Steroidal Saponins from *Chlorophytum orchidastrum*

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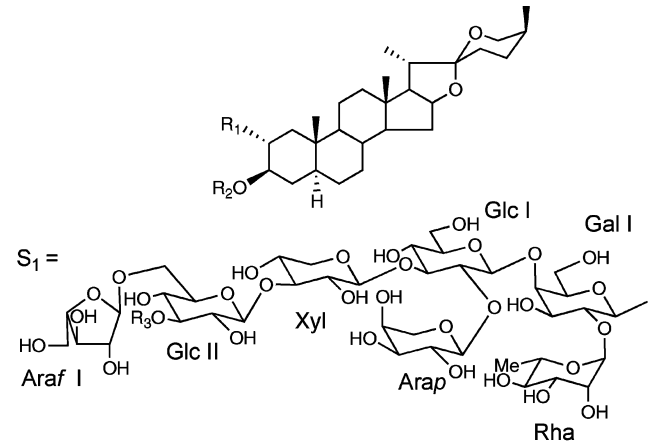
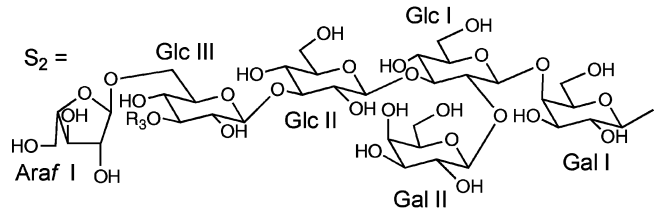
Six new spirostane-type saponins (**1**−**6**), named orchidastrosides A−F, and chloromaloside D were isolated from an ethanol extract of the roots of *Chlorophytum orchidastrum*. The saponins have neotigogenin or neogitogenin as the aglycon and oligosaccharidic chains possessing seven to nine sugar units. Their structures were elucidated mainly by 2D NMR spectroscopic analyses (COSY, TOCSY, NOESY, HSQC, and HMBC) and FABMS and HRESIMS. Compounds **1**−**6** were tested for cytotoxicity against two human colon cancer cell lines, HCT 116 and HT-29.

The genus *Chlorophytum* (Liliaceae) has more than 215 species and has a wide distribution, mainly in pantropical areas. Several *Chlorophytum* species have been found to contain steroidal saponins.[1] As part of our continuing search for biologically active steroidal saponins,[2,3] we have now examined underground parts of *Chlorophytum orchidastrum* Lindl (Syns. *C. nimonii* (Grah.) Dalz in Hook, *Antherium nimonii* Grah.), which is found mainly in tropical and subtropical countries. This plant was reported to contain a tigogenin saponin.[4] The present paper reports the isolation and characterization of six new spirostane-type saponins (**1**−**6**), named orchidastrosides A−F, and (25*S*)-5α-spirostan-3*β*-ol 3-*O*-*β*-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside (chloromaloside D).[5] Cytotoxic effects of **1**−**6** were examined against two human colon cancer cell lines, HCT 116 and HT-29.





	R_1	R_2	R_3
1	H	S_1	H
2	H	S_1	Glc III
3	H	S_1	Ara-*f* II, Glc III
4	OH	S_1	H
5	OH	S_2	Ara-*f* II, Glc IV
6	OH	S_2	Xyl

Results and Discussion

The BuOH fraction of an EtOH extract of roots of *C.orchidastrum* was submitted to multiple chromatographic steps including vacuum-liquid chromatography (VLC; reversed-phase silica gel RP-18) and medium-pressure liquid chromatography (MPLC; silica gel and reversed-phase silica gel RP-18), yielding orchidastrosides A−F (**1**−**6**) and chloromaloside D.[5] Compounds **1**−**6** were isolated as white, amorphous powders. The sugars obtained by aqueous acid hydrolysis of each compound were identified as D-glucose, D-galactose, D-xylose, L-rhamnose, and L-arabinose, by TLC and GC analysis (see Experimental Section).

