5.0)	5.37 br s
7	2.02 m[c]	2.07 m[c]	2.02 m	2.35 m[c]	2.34 dd (13.0, 4.5)	2.32 dd (12.7, 4.5)	1.90 m	1.90 m
	1.18 m[c]	1.20 m[c]	1.13 m[c]	1.97 t (12.8)	1.96 t (12.7)	1.97 t (12.7)	1.52 m[c]	1.53 m
8	2.11 m[c]	2.13 m[c]	1.94 m[c]	1.79 m	1.78 qd (11.4, 4.7)	1.45 m[c]	1.52 m[c]	1.47 m[c]
9	0.67 td (11.3, 4.1)	0.70 td (11.4, 4.2)	0.61 td (10.9, 4.1)	1.12 m[c]	1.12 m[c]	1.12 m[c]	0.92 m[c]	0.92 m[c]
11	1.43 m[c]	1.48 m[c]	1.42 m	1.42 m	1.42 m	1.43 m[c]	1.44 m[c]	1.43 m[c]
	1.43 m[c]	1.40 qd (13.0, 3.6)	1.31 m[c]	1.21 m[c]	1.21 m[c]	1.12 m[c]	1.44 m[c]	1.43 m[c]
12	1.96 m[c]	1.98 m	1.95 m[c]	1.90 m[c]	1.90 m	1.92 m	2.05 m	2.02 m
	1.08 m[c]	1.10 m[c]	1.05 m[c]	1.04 m[c]	1.04 m[c]	1.06 m	1.16 m[c]	1.08 m[c]
14	0.89 m	0.91 m	0.82 m[c]	0.96 m[c]	0.95 m[c]	0.94 m	0.92 m[c]	0.78 m
15	2.27 m[c]	2.30 m[c]	2.37 m[c]	2.10 m[c]	2.07 dt (12.6, 7.5)	2.20 m[c]	2.29 dt (12.5, 7.7)	2.25 m[c]
	1.45 m[c]	1.51 td (13.2, 4.5)	1.83 td (13.7, 4.6)	1.36 m	1.34 td (13.1, 4.5)	1.76 m[c]	1.52 m[c]	1.45 m[c]
16	4.65 m	4.65 td (7.2, 4.7)	4.29 m[c]	4.58 m	4.56 m	4.30 m[c]	4.77 td (7.4, 4.5)	4.63 m
17	1.09 m[c]	1.10 m[c]	1.24 m[c]	1.09 m	1.04 m[c]	1.22 m[c]	1.68 dd (11.0, 7.0)	1.04 m[c]
18	1.06 s	1.07 s	0.83 s	0.97 s	0.95 s	0.79 s	1.16 s	1.09 s
19	1.27 s	1.27 s	1.22 s	0.65 s	0.65 s	0.57 s	0.95 s	0.95 s
20	2.53 m	2.53 m	2.78 m	2.53 m	2.51 m	2.79 m	2.57 m	2.26 m[c]
21	1.14 d (6.6)	1.16 d (6.6)	1.12 d (6.8)	1.17 d (6.4)	1.13 d (6.6)	1.15 d (6.1)	1.21 d (7.0)	1.10 d (6.5)
22	2.34 br t (12.8)	2.34 m[c]	2.29 m[c]	2.10 m[c]	2.27 dd (14.0, 11.6)	2.30 br t (12.1)	4.21 m[c]	2.30 m[c]
	1.64 dd (12.7, 9.3)	1.61 m[c]	1.74 br t (11.6)	1.88 m[c]	1.66 m	1.78 m[c]		1.39 m[c]
23	4.19 m[c]	4.23 m[c]	4.20 m[c]	4.08 m[c]	4.18 m[c]	4.24 m[c]	1.78 m[c]	1.86 m
							1.78 m[c]	1.75 m
24	3.76 dd (6.9, 3.7)	3.83 dd (6.9, 3.6)	3.79 q (5.6)	3.68 m	3.77 m[c]	3.83 dd (6.4, 4.8)	2.12 m[c]	3.81 m
							1.35 m	
25	2.10 m[c]	2.11 m[c]	2.39 m[c]	2.37 m[c]	2.11 m	2.43 m	2.00 octet (6.6)	2.17 m
26	1.06 d (6.5)	1.09 d (6.8)	1.12 d (6.8)	1.15 d (6.8)	1.09 d (6.9)	1.16 d (6.8)	3.95 m[c]	1.02 d (6.8)
							3.65 dd (9.4, 6.0)	
27	1.27 d (6.4)	1.29 d (6.7)	1.27 d (6.7)	1.27 d (6.6)	1.29 d (6.7)	1.29 d (6.7)	1.02 d (6.7)	1.05 d (6.3)

[a]Acquired in pyridine-d_5. [b]Acquired in pyridine-d_5/D_2O (~9:1). [c]Indicates overlapping signals within column.

doublet signal observed at δ_H 1.30 (d, J = 6.4 Hz, H_3-6‴) was consistent with the presence of one deoxyhexose residue, while a methyl singlet at δ_H 2.00 (s, Fuc-$COCH_3$) was consistent with the presence of one acetyl group. These methyl group signals were correlated in the HSQC spectrum of **6** with signals at δ_C 13.5 (C-18), 16.1 (C-19), 19.9 (C-26/27), 20.0 (C-21), 19.7 (C-26/27), and 20.9 (Fuc-$COCH_3$), respectively. HMBC correlations of the characteristic steroid methyl groups provided rapid assignment of the aglycone of **6**. For example, correlations from H_3-26/27 (δ_H 1.06 and 1.27) revealed the chemical shifts of C-24 (δ_C 90.1) and C-25 (δ_C 30.2), with the former indicating oxygenation at C-24. The ^{13}C NMR spectrum of **6** displayed 47 signals, including seven methyl groups, 10 methylenes, 27 methines, and only three quaternary carbons [δ_C 36.1 (C-10), 42.9 (C-13), and 171.1 (Fuc-$COCH_3$)]. The ^{1}H and ^{13}C NMR spectra of **6** (Tables 1 and 2), including COSY, TOCSY, HSQC, and HMBC experiments, revealed chemical shifts in close agreement with those of chamaeliroside B (**2**).[11] Secondary OH groups were found at C-3, C-6, C-16, C-23, and C-24, with downfield chemical shifts of both C-3 ($\Delta\delta_C$ = +6.5 ppm as compared with compound **3**)[11] and C-24 ($\Delta\delta_C$ = +10.4 ppm)[11] consistent with *O*-glycosylation. The stereochemistry of the different ring junctions and substituents in **6** was determined via a 2D ROESY experiment. For example, cross-peaks observed between H_3-19 (δ_H 1.27) and both H-1β (δ_H 1.64) and H-2β (δ_H 1.79) and between H-3 (δ_H 4.19) and both H-1α (δ_H 1.02) and H-2α (δ_H 2.19) indicated β-orientation of the *O*-glycosyl moiety at C-3. Additional cross-peaks observed between H-5/H-6, H-5/H-9, H-8/H_3-18/H_3-19, H-9/H-14, H_3-18/H-15β, H-14/H-15α, H-15α/H-16, and H-16/H-17 established the β-orientation of the substituents at C-6, C-16, and C-17 and *trans* fusion of the steroid A/B, B/C, and C/D ring junctions (Figure 1).

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Table 2. ^{13}C NMR Data for the Aglycone of Compounds 6−9, 11−13, and 15

	6[a,b]	7[c,d]	8[a,b]	9[a,b]	11[a,b]	12[a,d]	13[a,d]	15[c,d]
1	38.9, CH_2	38.9, CH_2	38.9, CH_2	36.8, CH_2	36.8, CH_2	36.7, CH_2	37.5, CH_2	37.5, CH_2
2	30.3, CH_2	30.2, CH_2	30.3, CH_2	29.6, CH_2	29.6, CH_2	29.5, CH_2	30.2, CH_2	30.2, CH_2
3	78.0, CH	78.2, CH	78.0, CH	76.8, CH	76.8, CH	77.0, CH	78.4, CH	78.8, CH
4	32.9, CH_2	32.7, CH_2	32.9, CH_2	27.0, CH_2	27.0, CH_2	27.0, CH_2	39.3, CH_2	39.4, CH_2
5	47.9, CH	47.8, CH	47.8, CH	56.5, CH	56.5, CH	56.4, CH	141.0, C	141.0, C
6	71.0, CH	70.9, CH	70.9, CH	209.9, C	209.9, C	210.5, C	121.9, CH	121.9, CH
7	40.7, CH_2	40.5, CH_2	40.7, CH_2	46.7, CH_2	46.7, CH_2	46.6, CH_2	32.2, CH_2	32.1, CH_2
8	30.7, CH	30.7, CH	30.6, CH	37.5, CH	37.5, CH	37.4, CH	31.9, CH	31.8, CH
9	54.7, CH	54.6, CH	54.6, CH	53.8, CH	53.8, CH	53.5, CH	50.5, CH	50.3, CH
10	36.1, C	36.0, C	36.0, C	40.9, C	40.9, C	40.9, C	37.0[e], C	36.9, C
11	21.1, CH_2	21.1, CH_2	21.3, CH_2	21.4, CH_2	21.4, CH_2	21.4, CH_2	21.1, CH_2	21.0, CH_2
12	40.4, CH_2	40.4, CH_2	40.3, CH_2	39.8, CH_2	39.8, CH_2	39.6, CH_2	40.4, CH_2	40.2, CH_2
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16	71.8, CH	71.7, CH	83.1, CH	71.6, CH	71.5, CH	82.8, CH	71.3, CH	71.1, CH
17	62.7, CH	62.6, CH	62.9, CH	62.4, CH	62.4, CH	62.4, CH	58.0, CH	62.3, CH
18	13.5, CH_3	13.5, CH_3	13.6, CH_3	13.4, CH_3	13.4, CH_3	13.6, CH_3	13.5, CH_3	13.3, CH_3
19	16.1, CH_3	16.0, CH_3	16.0, CH_3	13.1, CH_3	13.1, CH_3	13.0, CH_3	19.4, CH_3	19.4, CH_3
20	27.4, CH	27.4, CH	26.4, CH	27.6, CH	27.4, CH	26.3, CH	36.0, CH	30.6, CH
21	20.0, CH_3	20.0,[e] CH_3	18.1, CH_3	20.5, CH_3	20.0, CH_3	18.1, CH_3	15.0, CH_3	18.4, CH_3
22	38.3, CH_2	38.3, CH_2	39.2, CH_2	40.3, CH_2	38.3, CH_2	39.1, CH_2	75.1, CH	30.2, CH_2
23	71.1, CH	71.0, CH	69.5, CH	71.6, CH	71.1, CH	69.3, CH	31.9, CH_2	27.2, CH_2
24	90.1, CH	89.2, CH	80.4, CH	79.5, CH	89.8, CH	80.2, CH	31.5, CH_2	84.2, CH
25	30.2, CH	30.1, CH	30.2, CH	30.0, CH	30.2, CH	30.0, CH	34.2, CH	31.6, CH
26	19.9, CH_3	19.9,[e] CH_3	20.5, CH_3	20.5, CH_3	19.9, CH_3	20.5, CH_3	75.4, CH_2	18.4, CH_3
27	19.7, CH_3	19.7, CH_3	17.4, CH_3	17.2, CH_3	19.7, CH_3	17.2, CH_3	17.6, CH_3	19.4, CH_3

[a]Recorded at 188 MHz. [b]Acquired in pyridine-d_5. [c]Recorded at 225 MHz. [d]Acquired in pyridine-d_5/D_2O (~9:1). [e]Assignments are interchangeable within column.

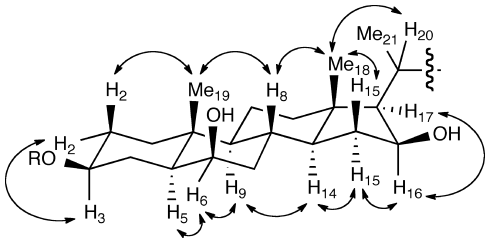

Figure 1. Key ROESY correlations of chamaeliroside C (**6**) (R = 1→6 linked diglucose moiety, 750 MHz, pyridine-d_5).

A further cross-peak between H_3-18 and H-20 suggested the α-orientation of the C-21 methyl group. Due to the flexible nature of the steroidal side chain, the absolute configuration of the vicinal C-23/C-24 diol was unable to be determined via NMR spectroscopy. Comparison of ^{1}H and ^{13}C NMR spectroscopic data for the side chain of **6** with those of **2**, a closely related saponin whose (23*R*,24*S*) stereochemistry was deduced on the basis of X-ray crystallography,[11] revealed chemical shifts for positions 23 and 24 that were in close agreement ($\Delta\delta_H \leq 0.02$ ppm; $\Delta\delta_C \leq 0.4$ ppm).[11] Compound **6** was thus assumed to possess the same steroidal skeleton, (23*R*,24*S*)-chiograsterol B (**3**), that is found in chamaelirosides A and B (**1** and **2**) previously reported from *C. luteum*.[11]

The ^{1}H NMR spectrum of **6** also contained signals typical of anomeric protons of a glycoside, at δ_H 5.04 (d, J = 7.7 Hz, H-1′ and H-1‴ isochronous) and 5.18 (d, J = 7.8 Hz, H-1″), which displayed HSQC correlations with signals at δ_C 102.1 (C-1′), 106.0 (C-1‴), and 105.4 (C-1″), respectively. The 1D TOCSY experiments indicated one β-fucopyranosyl and two β-glucopyranosyl units, and the COSY, TOCSY, HSQC, and HMBC spectra allowed the complete assignment of the sugar units of **6** (Tables 3 and 4). The absolute configurations of the sugars were determined to be D-fucose and D-glucose via enantioselective GC analysis. Correlations observed in the HMBC spectrum of **6** between δ_H 3.76 (H-24, aglycone) and δ_C 106.0 (C-1‴, 24-*O*-β-D-fucose), δ_H 4.19 (H-3, aglycone) and δ_C 102.1 (C-1′, 3-*O*-β-D-glucose), and δ_H 5.18 (H-1″, 6′-*O*-β-D-glucose) and δ_C 70.1 (C-6′, 3-*O*-β-D-glucose) established the attachment of a (1→6) linked diglucose moiety at position C-3 and a single fucose residue at C-24. The chemical shift of C-24 (δ_C 90.1) was consistent with *O*-glycosylation at this position and was in agreement with its value in **2** (δ_C 89.7, $\Delta\delta_C$ = 0.4 ppm).[11] In addition, the downfield shifts of H-4‴ (δ_H 5.56) and C-4‴ (δ_C 74.4) when compared with their values in **2** (δ_H 3.99, $\Delta\delta_H$ = 1.57 ppm; δ_C 72.8, $\Delta\delta_C$ = 1.6 ppm), along with an HMBC correlation between δ_H 5.56 and δ_C 171.1, indicated acetylation at position C-4‴. Specific acetylation of a fucose residue has been reported previously in triterpenoid saponins isolated from *Quillaja saponaria* Molina,[23] *Acanthophyllum squarrosum* Boiss.[24] and *A. glandulosum* Bunge ex Boiss.,[25] and *Bellis perennis* L.[26] The structure of **6** was therefore elucidated as (23*R*,24*S*)-24-[(4-*O*-acetyl-β-D-fucopyranosyl)oxy]-6β,16β,23-trihydroxy-5α-cholestan-3β-yl-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (chamaeliroside C).

Compound **7** displayed a $[M + Na]^+$ ion at m/z 931.4864 (by HRESIMS), giving a molecular formula of $C_{44}H_{76}O_{19}$. Fragmentation in the negative-ion ESIMS^n yielded neutral losses of 132 (m/z 775), 150 (m/z 757), and 162 Da (m/z

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Table 3. ^{1}H NMR Data for the Sugar Units of Compounds 6−9, 11−13, and 15 (750 MHz)