Orchidastroside A (**1**) exhibited in the HRESIMS the $[M + Na]^+$ peak at *m/z* 1467.6615, consistent with the molecular formula $C_{66}H_{108}O_{34}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m/z* 1443 $[M - H]^-$, indicating a molecular weight of 1444. Other fragment-ion peaks were observed at *m/z* 1311, 1149, 1017, 885, 723, 577, and 415, suggesting the elimination of three hexosyl, three pentosyl, and one desoxyhexosyl moiety. The aglycon of **1** was identified as (25*S*)-5α-spirostan-3*β*-ol (neotigogenin) by comparison of its NMR data based on correlations observed in the COSY, NOESY, HSQC, and HMBC spectra and comparison with spectra reported in the literature.[5,6] The 25*S* configuration of the aglycon (Agly) was deduced after considering the chemical shifts

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Table 1. ^{1}H and ^{13}C NMR Data of the Aglycon Part of **1**−**6** in Pyridine-d_5 (δ in ppm, J in Hz)[a]

	1		**2**		**3**		**4**		**5**		**6**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	36.9	0.74, 1.49	36.8	0.75, 1.50	36.8	0.73, 1.48	44.9	1.00, 2.07	44.9	1.00, 2.07	44.9	1.06, nd
2	29.6	1.68, 1.96	29.5	1.68, 1.96	29.6	1.68, 1.94	70.0	3.84	70.1	3.86	70.8	3.82
3	76.9	3.83	76.9	3.84	76.9	3.83	83.4	3.80	83.5	3.82	83.4	3.82
4	34.1	1.57, 1.84	34.0	1.83, nd	34.1	1.82, nd	33.3	1.37, 1.76	33.3	1.37, 1.76	33.5	1.88, nd
5	44.3	0.85	44.2	0.84	44.3	0.83	44.1	0.95	44.1	0.95	44.0	0.90
6	28.5	1.10, nd	28.5	1.11, nd	28.5	1.08, nd	27.6	0.92, 1.06	27.6	0.92, 1.06	27.4	0.92, 1.06
7	31.7	1.34, 1.94	31.6	1.30, 1.92	31.6	1.30, 1.90	31.6	1.31, 1.94	31.6	1.31, 1.94	31.3	1.38, 1.93
8	34.9	1.34	34.8	1.33	34.9	1.31	34.2	1.26	34.2	1.26	34.2	1.26
9	54.1	0.46	54.0	0.45	54.0	0.44	53.9	0.48	53.9	0.48	53.9	0.48
10	35.5		35.5		35.5		36.5		36.5		36.8	
11	20.9	1.32, 1.40	20.9	1.32, 1.40	20.9	1.30, nd	21.0	1.08, 1.32	21.0	1.08, 1.32	20.9	1.32, nd
12	39.8	0.97, 1.60	39.8	0.98, 1.60	39.8	0.97, 1.59	39.6	0.92, 1.54	39.6	0.92, 1.54	39.5	0.92, 1.52
13	40.4		40.4		40.4		40.4		40.4		40.5	
14	56.1	0.96	56.0	0.96	56.0	0.93	55.9	0.92	55.9	0.92	55.6	0.92
15	32.0	0.72, 1.46	32.0	0.76, 1.42	32.0	0.72, 1.46	31.8	0.68, 1.42	31.8	0.68, 1.42	31.2	1.42, nd
16	81.0	4.46	80.9	4.46	81.0	4.45	80.9	4.44	80.9	4.44	80.8	4.44
17	62.4	1.73	62.3	1.74	62.3	1.72	62.3	1.72	62.3	1.72	62.3	1.72
18	16.2	0.74 s	16.2	0.74 s	16.2	0.72 s	16.2	0.71 s	16.2	0.71 s	16.2	0.71 s
19	12.1	0.77 s	12.0	0.77 s	12.1	0.76 s	12.9	0.59 s	12.9	0.59 s	12.9	0.59 s
20	42.1	1.84	42.1	1.84	42.1	1.83	42.1	1.82	42.1	1.82	42.1	1.82
21	14.5	1.07 d (6.6)	14.4	1.07 d (6.7)	14.4	1.06 d (6.6)	14.4	1.06 d (6.4)	14.4	1.06 d (6.4)	14.5	1.06 d (6.5)
22	109.6		109.6		109.6		109.6		109.6		109.6	
23	26.0	1.40, 1.83	25.9	1.39, 1.82	25.9	1.38, 1.81	25.9	1.37, 1.82	25.9	1.37, 1.82	25.8	1.36, 1.81
24	25.7	1.29, 2.08	25.7	1.30, 2.08	25.7	1.28, 2.06	25.7	1.28, 2.06	25.7	1.28, 2.06	25.6	1.26, 2.05
25	27.1	1.52	27.1	1.54	27.1	1.52	27.1	1.53	27.1	1.53	27.1	1.52
26	64.8	3.32, 3.98	64.8	3.32, 3.98	64.8	3.32, 3.98	64.8	3.32, 3.98	64.8	3.32, 3.98	64.8	3.32, 3.98
27	15.9	1.00 d (6.9)	15.9	1.00 d (6.9)	15.9	0.99 d (6.6)	15.9	1.00 d (6.7)	15.9	1.00 d (6.7)	15.9	1.00 d (6.6)