	6[a]	7[b]	8[a]	9[a]	11[a]	12[b]	13[b]	15[b]
	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose
1′	5.04 d (7.7)	5.06 d (7.8)	5.04 d (7.7)	4.99 d (7.7)	4.99 d (7.7)	5.00 d (7.8)	5.06 d (7.8)	5.00 d (7.7)
2′	3.99 t (8.4)	4.03 dd (9.1, 7.8)	3.99 t (8.4)	4.00 t (8.4)	4.00 t (8.3)	4.02 t (8.4)	4.08 dd (8.9, 7.8)	4.02 br t (7.7)
3′	4.22 t (8.5)	4.28 t (9.1)	4.23 t (8.4)	4.21 t (8.4)	4.21 t (9.3)	4.26 m	4.34 t (9.0)	4.29 t (9.2)
4′	4.16 t (8.8)	4.20 t (9.2)	4.17 t (8.4)	4.12 m[c]	4.12 m[c]	4.14 m[c]	4.27 t (9.0)	4.22 t (9.2)
5′	4.14 m	4.15 m	4.16 m	4.12 m[c]	4.12 m[c]	4.14 m[c]	4.01 ddd (9.0, 5.7, 2.2)	4.11 m
6a′	4.86 br d (11.7)	4.88 br d (11.4)	4.86 d (11.6)	4.86 br d (11.4)	4.86 br d (11.2)	4.87 br d (11.2)	4.57 dd (11.9, 2.1)	4.82 br d (11.4)
6b′	4.40 dd (11.5, 5.8)	4.39 dd (11.4, 5.9)	4.39 dd (11.6, 5.7)	4.37 dd (5.3, 11.4)	4.37 dd (11.2, 5.4)	4.36 dd (11.2, 5.7)	4.40 dd (11.9, 5.7)	4.37 dd (11.4, 5.1)
	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	26-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose
1″	5.18 d (7.8)	5.15 d (7.8)	5.18 d (7.8)	5.19 d (7.8)	5.19 d (7.8)	5.16 d (7.8)	4.83 d (7.8)	5.10 d (7.7)
2″	4.06 t (8.7)	4.07 dd (9.0, 7.9)	4.05 t (7.1)	4.05 t (7.0)	4.06 t (7.4)	4.07 t (8.5)	4.05 dd (8.9, 7.8)	4.06 br t (8.3)
3″	4.23 m[c]	4.26 t (9.0)	4.23 m[c]	4.23 m[c]	4.24 m[c]	4.26 t (9.0)	4.27 t (9.0)	4.25 t (9.2)
4″	4.23 m[c]	4.20 t (9.2)	4.23 m[c]	4.23 m[c]	4.24 m[c]	4.20 t (9.0)	4.22 t (9.0)	4.17 t (9.2)
5″	3.93 m	3.95 ddd (9.6, 5.5, 2.4)	3.93 m	3.93 m	3.94 m	3.95 ddd (9.0, 5.8, 2.2)	3.96 ddd (9.0, 5.7, 2.3)	3.95 m
6a″	4.51 dd (11.8, 2.4)	4.51 dd (11.9, 2.3)	4.51 ddd (11.6, 5.4, 2.2)	4.51 br d (11.4)	4.51 ddd (11.8, 5.7, 2.4)	4.51 dd (11.8, 2.1)	4.56 dd (11.9, 2.2)	4.51 br d (11.9)
6b″	4.37 dd (11.8, 5.2)	4.34 dd (11.9, 5.5)	4.37 dd (11.7, 6.0)	4.37 dd (5.8, 11.4)	4.37 dd (11.9, 5.4)	4.34 dd (11.8, 5.8)	4.37 dd (11.9, 5.7)	4.32 dd (11.9, 5.4)
	24-*O*-*β*-D-fucose	24-*O*-*α*-L-arabinose	16-*O*-*β*-D-glucose		24-*O*-*β*-D-fucose	16-*O*-*β*-D-glucose		24-*O*-*β*-D-glucose
1‴	5.04 d (7.7)	5.08 d (7.4)	4.71 d (7.7)		5.00 d (7.8)	4.72 d (7.8)		4.96 d (7.7)
2‴	4.29 br t (8.7)	4.49 dd (9.1, 7.4)	3.99 t (8.4)		4.37 br t (7.6)	4.03 t (8.2)		4.04 br t (7.8)
3‴	4.20 dd (3.2, 9.8)	4.15 dd (9.1, 3.6)	4.17 t (8.8)		4.05 dd (3.5, 9.2)	4.22 m[a]		4.28 t (9.0)
4‴	5.56 d (3.1)	4.28 m	4.24 t (8.8)		3.99 br d (3.5)	4.22 m[a]		4.18 t (9.0)
5a‴	3.86 br q (6.4)	4.31 dd (12.3, 2.5)	3.84 ddd (9.4, 5.0, 2.9)		3.77 br q (6.4)	3.89 ddd (9.1, 5.7, 2.2)		3.96 m
5b‴		3.74 dd (12.3, 1.4)						
6a‴	1.30 d (6.4)		4.43 br d (11.5)		1.53 d (6.4)	4.48 dd (11.8, 2.2)		4.52 br d (11.9)
6b‴			4.32 dd (11.5, 5.4)			4.32 dd (11.8, 5.7)		4.34 dd (11.9, 6.9)
4‴-OAc	2.00 s							

[a]Acquired in pyridine-d_5. [b]Acquired in pyridine-d_5/D_2O (~9:1). [c]Overlapping signals within column.

745) from the quasi-molecular ion at m/z 907 $[M - H]^-$, suggesting the presence of both terminal pentose and hexose residues. The further neutral loss of two 162 Da units from the $[M - 132 - H]^-$ precursor ion (m/z 775) to yield m/z 613 and 451 product ions suggested that the sugar portion of **7** was comprised of one pentose and two hexose monosaccharides. The ^{1}H NMR spectrum of **7** (in pyridine-d_5/D_2O, ~9:1) displayed two signals typical of the angular methyl group of a steroid at δ_H 1.07 (s, H_3-18) and 1.27 (s, H_3-19), along with signals for three methyl groups attached to methine carbons at δ_H 1.09 (d, J = 6.8 Hz, H_3-26/27), 1.16 (d, J = 6.6 Hz, H_3-21), and 1.29 (d, J = 6.7 Hz, H_3-26/27). These were correlated in the HSQC spectrum of **7** with signals at δ_C 13.5 (C-18), 16.0 (C-19), 19.9 (C-26/27), 20.0 (C-21), and 19.7 (C-26/27), respectively. Along with the seven double-bond equivalents required by the molecular formula for **7**, the presence of signals for three methyl groups attached to methine carbons suggested a saturated cholestane aglycone. The ^{13}C NMR spectrum of **7** displayed 44 signals including five methyl groups and 11 methylene, 26 methine, and only two quaternary carbons [δ_C 36.0 (C-10) and 42.8 (C-13)], further supporting a cholestane aglycone structure. The ^{1}H and ^{13}C NMR spectra of the aglycone of **7** (Tables 1 and 2) confirmed that it possessed the same aglycone as compounds **1**, **2**, and **6**. Comparison of the ^{1}H and ^{13}C NMR spectra of **7** with those of **2** and **6** (both bearing 24-*O*-glycosyl substituents, vide infra) revealed chemical shifts consistent with a 23*R*,24*S* configuration in the aglycone in **7**.

The ^{1}H NMR spectrum of **7** also displayed three signals typical of the anomeric proton of a glycoside. NMR experiments as described previously and enantioselective GC analysis of the isolated sugar units revealed two *β*-D-glucopyranosyl residues and one *α*-L-arabinopyranosyl residue (Tables 3 and 4). Correlations observed in the HMBC spectrum of **7** between δ_H 4.18 (H-3, aglycone) and δ_C 102.0 (C-1′, 3-*O*-*β*-D-glucose), δ_H 5.15 (H-1″, 6′-*O*-*β*-D-glucose) and δ_C 69.9 (C-6′, 3-*O*-*β*-D-glucose), and δ_H 5.08 (H-1‴, 24-*O*-*α*-L-arabinose) and δ_C 89.2 (C-24, aglycone) established the attachment of a (1→6) linked diglucose moiety at position C-3 and a single arabinose residue at position C-24 of the aglycone. The structure of **7** was therefore elucidated as (23*R*,24*S*)-24-*O*-*α*-L-arabinopyranosyloxy-6*β*,16*β*,23-trihydroxy-5*α*-cholestan-

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Table 4. ^{13}C NMR Data for the Sugar Units of Compounds 6−9, 11−13, and 15

	6[a,b]	7[c,d]	8[a,b]	9[a,b]	11[a,b]	12[a,d]	13[a,d]	15[c,d]
	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose	3-*O*-*β*-D-glucose
1′	102.1, CH	102.0, CH	102.1, CH	102.2, CH	102.2, CH	102.1, CH	102.5, CH	102.7, CH
2′	75.2, CH	74.9, CH	75.2, CH	75.2,[e] CH	75.2,[e] CH	75.0, CH	75.2, CH	74.9, CH
3′	78.5, CH	78.2, CH	78.5,[e] CH	78.6, CH	78.6, CH	78.1,[e] CH	78.4, CH	78.0, CH
4′	71.7, CH	71.4, CH	71.7;[f] CH	71.7;[f] CH	71.7;[f] CH	71.4, CH	71.6, CH	71.1, CH
5′	77.4, CH	77.1, CH	77.4, CH	77.5, CH	77.5, CH	77.2, CH	78.4, CH	76.9, CH
6′	70.1, CH_2	69.9, CH_2	70.1, CH_2	70.1, CH_2	70.1, CH_2	69.9, CH_2	62.7, CH_2	69.8, CH_2
	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose	26-*O*-*β*-D-glucose	6′-*O*-*β*-D-glucose
1″	105.4, CH	105.1, CH	105.4, CH	105.4, CH	105.4, CH	105.1, CH	104.8, CH	104.9, CH
2″	75.3, CH	75.0, CH	75.3, CH	75.3,[e] CH	75.3,[e] CH	75.0, CH	75.1, CH	74.9, CH
3″	78.5, CH	78.2, CH	78.5,[e] CH	78.6, CH	78.6, CH	78.2,[e] CH	78.4, CH	78.0, CH
4″	71.7, CH	71.5, CH	71.8,[e] CH	71.8;[f] CH	71.8;[f] CH	71.5, CH	71.6, CH	71.4, CH
5″	78.5, CH	78.3, CH	78.6,[e] CH	78.6, CH	78.6, CH	78.3, CH	78.4, CH	78.2, CH
6″	62.8, CH_2	62.5, CH_2	62.8, CH_2	62.8, CH_2	62.8, CH_2	62.5, CH_2	62.7, CH_2	62.5, CH_2
	24-*O*-*β*-D-fucose	24-*O*-*α*-L-arabinose	16-*O*-*β*-D-glucose		24-*O*-*β*-D-fucose	16-*O*-*β*-D-glucose		24-*O*-*β*-D-glucose
1‴	106.0, CH	106.0, CH	107.4, CH		106.2, CH	107.3, CH		103.4, CH
2‴	73.4, CH	73.2, CH	75.8, CH		73.4, CH	75.6, CH		75.2, CH
3‴	73.2, CH	74.6, CH	78.5,[e], CH		75.6, CH	78.1, CH		78.2, CH
4‴	74.4, CH	69.5, CH	71.8;[f] CH		72.8, CH	71.5, CH		71.7, CH
5‴	69.7, CH	67.2, CH_2	78.1, CH		71.5, CH	78.1, CH		78.0, CH
6‴	16.9, CH_3		63.1, CH_2		17.3, CH_3	62.6, CH_2		62.7, CH_2
4‴-OAc	20.9, CH_3							
4‴-OAc	171.1, C							