[a] Overlapped ^{1}H NMR signals are reported without designated multiplicity. nd: not determined.

at δ_C 26.0 (C-23), 25.7 (C-24), 27.1 (C-25), 64.8 (C-26), and 15.9 (C-27), which had higher field resonances as compared to 25*R* spirostanes.[7,8] The ^{1}H NMR spectrum of **1** displayed signals for seven anomeric protons [δ_H 4.74 (d, J = 7.6 Hz), 4.82 (d, J = 7.6 Hz), 4.98 (d, J = 8.3 Hz), 5.16 (d, J = 7.6 Hz), 5.22 (d, J = 7.4 Hz), 5.49 (br s), and 6.10 (br s)], which gave correlations in the HSQC spectrum with seven anomeric carbon signals [δ_C 99.7, 104.7, 104.6, 103.5, 104.9, 109.8, and 101.3, respectively]. The evaluation of chemical shifts and spin−spin couplings obtained from the COSY, TOCSY, HSQC, and HMBC data allowed the identification of two β-glucopyranosyl (Glc I, Glc II), one β-galactopyranosyl (Gal I), one β-xylopyranosyl (Xyl), one α-arabinopyranosyl (Ara*p*), one α-rhamnopyranosyl (Rha), and one α-arabinofuranosyl (Ara*f* I) unit.[9] The relatively large $^3J_{H\text{-}1,H\text{-}2}$ values (7.4−8.3 Hz) indicated a β-anomeric orientation for Glc, Gal, and Xyl and an α-anomeric orientation for Ara*p*. The multiplicity of the anomeric ^{1}H NMR signals of Rha and Ara*f* (br s) indicated an α-orientation. The linkage of Gal I at C-3 of the aglycon was supported by the HMBC correlation between the anomeric proton signal at δ_H 4.74 (Gal I H-1) and Agly (C-3) at δ_C 76.9 and by the NOESY correlation between δ_H 4.74 (Gal I H-1) and δ_H 3.83 (Agly H-3). Furthermore, the sequence of the oligosaccharidic chain linked at the C-3 position of the Agly was deduced by the HMBC correlations between δ_H 4.82 (Glc I H-1) and δ_C 80.8 (Gal I C-4), between δ_H 6.10 (Rha H-1) and δ_C 77.1 (Gal I C-2), between δ_H 5.22 (Ara*p* H-1) and δ_C 80.9 (Glc I C-2), between δ_H 5.16 (Xyl H-1) and δ_C 87.0 (Glc I C-3), and between δ_H 4.98 (Glc II H-1) and δ_C 87.8 (Xyl C-3). This was completed by the NOESY correlations between δ_H 4.82 (Glc I H-1) and δ_H 4.40 (Gal I H-4), between δ_H 6.10 (Rha H-1) and δ_H 4.24 (Gal I H-2), between δ_H 5.22 (Ara*p* H-1) and δ_H 4.04 (Glc I H-2), between δ_H 5.16 (Xyl H-1) and δ_H 3.96 (Glc I H-3), between δ_H 4.98 (Glc II H-1) and δ_H 3.98 (Xyl H-3), and between δ_H 5.49 (Ara*f* I H-1) and δ_H 4.56 (Glc II H-6). Thus, the structure of **1** was elucidated as (25*S*)-5α-spirostan-3β-ol 3-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside.

Orchidastroside B (**2**) exhibited in the HRESIMS the $[M + Na]^+$ peak at *m*/*z* 1629.7153, consistent with the molecular formula $C_{72}H_{118}O_{39}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m*/*z* 1605 $[M - H]^-$, indicating a molecular weight of 1606, 162 mass units higher than that of **1**. Other fragment-ion peaks were observed indicating the loss of four hexosyl, three pentosyl, and one desoxyhexosyl moiety. The ^{1}H and ^{13}C NMR data for **2** were almost superimposable with those of **1** except for the oligosaccharidic chain linked at the C-3 position of the neotigogenin, which contained a supplementary β-glucopyranosyl moiety (Glc III) (Table 2). The linkage of Glc III at the 3-position of Glc II was suggested by the HMBC correlation between δ_H 5.10 (Glc III H-1) and δ_C 86.9 (Glc II C-3) and confirmed by the NOESY correlation between δ_H 5.10 (Glc III H-1) and δ_H 4.03 (Glc II H-3). Thus, the structure of **2** was established as (25*S*)-5α-spirostan-3β-ol 3-*O*-α-L-arabinofuranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside.