[a]Recorded at 188 MHz. [b]Acquired in pyridine-d_5. [c]Recorded at 225 MHz. [d]Acquired in pyridine-d_5/D_2O (~9:1). [e,f]Assignments are interchangeable within column.

3*β*-yl-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (chamaeliroside D).

Compound **8** had a molecular formula of $C_{45}H_{78}O_{20}$ (HRESIMS, calculated for $[M + Na]^+$). Analysis of the ESIMS^n fragmentation of the $[M - H]^-$ quasi-molecular ion, along with the molecular formula for **8**, suggested the presence of three hexose monosaccharides accompanied by a C_{27} skeleton bearing five oxygens. The ^{1}H NMR spectrum of **8** displayed five methyl group signals [δ_H 0.83 (s, H_3-18), 1.12 (d, J = 6.8 Hz, H_3-21), 1.12 (d, J = 6.8 Hz, H_3-26/27), 1.22 (s, H_3-19), and 1.27 (d, J = 6.7 Hz, H_3-26/27)], which were correlated in the HSQC spectrum with signals at δ_C 13.6 (C-18), 18.1 (C-21), 20.5 (C-26/27), 16.0 (C-19), and 17.4 (C-26/27), respectively. The two isochronous methyl doublet signals at δ_H 1.12 (d, J = 6.8 Hz, 6H) were each collapsed via homodecoupling experiments with irradiation of δ_H 2.39 (m, H-25) and 2.78 (m, H-20), showing that this apparent doublet comprised signals arising from both H_3-21 and H_3-26/27. Complete ^{1}H and ^{13}C NMR assignment of the steroidal skeleton of **8** revealed that this saponin possessed an aglycone with the same planar structure as that found in **1**, **2**, **6**, and **7** (Tables 1 and 2). However, the chemical shifts for the steroidal D ring and side chain of **8** were significantly different from those of **1**, **2**, **6**, and **7**. Assignment of configuration at C-23 and C-24 was made difficult since the ^{1}H and ^{13}C NMR data for the side chain (C-20 to C-27) of **8** matched neither those of **1**, which possesses a free OH group at C-24, nor those of **2**, **6**, and **7**, which all possess *O*-glycosyl substitution at C-24.[11] To determine the absolute configuration of C-23 and C-24 in **8**, the pure saponin was subjected to acid-catalyzed hydrolysis to yield an aglycone with spectroscopic (^{1}H and ^{13}C NMR) properties identical with those of (23*R*,24*S*)-chiograsterol B (**3**).[11]

The ^{1}H and ^{13}C NMR spectra of **8** and enantioselective GC analysis of the hydrolysate as before revealed that three *β*-D-glucose units were present. Correlations observed in the HMBC spectrum of **8** between δ_H 5.04 (H-1′, 3-*O*-*β*-D-glucose) and δ_C 78.0 (C-3, aglycone), δ_H 5.18 (H-1″, 6′-*O*-*β*-D-glucose) and δ_C 70.1 (C-6′, 3-*O*-*β*-D-glucose), and δ_H 4.71 (H-1‴, 16-*O*-*β*-D-glucose) and δ_C 83.1 (C-16, aglycone) revealed the same (1→6) linked diglucose moiety attached at C-3 that is present in **1**, **2**, **6**, and **7**, along with a single glucose residue linked at position C-16. The chemical shift of C-24 (δ_C 80.4) when compared with its chemical shift in **2** (δ_C 89.7),[11] **6** (δ_C 90.1), and **7** (δ_C 89.2) was consistent with a free OH at C-24. Thus, compound **8** was determined to be (23*R*,24*S*)-16-*O*-*β*-D-glucopyranosyloxy-6*β*,23,24-trihydroxy-5*α*-cholestan-3*β*-yl-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (chamaeliroside E).

Compound **9** had a molecular formula of $C_{39}H_{66}O_{15}$, and ESIMS^n fragmentation of the $[M + Na]^+$ quasi-molecular ion at m/z 797 showed two successive neutral losses of 162 Da, suggesting the presence of two hexose units. The molecular formula indicated seven double-bond equivalents, which suggested either a cholesterol-derived or pentacyclic furostanol skeleton. The ^{1}H NMR spectrum of **9** displayed two signals for methyl groups attached to quaternary carbons [δ_H 0.65 (s, H_3-19) and 0.97 (s, H_3-18)] and three signals for methyl groups attached to methine carbons [δ_H 1.15 (d, J = 6.8 Hz, H_3-26/27), 1.17 (d, J = 6.4 Hz, H_3-21), and 1.27 (d, J = 6.6 Hz, H_3-26/27)], further supporting an open-chain steroidal aglycone for **9**. The methyl signals showed HSQC correlations with signals at δ_C 13.1 (C-19), 13.4 (C-18), 20.5 (2C, C-21 and C-26/27), and 17.2 (C-26/27), respectively. The ^{13}C NMR spectrum of **9** also displayed signals for 10 methylene, 21 methine, and three quaternary carbons [δ_C 40.9 (C-10), 43.0 (C-13), and 209.9

(C-6)]. The complete ^{1}H and ^{13}C NMR assignments of the aglycone of **9** (Tables 1 and 2) indicated the same planar structure as chiograsterol A, a steroidal aglycone reported once before from *Chionographis japonica*.[12] The two sugars were determined to both be *β*-D-glucopyranose units in **9**, using procedures described above, and the NMR spectra revealed the same attachment of a (1→6) linked diglucose moiety as seen in **1**, **2**, and **6**−**8** (Tables 3 and 4).

Owing to the flexible nature of the steroidal side chain (C-20 to C-27) in **9**, the absolute configuration of the vicinal C-23/C-24 diol was unable to be determined unambiguously via NMR spectroscopy. The ^{1}H and ^{13}C NMR chemical shifts of positions 20 to 27 closely matched those of chamaeliroside A (**1**) ($\Delta\delta_H \leq 0.02$ ppm; $\Delta\delta_C \leq 0.2$ ppm),[11] a saponin that differs from **9** only in possessing a *β*-OH group at C-6 rather than a ketone moiety. We thus presumed that **9** had the same 23*R*,24*S* absolute configuration. In order to confirm this, aglycone **10** was isolated from the acid hydrolysate of a crude *C. luteum* extract via semipreparative RPHPLC. Reduction of ketone **10** with sodium borohydride yielded a pentahydroxy sterol with spectroscopic properties identical with those of (23*R*,24*S*)-chiograsterol B, with hydride delivery from the least hindered *α*-face of the steroid yielding the axially orientated 6*β*-OH group as expected.[11] The melting point and optical rotation of **10** (mp 124−126 °C; $[\alpha]^{23}_D$ −7.2, *c* 0.12, MeOH) were in good agreement with the limited physical properties reported in the literature for chiograsterol A (mp 125.5−128/206−210.5 °C; $[\alpha]^{24}_D$ −6.8 ± 6, *c* 0.311, MeOH),[12,27] strongly suggesting that the chiograsterol A aglycone of the original literature report also possesses a 23*R*,24*S* configuration. This also lends strong support to our previous, tentative assignment of 23*R*,24*S* to chiograsterol B of the original report.[11,12] Compound **9** was therefore determined to be (23*R*,24*S*)-16*β*,23,24-trihydroxy-6-oxo-5*α*-cholestan-3*β*-yl-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (6-dehydrochamaeliroside A).