Orchidastroside C (**3**) exhibited in the HRESIMS the $[M + Na]^+$ peak at *m*/*z* 1761.7564, consistent with the molecular formula $C_{77}H_{126}O_{43}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m*/*z* 1737 $[M - H]^-$, indicating a molecular weight of 1738, 132 mass units higher than that of **2**. Other fragment-ion peaks were observed indicating the loss of four hexosyl, four pentosyl, and one desoxyhexosyl moiety. This time, signals of an additional α-arabinofuranosyl (Ara*f* II) moiety remained after comparison of the NMR data of **3** and **2** (Table 2). The HMBC correlation between δ_H 5.47 (Ara*f* II H-1) and δ_C 68.0 (Glc III C-6), and the NOESY correlation between δ_H 5.47 (Ara*f* II H-1) and δ_H 4.41 (Glc III H-6), proved the linkage of Ara*f* II to the Glc III unit at C-6. Thus, the structure of **3** was elucidated as (25*S*)-5α-spirostan-3β-ol 3-*O*-α-L-arabinofuranosyl-(1→6)-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside.

Orchidastroside D (**4**) exhibited the $[M + Na]^+$ peak at *m*/*z* 1483.6573, consistent with the molecular formula $C_{66}H_{108}O_{35}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m*/*z* 1459 $[M - H]^-$, indicating a molecular weight of 1460, 16 mass

Table 2. ^{1}H and ^{13}C NMR Data of the Sugar Moieties of **1**−**6** in Pyridine-d_5 (δ in ppm, J in Hz)[a]

	1		**2**		**3**		**4**		**5**		**6**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
GalI-1	99.7	4.74 d (7.6)	99.8	4.74 d (7.6)	99.8	4.74 d (7.6)	100.2	4.78 d (7.6)	102.5	4.84 d (7.6)	102.2	4.84 d (7.6)
2	77.1	4.24	77.2	4.23	77.3	4.23	77.1	4.29	72.1	4.42	72.0	4.41
3	75.6	4.04	75.6	4.06	76.0	4.03	75.5	4.06	74.6	4.06	74.8	4.07
4	80.8	4.40	80.7	4.41	80.8	4.40	80.2	4.39	79.3	4.44	79.0	4.43
5	74.7	3.92	74.6	3.91	74.6	3.92	74.7	3.91	74.9	4.02	75.0	4.02
6	60.1	4.11, 4.57	60.1	4.13, 4.55	60.3	4.12, 4.55	60.4	4.20, 4.44	60.5	4.17, 4.43	60.4	4.18, 4.42
GlcI-1	104.7	4.82 d (7.6)	104.6	4.81 d (7.6)	104.7	4.81 d (7.6)	104.2	4.82 d (7.6)	103.8	4.99 d (7.6)	103.6	5.00 d (7.6)
2	80.9	4.04	80.8	4.04	80.7	4.03	80.3	4.06	80.3	4.13	80.2	4.15
3	87.0	3.96	87.0	3.95	87.2	3.95	87.0	3.96	87.2	4.09	86.2	4.09
4	69.5	3.86	69.6	3.87	69.7	3.86	69.5	3.85	71.2	3.86	70.2	3.84
5	76.9	3.68	76.7	3.68	76.7	3.67	76.9	3.68	76.8	3.66	76.8	3.66
6	62.3	3.92, 4.39	62.2	3.92, 4.39	62.3	3.91, 4.38	62.0	3.92, 4.38	62.1	3.88, 4.29	62.1	3.88, 4.29
GlcII-1	104.6	4.98 d (8.3)	104.0	5.05 d (7.9)	104.0	5.05 d (7.8)	104.4	4.97 d (8.3)	103.0	5.21 d (7.9)	102.6	5.21 d (7.9)
2	75.0	3.94	75.1	3.92	75.1	3.92	75.0	3.94	75.0	3.90	74.7	3.90
3	77.2	4.04	86.9	4.03	87.5	4.01	77.0	4.04	87.6	4.07	87.3	4.07
4	71.3	3.82	71.2	3.83	71.3	3.83	71.2	3.82	70.0	3.64	70.8	3.98
5	76.1	3.96	76.2	3.96	76.2	3.97	76.2	3.96	77.0	3.85	77.7	3.85
6	68.4	3.83, 4.56	68.4	3.85, 4.57	68.5	3.85, 4.54	68.4	3.85, 4.56	62.0	3.90, 4.38	62.0	3.90, 4.38
Rha-1	101.3	6.10 br s	101.3	6.08 br s	101.4	6.08 br s	101.1	6.08 br s				
2	71.7	4.67	71.6	4.67	72.0	4.68	71.6	4.69				
3	72.0	4.42	72.0	4.42	72.3	4.41	72.0	4.42				
4	73.3	4.21	73.0	4.21	73.3	4.21	73.2	4.20				
5	69.2	4.78	69.2	4.78	69.5	4.76	69.3	4.75				
6	18.0	1.62 d (6.1)	18.0	1.63 d (6.1)	18.0	1.62 d (6.0)	17.8	1.58 d (6.0)				
Xyl-1	103.5	5.16 d (7.6)	103.6	5.15 d (7.6)	103.5	5.15 d (7.6)	103.4	5.16 d (7.6)			103.4	5.15 d (7.6)
2	74.9	3.87	74.8	3.87	74.8	3.85	74.7	3.86			74.7	3.93
3	87.8	3.98	87.1	4.01	87.6	4.00	87.5	4.00			76.1	3.99
4	70.0	3.68	69.5	3.67	69.7	3.66	69.8	3.69			70.3	3.88
5	65.6	3.56 tl (10.0), 4.06	65.7	3.57 tl(10.0), 4.07	66.6	3.55 tl (10.1), 4.04	65.6	3.56 tl (10.1), 4.05			65.5	3.55 tl (10.1), 4.06
Ara*p*-1	104.9	5.22 d (7.4)	105.0	5.21 d (7.5)	104.8	5.21 d (7.5)	104.3	5.32 d (7.4)				
2	72.5	4.29	72.3	4.28	73.0	4.28	72.3	4.31				
3	74.1	3.95	74.2	3.96	74.0	3.95	74.1	3.95				
4	69.1	4.02	69.0	4.04	69.3	4.03	69.0	4.04				
5	67.0	3.50 ld (12.1), 4.44	66.6	3.52 ld (12.0), 4.45	67.0	3.50 ld (12.1), 4.43	66.5	4.47, nd				
Ara*f*I-1	109.8	5.49 br s	109.8	5.48 br s	109.8	5.49 br s	109.8	5.49 br s	109.7	5.47 br s	109.5	5.48 br s
2	82.6	4.77	82.6	4.76	82.7	4.74	82.6	4.77	82.6	4.74	82.5	4.77
3	78.1	4.63	77.7	4.62	78.2	4.60	78.0	4.64	78.0	4.59	78.0	4.64
4	85.4	4.66	85.2	4.64	85.5	4.64	85.4	4.65	85.3	4.64	85.5	4.66
5	62.1	4.09,4.21	62.0	4.08, 4.20	62.1	4.09, 4.19	62.2	4.10, 4.20	62.1	4.09, 4.19	60.4	4.21, 4.44
GlcIII-1			104.8	5.10 d (7.8)	104.6	5.02 d (7.7)			104.0	5.02 d (7.6)	104.3	5.02 d (7.6)
2			75.4	3.94	75.6	3.92			74.5	3.88	75.0	3.89
3			77.2	4.12	77.4	4.06			87.0	3.94	88.1	4.05
4			71.1	3.82	71.3	3.82			69.8	3.74	69.8	3.69
5			77.0	3.72	77.0	3.70			76.1	3.99	77.7	3.82
6			61.9	4.24, 4.44	68.0	3.82, 4.41			67.9	3.82, 4.42	68.0	3.85, 4.54
GalII-1									103.7	5.45	103.4	5.47
2									73.5	3.90	73.5	3.90
3									75.1	4.00	75.1	4.00
4									69.3	3.84	69.6	3.65
5									75.5	3.97	75.5	3.97
6									61.4	4.01, 4.31	61.4	3.99, 4.30
GlcIV-1									104.6	5.02 d (7.8)		
2									74.8	3.92		
3									77.3	4.06		
4									70.7	3.99		
5									77.4	3.80		
6									68.1	3.88, 4.55		
Ara*f*II-1					109.9	5.47 br s			109.8	5.49 br s		
2					82.6	4.76			82.5	4.75		
3					78.0	4.62			78.0	4.62		
4					85.6	4.65			85.2	4.64		
5					62.2	4.10, 4.20			62.2	4.10, 4.20		