Compound **11** had the molecular formula $C_{45}H_{76}O_{19}$ and underwent fragmentation in negative-ion ESIMS^n to give product ions corresponding to the presence of both terminal deoxyhexose and hexose residues attached to a steroidal skeleton. The ^{1}H and ^{13}C NMR spectra of **11** were similar to those of **9**, and assignment of the aglycone signals of **11** via examination of COSY, TOCSY, HSQC, and HMBC spectra (Tables 1 and 2) revealed that this saponin also contained the (23*R*,24*S*)-chiograsterol A (**10**) aglycone. Analysis of the sugars as described before revealed two units of *β*-D-glucose and one unit of *β*-D-fucose (Tables 3 and 4). Correlations observed in the HMBC spectrum of **11** revealed these to be configured in the same linkage pattern as that found in **2** and **6**, comprising a (1→6) linked diglucose moiety linked at C-3 of the aglycone and a single fucose residue at C-24. Compound **11** was therefore elucidated as (23*R*,24*S*)-24-*O*-*β*-D-fucopyranosyloxy-16*β*,23-dihydroxy-6-oxo-5*α*-cholestan-3*β*-yl-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (6-dehydrochamaeliroside B).

Compound **12** had the molecular formula $C_{45}H_{76}O_{20}$, and fragmentation in negative-ion ESIMS^n indicated the presence of one deoxyhexose and two hexose units. The ^{1}H and ^{13}C NMR spectra of **12** were similar to those of **9**, with the exception that the chemical shift of C-16 (δ_C 82.8) was significantly downfield compared with C-16 (δ_C 71.6) in **9** ($\Delta\delta_C$ = +11.2 ppm), typical of *O*-glycosylation. Three anomeric proton signals were evident in the ^{1}H NMR spectrum of **12** [δ_H 4.72 (d, *J* = 7.8 Hz, H-1‴), 5.00 (d, *J* = 7.8 Hz, H-1′), and 5.16 ppm (d, *J* = 7.8 Hz, H-1″)]. ^{1}H and ^{13}C NMR assignments of the sugar units of **12** (Tables 3 and 4) and enantioselective GC analysis of its hydrolysate revealed that three isolated *β*-D-glucose moieties were present. Correlations observed in the HMBC spectrum between δ_H 5.00 (H-1′, 3-*O*-*β*-D-glucose) and δ_C 77.0 (C-3, aglycone), δ_H 5.16 (H-1″, 6′-*O*-*β*-D-glucose) and δ_C 69.9 (C-6′, 3-*O*-*β*-D-glucose), and δ_H 4.72 (H-1‴, 16-*O*-*β*-D-glucose) and δ_C 82.8 (C-16, aglycone) confirmed that **12** possessed a structure analogous to that of **8**. The structure of **12** was therefore determined to be (23*R*,24*S*)-16-*O*-*β*-D-glucopyranosyloxy-23,24-dihydroxy-6-oxo-5*α*-cholestan-3*β*-yl-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (6-dehydrochamaeliroside E).

Positive-ion HRESIMS of **13** provided a molecular formula of $C_{39}H_{66}O_{14}$. Fragmentation of the quasi-molecular precursor ion $[M - H]^-$ at *m*/*z* 757 in negative-ion ESIMS^n gave product ions of *m*/*z* 595 ($[M - 162 - H]^-$) and *m*/*z* 433 ($[M - 162 - 162 - H]^-$), indicating the presence of two hexose moieties. The ^{1}H NMR spectrum of **13** displayed signals for two methyl groups attached to quaternary carbons, at δ_H 0.95 (s, H_3-19) and 1.16 (s, H_3-18), and two signals for methyl groups attached to methine carbons, at δ_H 1.02 (d, *J* = 6.7 Hz, H_3-27) and 1.21 (d, *J* = 7.0 Hz, H_3-21). These displayed HSQC correlations with signals at δ_C 19.4 (C-19), 13.5 (C-18), 17.6 (C-27), and 15.0 (C-21), respectively. The HMBC correlations of δ_H 0.95 (H_3-19) revealed a chemical shift for C-5 (δ_C 141.0) that was typical of $\Delta^{5(6)}$-unsaturation; along with the double-bond equivalents required by the molecular formula for **13**, this indicated a cholestene-type aglycone. HMBC correlations between δ_H 1.02 (H_3-27) and δ_C 75.4 (C-26) and between δ_H 1.21 (H_3-21) and δ_C 75.1 (C-22) clearly indicated oxygenation of positions C-22 and C-26 in the steroidal side chain. Examination of TOCSY, COSY, HSQC, and HMBC spectra allowed identification of the aglycone as 3,16,22,26-tetrahydroxycholest-5-ene, previously reported in saponins isolated from *Solanum lyratum* Thunb. and *S. anguivi* Lam.,[28,29] *Allium tubersosum* Rottler ex Spreng.,[30] and *Trillium erectum* L.[19] The stereochemistry of the ring junctions and substituents of the aglycone of **13** were determined via cross-peaks observed in a 2D ROESY spectrum. The *β*-orientation of the substituents at positions C-3, C-16, and C-17 and the *trans* fusion of the steroid B/C and C/D ring junctions were established via cross-peaks observed between H_3-19/H-2*β*/H-4*β*, H-3/H-2*α*/H-4*α*, H_3-18/H-8/H-15*β*, H-15*α*/H-7*α*/H-14/H-16, H-16/H-17, and H-7*α*/H-9. The flexibility of the steroidal side chain **13** meant that the absolute configurations of C-22 and C-25 could not be unambiguously determined using NMR spectroscopy. The chemical shifts for positions 20 to 27 in **13** were in good agreement with those of the revised structure of bethoside B isolated from *T. erectum* ($\Delta\delta_H \leq 0.04$; $\Delta\delta_C \leq 0.3$)[19,31] as well the closely related and co-occurring heloside A (**4**) ($\Delta\delta_H \leq 0.06$; $\Delta\delta_C \leq 0.1$),[13] both of which possess a 22*S*,25*R* configuration. The fact that **13** co-occurs in *C. luteum* with the closely related helosides A and B lends support to the 22*S*,25*R* assignment, since these three saponins presumably share a common biosynthetic origin. Thus, the aglycone of this saponin was identified as **14**, the same one that is present in bethoside B.[19,31]

The sugar units of **13** were identified as two *β*-D-glucopyranosyl residues via examination of 1D TOCSY spectra and enantioselective GC analysis (Tables 3 and 4). Correlations in the HMBC spectrum of **13** between δ_H 3.95 (H-3, aglycone) and δ_C 102.5 (C-1′, 3-*O*-*β*-D-glucose), and δ_H 4.83 (H-1″, 26-*O*-*β*-D-glucose) and δ_C 75.4 (C-26, aglycone), established the

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attachment of a single glucose residue at both positions C-3 and C-26. The structure of **13** was therefore established as (22*S*,25*R*)-26-*O*-*β*-D-glucopyranosyloxy-16*β*,22-dihydroxycholest-5-en-3*β*-yl-*O*-*β*-D-glucopyranoside (11-deoxyheloside A).

Compound **15** had the molecular formula $C_{45}H_{76}O_{18}$ (calculated for $[M + Na]^+$: 927.4924), and negative-ion ESIMS^n fragmentation of **15** suggested the presence of at least two hexose moieties. The molecular formula suggested the presence of an additional C_6 sugar along with a C_{27} aglycone. The ^{1}H NMR spectrum of **15** displayed two signals typical of the angular methyl group of a steroid at δ_H 0.95 (s, H_3-19) and 1.09 (s, H_3-18), which were correlated in the HSQC spectrum of **15** with signals at δ_C 19.4 (C-19) and 13.3 (C-18), respectively. The ^{1}H NMR spectrum also contained three signals that corresponded to methyl groups attached to methine carbons. The presence of three secondary methyl groups along with the eight double-bond equivalents given by the molecular formula was consistent with a cholestene-type aglycone for **15**. Correlations observed in the HMBC spectrum between δ_H 1.02 and 1.05 (H_3-26/27) and δ_C 84.2 (C-24) indicated oxygenation at C-24. COSY and HMBC correlations of δ_H 3.81 (H-24) revealed the presence of a C-23 methylene carbon, in contrast to chamaeliroside-derived saponins **1**, **2**, **6**–**9**, **11**, and **12**, which all contain a C-23/C-24 vicinal diol. The complete ^{1}H and ^{13}C NMR assignments of the aglycone of **15** (Tables 1 and 2) revealed it to have the previously unreported 3,16,24-trihydroxycholest-5-ene skeleton. Correlations observed in the ROESY spectrum of **15** between H_3-19/H-4*β*, H-3/H-4*α*, H_3-18/H-15*β*, H-15*α*/H-16, and H-16/H-14/H-17 established the *β*-orientation of the substituents at positions C-3 and C-16. Signal overlap in the 2D ROESY spectrum prevented unambiguous determination of the stereochemistry of the steroid B/C and C/D ring junctions, but they are tentatively assigned as *trans* on the basis of the agreement of the ^{13}C NMR data for the B, C, and D rings of **15** with those of **13**. The flexible nature of the steroidal side chain meant that ROESY correlations could not be used to determine the absolute configuration of C-24. The small quantity of **15** isolated also precluded determination of stereochemistry via X-ray crystallography or Mosher's analysis; the absolute configuration of C-24 remains undefined.

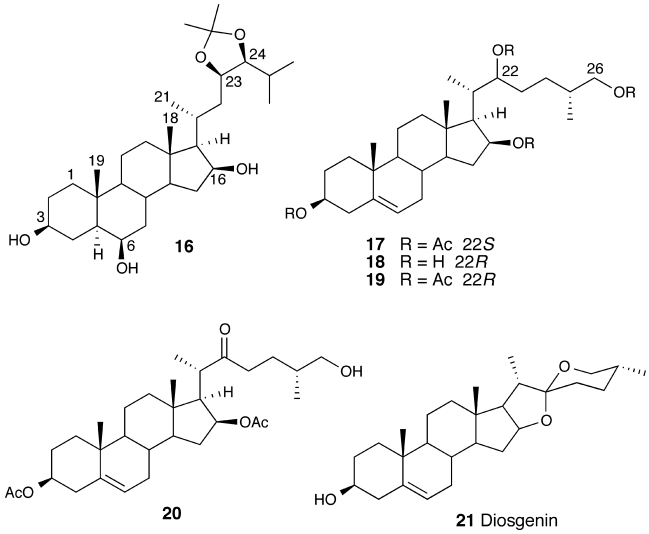
Three anomeric proton signals were also present in the ^{1}H NMR spectrum of **15**, and these displayed HSQC correlations with signals at δ_C 103.4 (C-1‴), 102.7 (C-1′), and 104.9 (C-1″). Correlations between δ_H 5.00 (H-1′, 3-*O*-*β*-D-glucose) and δ_C 78.8 (C-3, aglycone), δ_H 5.10 (H-1″, 6′-*O*-*β*-D-glucose) and δ_C 69.8 (C-6′, 3-*O*-*β*-D-glucose), and δ_H 4.96 (H-1‴, 24-*O*-*β*-D-glucose) and δ_C 84.2 (C-24, aglycone) in the HMBC spectrum of **15** revealed the presence of the same (1→6) linked diglucose moiety as seen in **1**, **2**, **6**–**9**, **11**, and **12** and a single glucose residue attached at C-24 (Tables 3 and 4). The structure of **15** was therefore determined as 24-*O*-*β*-D-glucopyranosyloxy-16*β*-hydroxycholest-5-en-3*β*-yl-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

The isolation of a structurally diverse range of saponins provides a means to investigate structure–activity relationships of the bioactive components of this medicinal herb. The isolated saponins and aglycones were screened for antiproliferative activity against human cancer cell lines, as this is a common bioactivity of many steroidal saponins.[14] The prolonged treatment time of sparsely seeded cultures allowed for comparison of clonogenic survival of treated cells. The analyzed compounds included five obtained in previous work (**1**–**5**),[11,13] six isolated in the present study (**6** and **8**–**12**), five derivatives generated previously (**14** and **16**–**20**), and diosgenin (**21**).[11,13,31] Compounds **3**, **10**, **16**, **17**, **19**, and **20** showed cytotoxic activity against all the tested cell lines (Table 5), while compounds **1**, **2**, **4**–**6**, **8**, **9**, **11**, **12**, **14**, **18**, and **21** were all inactive (IC_{50} > 50 μg/mL).

Table 5. IC_{50} of Compounds 3, 10, 16, 17, 19, and 20 against Normal Fibroblasts (NFF) and Cancer Cell Lines (μg/mL)[a]

	cell line					
compound	NFF	HeLa	HT29	MCF7	MM96L	K562
3	22	12	11	13	8.5	7
10	17	15	13	15	4.5	5
16	10	4.3	3.5	5	5	8.5
17	12	16	4.5	11	3.5	4.5
19	13	13	6.5	7.5	4.5	8.5
20	2	2.8	0.8	0.8	1	1.7

[a]The positive control, cisplatin, had an IC_{50} of 0.1–0.3 μg/mL in these cell lines.



None of the eight open-chain glycosides tested displayed antiproliferative activity, despite representing a range of structural types including monodesmosides (**1** and **9**), bisdesmosides (**2**, **4**, **6**, **8**, **11**, and **12**), 5*α*-cholestanes (**1**, **2**, **6**, **8**, **9**, **11**, and **12**), and $\Delta^{5(6)}$-unsaturated cholestenes (**4**). The open-chain aglycone helogenin (**5**) and the closely related aglycone **14** and its 22*R* epimer **18** were also inactive against the cell lines tested. In contrast, the presence of the C-23/C-24 vicinal diol and 6-oxo functionality in aglycones **3** and **10** appeared to increase antiproliferative activity, compared with the $\Delta^{5(6)}$-unsaturation and C-22/C-26 diol found in **5**, **14**, and **18**. Decreased polarity of the steroidal aglycone also appeared to increase antiproliferative activity; the per-acetylated derivatives of **14** and **18** (**17** and **19**, respectively) showed significantly increased activity compared with the free aglycones, while the acetonide derivative **16** of (23*R*,24*S*)-chiograsterol B (**3**) also displayed enhanced activity. The antiproliferative activity of the steroidal aglycones was not solely dependent on polarity however, since diacetate **20**, derived from the common steroidal aglycone diosgenin (**21**), displayed antiproliferative activity, while the less polar diosgenin did not.

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Phytochemical investigation of the widely used medicinal herb *C. luteum* resulted in the isolation of 15 steroidal saponins, eight of which are new compounds. Three of these possess the unusual (23*R*,24*S*)-chiograsterol A aglycone (**10**), which is here fully characterized spectroscopically for the first time. Compound **15** also possesses a new aglycone, 3β,16β,24-trihydroxycholest-5-ene. Unusual for a terrestrial plant, the major saponins of *C. luteum* are all open-chain steroidal glycosides. The isolated saponins fall into a number of structural subfamilies that are related by their parent aglycone: those derived from (23*R*,24*S*)-chiograsterol B (**1**, **2**, and **6**−**8**), (23*R*,24*S*)-chiograsterol A (**9**, **11**, and **12**), helogenin (**4**, heloside B, and **13**), 3β,16β,24-trihydroxycholest-5-ene (**15**), and pennogenin. All of the eight open-chain saponins (**1**, **2**, **4**, **6**, **8**, **9**, **11**, and **12**) and three of the aglycones (**5**, **14**, and **18**) tested did not inhibit cell proliferation in any of the cell lines tested. However, several of the aglycones and their derivatives (**3**, **10**, **16**, **17**, **19**, and **20**) displayed significant activity in these assays. The biogenesis of these compounds awaits experimental verification.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV500, AV750, or AV900 spectrometer with the pyridine-d_5 signal (δ_H 8.71 ppm, δ_C 149.9 ppm) or TMS (in pyridine-d_5/D_2O, ~9:1) as internal standard. *J* values are expressed in Hz. Low-resolution and tandem mass spectra were recorded on a Bruker ESQUIRE HCT instrument (positive- and negative-ion ESI). High-resolution mass spectra were acquired on a Bruker MicrOTOF-Q instrument (positive-ion ESI) with internal calibration using Agilent Tune-Mix. RPHPLC was performed on a Shimadzu LC-20AT liquid chromatograph equipped with an ELSD-LT detector (52 °C, N_2 pressure: 200 KPa), column oven (40 °C), and either a semi-preparative (Luna C-18, 5 μm, 250 × 10 mm) or analytical (Luna C-18, 5 μm, 250 × 4.60 mm) Phenomenex HPLC column. Melting points were performed on a melting-point apparatus (Dr Tottoli) and are uncorrected.

Plant Material. *C. luteum* was sourced from Botanical Liaisons Ltd. (USA) and Blessed Herbs Ltd. (USA). A specimen from Blessed Herbs Ltd. was deposited (accession PHARM-06275) at the Medicinal Plant Herbarium, Southern Cross University, Lismore, Australia, and verified by Dr. Hans Wohlmuth.