[a] Overlapped ^{1}H NMR signals are reported without designated multiplicity. nd: not determined.

units higher than that of **1**. Other fragment-ion peaks were observed referring to the loss of three hexosyl, three pentosyl, and one desoxyhexosyl moiety, as in compound **1**. A detailed comparison of the 2D NMR spectral data of **4** and **1** showed that both compounds shared the same oligosaccharidic chain (Table 2) and differed only by the aglycon part. An OH group at C-2 of the aglycon of **4** was revealed by a downfield shift to δ_C 70.0 (C-2) instead of 29.6 for **1** (Table 1). Cross-peaks in the NOESY spectrum, between the β-axial protons of the CH_3-19 angular group at δ_H 0.59 (s) and the H-2 at δ_H 3.84, indicated a β-axial orientation of this proton and, consequently, an α-equatorial orientation of the OH. Thus, the aglycon of **4** was identified as (25*S*)-5α-spirostan-2α,3β-diol (neogitogenin).[6] On the basis of the above results, the structure of **4** was established as (25*S*)-5α-spirostan-2α,3β-diol 3-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside.

Orchidastroside E (**5**) exhibited in the HRESIMS the $[M + Na]^+$ peak at *m*/*z* 1691.7102, consistent with the molecular formula $C_{73}H_{120}O_{42}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m*/*z* 1667 $[M - H]^-$, indicating a molecular weight of 1668. Other fragment-ion peaks were observed at *m*/*z* 1535, 1373, 1241, 1079, 917, 755, 593, and 431, suggesting the elimination of six hexosyl and two pentosyl moieties. The aglycon was identified

by NMR analysis as neogitogenin, as in **4**. The ^{1}H NMR spectrum of **5** displayed signals for eight anomeric protons [δ_H 4.84 (d, J = 7.6 Hz), 4.99 (d, J = 7.6 Hz), 5.02 (d, J = 7.6 Hz), 5.02 (d, J = 7.6 Hz), 5.21 (d, J = 7.8 Hz), 5.45 (overlapped), 5.47 (br s), and 5.49 (br s)], which gave correlations in the HSQC spectrum with eight anomeric carbon signals [δ_C 102.5, 103.8, 104.0, 104.6, 103.0, 103.7, 109.7, and 109.8, respectively]. Eight sugars were identified by extensive 2D NMR analysis as two arabinofuranosyl (Ara*f* I, Ara*f* II), four glucopyranosyl (Glc I, Glc II, Glc III, Glc IV), and two galactopyranosyl (Gal I, Gal II) moieties. The linkage of Gal I at C-3 of the aglycon was supported by the HMBC correlation between the anomeric proton signal at δ_H 4.84 (Gal I H-1) and Agly (C-3) at δ_C 83.5 and by the NOESY correlation between δ_H 4.84 (Gal I H-1) and δ_H 3.82 (Agly H-3). Furthermore, the structure of the oligosaccharide chain was determined by the HMBC correlations between δ_H 4.99 (Glc I H-1) and δ_C 79.3 (Gal I C-4), between δ_H 5.45 (Gal II H-1) and δ_C 80.3 (Glc I C-2), between δ_H 5.21 (Glc II H-1) and δ_C 87.2 (Glc I C-3), between δ_H 5.02 (Glc III H-1) and δ_C 87.6 (Glc II C-3), between δ_H 5.02 (Glc IV H-1) and δ_C 87.0 (Glc III C-3), between δ_H 5.47 (Ara*f* I H-1) and δ_C 67.9 (Glc III C-6), and between δ_H 5.49 (Ara*f* II H-1) and δ_C 68.1 (Glc IV C-6). This was confirmed by the NOESY correlations between δ_H 4.99 (Glc I H-1) and δ_H 4.44 (Gal I H-4), between δ_H 5.45 (Gal II H-1) and δ_H 4.13 (Glc I H-2), between δ_H 5.21 (Glc II H-1) and δ_H 4.09 (Glc I H-3), between δ_H 5.02 (Glc III H-1) and δ_H 4.07 (Glc II H-3), between δ_H 5.02 (Glc IV H-1) and δ_H 3.94 (Glc III H-3), between δ_H 5.47 (Ara*f* I H-1) and δ_H 3.82 (Glc III H-6), and between δ_H 5.49 (Ara*f* II H-1) and δ_H 4.55 (Glc IV H-6). Hence, the structure of **5** was elucidated as (25*S*)-5α-spirostan-2α,3β-diol 3-*O*-α-L-arabinofuranosyl-(1→6)-[α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Orchidastroside F (**6**) exhibited in the HRESIMS the $[M + Na]^+$ peak at *m/z* 1529.6919, consistent with the molecular formula $C_{67}H_{110}O_{37}$. Its negative-ion FABMS displayed a quasimolecular ion peak at *m/z* 1505 $[M - H]^-$, indicating a molecular weight of 1506. Other fragment-ion peaks were observed at *m/z* 1373, 1211, 1049, 917, 755, 593, and 431, suggesting the elimination of five hexosyl and two pentosyl moieties. The comparison of the NMR spectral data of **6** with **5** (Tables 1 and 2) revealed a common sequence, (25*S*)-5α-spirostan-2α,3β-diol 3-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. The difference was located at the C-3 position of the Glc III: NMR signals of one xylopyranosyl moiety (Xyl) remained in the spectra of **6**, instead of signals of an α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosyl moiety for **5**. The correlations in the HMBC spectrum between δ_H 5.15 (Xyl H-1) and δ_C 88.1 (Glc III C-3), and in the NOESY spectrum between δ_H 5.15 (Xyl H-1) and δ_H 4.05 (Glc III H-3), confirmed a 3-substitution of the Glc III by a Xyl unit. Thus, the structure of **6** was elucidated as (25*S*)-5α-spirostan-2α,3β-diol 3-*O*-α-L-arabinofuranosyl-(1→6)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Since steroidal saponins are reported to possess, to varying degrees, cytotoxic activity against various cancer cell lines,[1,3,10] we have tested orchidastrosides A−F (**1**−**6**) for cytotoxicity against the HCT 116 and HT-29 human colon cancer cell lines, by using the MTT assay[11] with paclitaxel as the positive contol. Among them, compound **1** was found to be the most active on both cell lines (IC_{50} 1.6 μM in HCT 116 and 1.5 μM in HT-29 cells), whereas **3**, **4**, and **6** displayed activity with a higher cytotoxic effect on HCT 116 (IC_{50} 1.35, 2.19, and 2.12 μM, respectively) than on HT-29 (IC_{50} 3.60, 9.15, and 8.87 μM, respectively). Compounds **2** and **5** were considered inactive on both cell lines with IC_{50} in the range 7−21 μM.

Experimental Section

General Experimental Procedures. The instruments used and the isolations were carried out using methods described elsewhere.[3,12] Medium-pressure liquid chromatography (MPLC) was on silica gel 60 (Merck, 15−40 μm) and reversed-phase silica gel RP-18 (Spherical C-18, 300A, 75−200 μm, Silicycle).

Plant Material. Roots of *C. orchidastrum* Lindl were purchased from a local herbal market in 2008 (Jardiland, Dijon, France) and identified by one of us (M.-A.L.-D.). A voucher specimen (No. 13092009) is deposited in the herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, Burgundy University, Dijon, France.

Extraction and Isolation. Powdered roots (270 g) of *C. orchidastrum* were refluxed with EtOH (3 × 2 L) for 50 min. After evaporation of the solvent under vacuum, the resulting EtOH extract (28.5 g) was suspended in H_2O (200 mL) and partitioned with H_2O-saturated *n*-BuOH (3 × 200 mL), yielding after evaporation of the solvent the corresponding *n*-BuOH (5.1 g) fraction. It was submitted to VLC [RP-18, MeOH−H_2O (0:10, 5:5, 10:0)], yielding three fractions (F1−F3). F3 (786.3 mg) was submitted to MPLC (silica gel 60, $CHCl_3$−MeOH−H_2O, 13:7:2, lower phase), yielding 13 subfractions (Fr. 1−13). Fr. 6 (86.1 mg) was rechromatographed by MPLC (RP-18, gradient MeOH−H_2O, 4:6 → 0:10), yielding Fr. 6.1−6.6. Fr. 6.3 (60.0 mg) was submitted to MPLC (RP-18, gradient MeOH−H_2O, 6:4 → 0:10), yielding **1** (8.2 mg). Fr. 8 (23.1 mg), Fr. 9 (33.2 mg), Fr. 7 (44.0 mg), Fr. 10 (30.1 mg), and Fr. 5 (21.2 mg) were submitted to MPLC (RP-18, gradient MeOH/H_2O, 7:3 → 0:10), yielding **2** (6.2 mg), **3** (9.4 mg), **4** (4.5 mg), **5** (8.5 mg), **6** (4.1 mg), and chloromaloside D (6.4 mg),[5] respectively.