Extraction and Isolation. Powdered roots of *C. luteum* (80 g) were extracted (80% MeOH(aq), 800 mL) with sonication (3 × 10 min). Following filtration and removal of the solvent in vacuo, the crude extract was partially purified by solid-phase extraction (Phenomenex Strata C-18E cartridge) eluting with H_2O followed by 20% MeOH(aq), then 100% MeOH. The 20% MeOH(aq) fraction was dried in vacuo, dissolved (90% MeOH(aq), 15 mL), filtered, and purified by semipreparative RPHPLC (gradient of 6% to 40% CH_3CN(aq) over 50 min, 2 mL/min). Two pure fractions were collected: heloside A (**4**)[13] (21.9 mg) and heloside B[13] (2.4 mg). The 100% MeOH fraction was dried in vacuo, dissolved (90% MeOH(aq), 30 mL), filtered, and purified by semipreparative RPHPLC (gradient of 20% to 53% CH_3CN(aq) over 65 min, increasing to 100% CH_3CN over 2 min, and held 10 min, 2 mL/min). Eleven different fractions were collected, numbered I−XI in order of elution. Fractions I, II, and IV−XI were pure and corresponded to **12** (5.3 mg), **8** (14.9 mg), chamaeliroside A (**1**)[11] (289 mg), **11** (15.2 mg), (25*S*)-27-hydroxypennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside[20,21] (40.8 mg), chamaeliroside B (**2**)[11] (66 mg), **15** (1.7 mg), **6** (6.3 mg), pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→4)-[*O*-α-L-rhamnopyranosyl-(1→2)]-*O*-β-D-glucopyanoside[21,22] (6.2 mg), and pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside[21,22] (8.0 mg), respectively. Fraction III was subjected to further fractionation by RPHPLC (isocratic conditions of 32% CH_3CN(aq)) to give subfraction IIIa and a second, pure fraction corresponding to compound **9** (15.0 mg). Subfraction IIIa was fractionated (isocratic conditions of 28% CH_3CN(aq)) to give pure compounds **13** (2.3 mg) and **7** (1.8 mg).

*Chamaeliroside C (**6**):* amorphous solid; $[\alpha]^{28}_D$ −22.3 (*c* 0.34, MeOH); ^{1}H (pyridine-d_5, 750 MHz) and ^{13}C NMR (pyridine-d_5, 188 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 987, 783; negative-ion ESIMS *m*/*z* 963, 921, 903, 775, 759, 613, 595; HRESIMS *m*/*z* 987.5120 $[M + Na]^+$ (calcd for $C_{47}H_{80}NaO_{20}$, 987.5135).

*Chamaeliroside D (**7**):* amorphous solid; $[\alpha]^{25}_D$ −13.6 (*c* 0.18, MeOH); ^{1}H (pyridine-d_5/D_2O ~9:1, 750 MHz) and ^{13}C NMR (pyridine-d_5/D_2O ~9:1, 225 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 931, 799, 781, 637; negative-ion ESIMS *m*/*z* 907, 775, 757, 745, 613, 595, 451; HRESIMS *m*/*z* 931.4864 $[M + Na]^+$ (calcd for $C_{44}H_{76}NaO_{19}$, 931.4873).

*Chamaeliroside E (**8**):* off-white solid; mp 186−188 °C (dec); $[\alpha]^{25}_D$ −18.4 (*c* 0.30, MeOH); ^{1}H (pyridine-d_5, 750 MHz) and ^{13}C NMR (pyridine-d_5, 188 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 961, 781, 619; negative-ion ESIMS *m*/*z* 937, 775, 759, 613, 451; HRESIMS *m*/*z* 961.5003 $[M + Na]^+$ (calcd for $C_{45}H_{78}NaO_{20}$, 961.4979).

*6-Dehydrochamaeliroside A (**9**):* Off-white solid; mp 180−181 °C (dec); $[\alpha]^{28}_D$ −33.0 (*c* 0.32, MeOH); ^{1}H (pyridine-d_5, 750 MHz) and ^{13}C NMR (pyridine-d_5, 188 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 797, 635, 473; negative-ion ESIMS *m*/*z* 773, 611, 449; HRESIMS *m*/*z* 797.4291 $[M + Na]^+$ (calcd for $C_{39}H_{66}NaO_{15}$, 797.4294).

*6-Dehydrochamaeliroside B (**11**):* white solid; mp 194−196 °C (dec); $[\alpha]^{26}_D$ −17.2 (*c* 0.18, MeOH); ^{1}H (pyridine-d_5, 750 MHz) and ^{13}C NMR (pyridine-d_5, 188 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 943; negative-ion ESIMS *m*/*z* 919, 773, 757, 593, 577; HRESIMS *m*/*z* 943.4854 $[M + Na]^+$ (calcd for $C_{45}H_{76}NaO_{19}$, 943.4873).

*6-Dehydrochamaeliroside E (**12**):* amorphous solid; $[\alpha]^{25}_D$ −37.4 (*c* 0.27, MeOH); ^{1}H (pyridine-d_5/D_2O ~9:1, 750 MHz) and ^{13}C NMR (pyridine-d_5/D_2O ~9:1, 188 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 959, 797, 779, 617; negative-ion ESIMS *m*/*z* 935, 773, 755, 611, 593; HRESIMS *m*/*z* 935.4825 $[M - H]^-$ (calcd for $C_{45}H_{75}O_{20}$, 935.4857).

*11-Deoxyheloside A (**13**):* amorphous solid; $[\alpha]^{25}_D$ −28.1 (*c* 0.23, MeOH); ^{1}H (pyridine-d_5/D_2O ~9:1, 750 MHz) and ^{13}C NMR (pyridine-d_5/D_2O ~9:1, 188 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 781, 619, 601; negative-ion ESIMS *m*/*z* 757, 595, 579, 433, 417; HRESIMS *m*/*z* 781.4333 $[M + Na]^+$ (calcd for $C_{39}H_{66}NaO_{14}$, 781.4345).

*24-O-β-D-Glucopyranosyloxy-16β-hydroxycholest-5-en-3β-yl-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**15**):* amorphous solid; $[\alpha]^{28}_D$ −17.6 (*c* 0.17, MeOH); ^{1}H (pyridine-d_5/D_2O ~9:1, 750 MHz) and ^{13}C NMR (pyridine-d_5/D_2O ~9:1, 225 MHz), see Tables 1−4; positive-ion ESIMS *m*/*z* 927, 765, 747; negative-ion ESIMS *m*/*z* 903, 741, 577; HRESIMS *m*/*z* 927.4903 $[M + Na]^+$ (calcd for $C_{45}H_{76}NaO_{18}$, 927.4924).

To obtain the aglycone **10**, crude extract prepared as above (from 20 g of plant material) was dissolved in EtOH (60 mL) and HCl(aq) (32%, 8 mL) and heated under reflux for 2.5 h. After cooling, water (60 mL) was added and the reaction was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with NaOH(aq) (5% w/v, 3 × 100 mL) and dried ($MgSO_4$) before removal of the solvent in vacuo. The crude residue was dissolved (90% MeOH(aq), 20 mL), filtered, and purified by semipreparative RPHPLC (gradient of 30% to 70% CH_3CN(aq) over 40 min, 2 mL/min). Compound **10** was collected as a pure fraction (11.2 mg).

*(23R,24S)-Chiograsterol A (**10**):* white solid; mp 124−126 °C; $[\alpha]^{23}_D$ −7.2 (*c* 0.12, MeOH); ^{1}H NMR (pyridine-d_5, 500 MHz) δ_H 6.47 (br s, 1H), 6.24 (br s, 1H), 5.85 (br s, 1H), 5.71 (br s, 1H), 4.59 (td, *J* = 7.3, 4.6 Hz, 1H, H-16), 4.09 (dd, *J* = 9.4, 7.1 Hz, 1H, H-23), 3.84 (m, 1H, H-3), 3.68 (m, 1H, H-24), 2.54 (m, 1H, H-20), 2.41 (dd, *J* = 13.1, 4.6 Hz, 1H, H-7a), 2.37 (m, 1H, H-25), 2.28−2.31 (m, 2H), 2.15 (dt, *J* = 12.8, 7.5 Hz, 1H, H-15a), 2.10 (dd, *J* = 14.3, 10.7 Hz, 1H, H-22a), 1.67−2.04 (m, 6H), 2.03 (t, *J* = 12.9 Hz, 1H, H-7b), 1.98 (m,

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1H, H-12a), 1.52 (m, 1H, H-11a), 1.39 (td, J = 13.2, 4.7 Hz, 1H, H-15b), 1.29 (m, 1H, H-11b), 1.27 (d, J = 6.7 Hz, 3H, H_3-26/27), 1.14−1.19 (m, 4H), 1.18 (d, J = 6.6 Hz, 3H, H_3-21), 1.15 (d, J = 6.9 Hz, 3H, H_3-26/27), 1.05 (m, 1H, H-14), 1.00 (s, 3H, H_3-18), 0.77 (s, 3H, H_3-19); ^{13}C NMR (pyridine-d_5, 125 MHz) δ_C 210.3 (C-6), 79.6 (C-24), 71.6 (2C, C-16 and C-23), 70.1 (C-3), 62.5 (C-17), 57.0 (C-5), 54.6 (C-14), 53.9 (C-9), 46.9 (C-7), 43.1 (C-13), 41.0 (C-10), 40.3 (C-22), 39.9 (C-12), 37.7 (C-8), 37.0 (C-1), 36.1 (C-15), 31.9 (C-2), 31.3 (C-4), 30.0 (C-25), 27.6 (C-20), 21.5 (C-11), 20.5 (2C, C-21 and C-26/27), 17.3 (C-26/27), 13.4 (C-18), 13.3 (C-19); positive-ion ESIMS m/z 489, 473; negative-ion ESIMS m/z 449; HRESIMS m/z 473.3247 $[M + Na]^+$ (calcd for $C_{27}H_{46}NaO_5$, 473.3237).