Orchidastroside A (1): white, amorphous powder; $[\alpha]_D^{20}$ −50.9 (*c* 0.25, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1443 $[M - H]^-$, 1311, 1149, 1017, 885, 723, 577, 415; HRESIMS (positive-ion mode) *m/z* 1467.6615 $[M + Na]^+$ (calcd for $C_{66}H_{108}O_{34}Na$, 1467.6620).

Orchidastroside B (2): white, amorphous powder; $[\alpha]_D^{20}$ −70.2 (*c* 0.19, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1605 $[M - H]^-$, 1443, 1311, 1149, 1017, 885, 723, 577, 415; HRESIMS (positive-ion mode) *m/z* 1629.7153 $[M + Na]^+$ (calcd for $C_{72}H_{118}O_{39}Na$, 1629.7148).

Orchidastroside C (3): white, amorphous powder;$[\alpha]_D^{20}$ −55.1 (*c* 0.14, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1737 $[M - H]^-$, 1605, 1443, 1311, 1149, 1017, 885, 723, 577, 415; HRESIMS (positive-ion mode) *m/z* 1761.7564 $[M + Na]^+$ (calcd for $C_{77}H_{126}O_{43}Na$, 1761.7571).

Orchidastroside D (4): white, amorphous powder; $[\alpha]_D^{20}$ −48.8 (*c* 0.20, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1459 $[M - H]^-$, 1327, 1165, 1033, 901, 739, 593, 431; HRESIMS (positive-ion mode) *m/z* 1483.6573 $[M + Na]^+$ (calcd for $C_{66}H_{108}O_{35}Na$, 1483.6569).

Orchidastroside E (5): white, amorphous powder;$[\alpha]_D^{20}$ −60.4 (*c* 0.30, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1667 $[M - H]^-$, 1535, 1373, 1241, 1079, 917, 755, 593, 431; HRESIMS (positive-ion mode) *m/z* 1691.7102 $[M + Na]^+$ (calcd for $C_{73}H_{120}O_{42}Na$, 1691.7152).

Orchidastroside F (6): white, amorphous powder; $[\alpha]_D^{20}$ −52.1 (*c* 0.19, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS (glycerol matrix) *m/z* 1505 $[M - H]^-$, 1373, 1211, 1049, 917, 755, 593, 431; HRESIMS (positive-ion mode) *m/z* 1529.6619 $[M + Na]^+$ (calcd for $C_{67}H_{110}O_{37}Na$, 1529.6624).

Acid Hydrolysis and GC Analysis. Each compound (3 mg) was hydrolyzed with 2 N aqueous CF_3COOH (5 mL) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 mL), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and then analyzed by TLC over silica gel ($CHCl_3$−MeOH−H_2O, 8:5:1) by

comparison with authentic samples. Furthermore, the residue of sugars was dissolved in anhydrous pyridine (100 μL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60 °C for 1 h. Then 150 μL of HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane, 3:1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under a N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 mL each), and the hexane layer (1 μL) was analyzed by GC.[13] D-glucose, D-galactose, D-xylose, L-rhamnose, and L-arabinose were detected by co-injection of the hydrolysate with standard silylated samples. Identification of D-glucose, D-galactose, D-xylose, L-rhamnose, and L-arabinose was carried out for **1**−**4**, giving peaks at 18.60, 17.21, 13.46, 13.16, and 11.88 min for **1**, 18.64, 17.21, 13.47, 13.13, and 11.89 min for **2**, 18.60, 17.20, 13.45, 13.11, and 11.86 min for **3**, and 18.62, 17.23, 13.48, 13.15, and 11.87 min for **4**, respectively. Identification of D-glucose, D-galactose, and L-arabinose was carried out for **5**, giving peaks at 18.61, 17.22, and 11.89 min, respectively, and D-glucose, D-galactose, D-xylose, and L-arabinose for **6**, giving peaks at 18.62, 17.21, 13.47, and 11.88 min, respectively.

MTT Cytotoxicity Assay. The bioassay was carried out as described elsewhere,[11] with two human colorectal cancer cell lines (HCT 116 and HT-29). The experiments were repeated twice. Paclitaxel was used as a positive control and showed IC_{50} values of 2.4 *n*M (HCT 116) and 2.1 *n*M (HT-29).

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of compounds **1**−**6** are available free of charge via the Internet at http://pubs.acs.org.

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