Reduction of 10 to (23*R*,24*S*)-Chiograsterol B (3). Compound **10** (~4 mg) was dissolved in MeOH (2 mL), and $NaBH_4$ (2 mg) was added. The mixture was stirred at room temperature for 16 h, before addition of 1 N HCl (2 mL) and extraction with EtOAc (3 × 2 mL). The EtOAc layer was concentrated in vacuo, and the resulting amorphous solid was dissolved in 90% MeOH(aq) (1 mL) and purified via RPHPLC using an analytical column (gradient of 30% to 70% CH_3CN(aq) over 40 min, held at 40% CH_3CN(aq) for 5 min) to give (23*R*,24*S*)-chiograsterol B (**3**), which possessed spectroscopic properties (^{1}H and ^{13}C NMR) identical with authentic material.[11]

General Methods for Determination of Sugar Absolute Configuration. The saponin (1 mg) was subjected to acid-catalyzed methanolysis, before per-trifluoroacetylation of the resultant methyl-glycosides and enantioselective GC analysis (Chirasil-L-Val capillary column) according to procedures used previously.[19] The retention times (min) of the TFAA-derivatized standards were as follows: D-glucose (26.09 and 29.87), L-glucose (26.06 and 29.77), D-fucose (19.82 and 24.06), L-fucose (20.04 and 24.43), D-arabinose (20.52 and 23.59), and L-arabinose (20.47 and 23.40). Co-injection of the different saponin hydrolysates with the TFAA-derivatized standards of authentic D-glucose, D-fucose, and L-arabinose gave coeluting peaks, consistent with the sugars identified. The multiple peaks observed correspond to formation of α- and/or β-anomers of the pyranose and/or furanose forms and are consistent with those previously reported for these sugars.[32]

Inhibition of Human Cell Proliferation Assay. Cells seeded at 3000−5000 cells/well in a 96-well plate were treated with compounds diluted from 10 mg/mL stock solutions in DMSO, giving a highest final concentration of DMSO (at 50 μg/mL of compound) of 0.5%. After incubation for 6 days, the plates were fixed in ethanol and cell content was compared with untreated controls by staining with sulforhodamine and quantitation at 540 nm in an ELISA reader. The IC_{50} values (dose at which cell growth was inhibited by 50%) were determined by interpolation on plots of percent control absorbance versus dose. For the nonadherent K562 cell line, MTS was added directly to the cultures and cell numbers were compared with the control after 2−4 h at 37 °C, from the absorbance at 490 nm. Compounds **10** and **17** caused cell lysis, as judged by the appearance of greatly enlarged cytoplasm. Cancer cell lines were cervical carcinoma (HeLa), colon tumor (HT29), breast tumor (MCF7), melanoma (MM96L), and leukemia (K562). The anticancer drug cisplatin, used as a positive control, had an IC_{50} of 0.1−0.3 μg/mL in the above cell lines.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **6**−**13** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Chevallier, A. *The Encyclopedia of Medincal Plants*; Dorling Kindersley: London, 1996.
(2) Burkhart, E. P.; Jacobson, M. G. *Agrofor. Syst.* **2009**, *76*, 437−453.
(3) United States Department of Agriculture Natural Resources Conservation Service, *The PLANTS Database*. http://plants.usda.gov (December 2, 2011).
(4) McKenzie, M.; Kirakosyan, A.; Kaufman, P. B. Risks Associated with Overcollection of Medicinal Plants in Natural Habitats. In *Recent Advances in Plant Biotechnology*; Kirakosyan, A.; Kaufman, P. B., Eds.; Springer: Berlin, 2009; Vol. 4, pp 363−387.
(5) Newton, K. M.; Reed, S. D.; Grothaus, L.; Ehrlich, K.; Guiltinan, J.; Ludman, E.; LaCroix, A. Z. *Maturitas* **2005**, *52*, 134−146.
(6) Ng, S. S. W.; Figg, W. D. *Anticancer Res.* **2003**, *23*, 3585−90.
(7) Greene, F. V. *Am. J. Pharm.* **1878**, *50*, 250−253.
(8) Greene, F. V. *Am. J. Pharm.* **1878**, *50*, 465−468.
(9) Cataline, E. L.; Francke, D. E. *J. Am. Pharm. Assoc. (1912−1977)* **1942**, *31*, 519−21.
(10) Marker, R. E.; Wagner, R. B.; Ulshafer, P. R. *J. Am. Chem. Soc.* **1942**, *64*, 1283−1285.
(11) Matovic, N. J.; Stuthe, J. M. U.; Challinor, V. L.; Bernhardt, P. V.; Lehmann, R. P.; Kitching, W.; De Voss, J. J. *Chem.—Eur. J.* **2011**, *17*, 7578−7591.
(12) Takeda, K.; Minato, H.; Shimaoka, A. *Chem. Commun. (London)* **1968**, 104−106.
(13) Challinor, V. L.; Stuthe, J. M. U.; Bernhardt, P. V.; Lehmann, R. P.; Kitching, W.; De Voss, J. J. *J. Nat. Prod.* **2011**, *74*, 1557−1560.
(14) Sparg, S. G.; Light, M. E.; van Staden, J. *J. Ethnopharmacol.* **2004**, *94*, 219−243.
(15) Minale, L.; Riccio, R.; Zollo, F. *Prog. Chem. Org. Nat. Prod.* **1993**, *62*, 75−308.
(16) Stonik, V. A. *Russ. Chem. Rev.* **2001**, *70*, 673−715.
(17) Stonik, V. A.; Ivanchina, N. V.; Kicha, A. A. *Nat. Prod. Commun.* **2008**, *3*, 1587−1610.
(18) Ivanchina, N. V.; Kicha, A. A.; Stonik, V. A. *Steroids* **2011**, *76*, 425−454.
(19) Hayes, P. Y.; Lehmann, R.; Penman, K.; Kitching, W.; De Voss, J. J. *Phytochemistry* **2009**, *70*, 105−113.
(20) Yokosuka, A., Mimaki, Y., Sashida, Y. In *The 51st Annual Meeting of the Japanese Society of Pharmacognosy*; Kobe, Japan, 2004; p 110.
(21) Ono, M.; Takamura, C.; Sugita, F.; Masuoka, C.; Yoshimitsu, H.; Ikeda, T.; Nohara, T. *Chem. Pharm. Bull.* **2007**, *55*, 551−556.
(22) Nohara, T.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1975**, *23*, 872−885.
(23) Guo, S. J.; Falk, E.; Kenne, L.; Ronnberg, B.; Sundquist, B. G. *Phytochemistry* **2000**, *53*, 861−868.
(24) Gaidi, G.; Miyamoto, T.; Rustaiyan, A.; Lacaille-Dubois, M. A. *J. Nat. Prod.* **2001**, *64*, 920−924.
(25) Gaidi, G.; Miyamoto, T.; Ramezani, M.; Lacaille-Dubois, M. A. *J. Nat. Prod.* **2004**, *67*, 1114−1118.
(26) Li, W.; Asada, Y.; Koike, K.; Nikaido, T.; Furuya, T.; Yoshikawa, T. *Tetrahedron* **2005**, *61*, 2921−2929.
(27) Takeda, K.; Shimaoka, A.; Iwasaki, M.; Minato, H. *Chem. Pharm. Bull.* **1965**, *13*, 691−694.
(28) Yahara, S.; Ohtsuka, M.; Nakano, K.; Nohara, T. *Chem. Pharm. Bull.* **1989**, *37*, 1802−1804.
(29) Zhu, X. H.; Tsumagari, H.; Honbu, T.; Ikeda, T.; Ono, M.; Nohara, T. *Tetrahedron Lett.* **2001**, *42*, 8043−8046.
(30) Sang, S. M.; Mao, S. L.; Lao, A. N.; Chen, Z. L.; Ho, C. T. *Food Chem.* **2003**, *83*, 499−506.
(31) Challinor, V. L.; Hayes, P. Y.; Bernhardt, P. V.; Kitching, W.; Lehmann, R. P.; De Voss, J. J. *J. Org. Chem.* **2011**, *76*, 7275−7280.

(32) Konig, W. A.; Benecke, I.; Bretting, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 693−694.