Steroidal Saponins from Two Species of *Dracaena*

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Four new steroidal saponins (**1**−**4**) were isolated from the stem and bark of two species of *Dracaena*: deistelianosides A and B (**1** and **2**) from *D. deisteliana* and arboreasaponins A and B (**3** and **4**) from *D. arborea*. Six known saponins and one known sapogenin were also isolated. The structures of **1**−**4** were established as diosgenin 3-*O*-[3-*O*-sulfate-α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**1**), 1-*O*-*β*-D-xylopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-*β*-D-fucopyranosyl(23*S*,24*S*)-spirosta-5,25(27)-diene-1*β*,3*β*,23α,24α-tetrol 24-*O*-α-L-arabinopyranoside (**2**), pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-[6-*O*-acetyl]-*β*-D-glucopyranoside (**3**), and 24α-hydroxypennogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranoside (**4**) using extensive 1D and 2D NMR spectroscopic analyses and mass spectrometry. Cytotoxic activity of several of these compounds was evaluated against the HT-29 and HCT 116 human colon cancer cell lines.

The genus *Dracaena* (Dracaenaceae) includes more than 480 species distributed in tropical and subtropical dry climate regions throughout the world.[1] *Dracaena deisteliana* Engl. and *D. arborea* (Willd.) Link are used in African folk medicine for the treatment of heart and kidney problems, measles, eye injury, paralysis, epilepsy, convulsions, spasms, venereal diseases, diarrhea, dizziness, and infertility in women, and for social purposes.[1,2] Previous phytochemical investigations on the genus *Dracaena* revealed the presence of steroidal saponins[3−5] and flavonoids.[6,7] A survey of the literature showed that no significant chemical and biological work had been done on these two species. In a continuation of our studies of Cameroonian plants used in folk medicine[8] we herein describe the isolation and structure elucidation of two new steroidal saponins and three known saponins from *D. deisteliana* stems and two new saponins and four known compounds from *D. arborea* bark. The cytotoxicity of several of these compounds was also evaluated against the HT-29 and HCT 116 human colon cancer cell lines.

Results and Discussion

A methanol extract of dried stems of *D. deisteliana* was subjected to multiple chromatographic steps (VLC and MPLC, see Experimental Section), affording two new steroidal saponins, which were named deistelianosides A (**1**) and B (**2**), and three known saponins, identified as neoruscogenin 1-*O*-α-L-arabinopyranoside,[9] neoruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside, and neoruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.[10] Using the same method of extraction and purification, two new steroidal saponins, which were named arboreasaponins A (**3**) and B (**4**), three known steroidal saponins, pennogenin-3-*O*-*β*-D-glucopyranoside (floribundasaponin A),[11] pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranoside (manioside A),[3] and pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranoside (spiroconazole A),[4] and one known sapogenin, (25*R*)-spirost-5-ene-1*β*,3*β*,14α,17α-tetrol (namogenin A),[12] were isolated from bark of *D. arborea*. Their structures were elucidated using chemical and spectroscopic methods including 1D and 2D NMR experiments (COSY, TOCSY, NOESY, HSQC, and HMBC), HRESIMS, and FABMS.

Compounds **1**−**4** were isolated as white, amorphous powders. The sugars obtained by aqueous acid hydrolysis of each compound were identified as D-glucose and L-rhamnose (in the case of **1**, **3**, and **4**) and D-fucose, D-xylose, L-arabinose, and L-rhamnose (in the case of **2**) by TLC and GC (see Experimental Section). In the ^{1}H NMR spectra of **1**−**4**, the relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of the anomeric protons of the glucose, xylose, and arabinose moieties (between 7.0 and 8.3 Hz) indicated *β*-anomeric orientation for glucose, xylose, fucose, and α-anomeric orientation for arabinose. The broad singlet of the anomeric proton of rhamnose indicated α-orientation.

Deistelianoside A (**1**) had the molecular formula $C_{39}H_{62}O_{15}S$ as determined by HRESIMS (positive-ion mode), showing a pseudomolecular ion peak at *m*/*z* 825.9560 $[M + Na]^+$ (calcd for 825.9553). Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m*/*z* 801 $[M - H]^-$, indicating a molecular weight of 802. The presence of a sulfate group was ascertained by the presence of peaks at *m*/*z* 97 $[OSO_3H]^-$ and *m*/*z* 80 $[SO_3]^-$. The aglycone of **1** was identified as diosgenin by comparison of ^{1}H and ^{13}C NMR spectroscopic data obtained from the 2D NMR (COSY, TOCSY, NOESY, HSQC, and HMBC) spectra of **1** with those reported in the literature.[13] The presence of two sugar residues was confirmed from the observation of two anomeric proton signals [*δ* 4.83 (d, *J* = 7.6 Hz), 5.74 (br s)], giving HSQC correlations with two anomeric ^{13}C NMR signals at δ_C 101.8 and 102.1, respectively. Complete assignments of each sugar were achieved by extensive 1D and 2D NMR analyses. Evaluation of chemical shifts and spin−spin couplings allowed the identification of one *β*-glucopyranosyl (Glc) and one α-rhamnopyranosyl (Rha) unit in **1**. The sequence of the oligosaccharidic chain of **1** was achieved by HMBC and NOESY experiments. The HMBC correlations between the anomeric ^{1}H NMR signal at δ_H 5.74 (br s, Rha H-1) and δ_C 77.8 (Glc C-4) and between δ_H 4.83 (d, *J* = 7.6 Hz, Glc H-1) and δ_C 78.1 (Agly C-3) proved the sequence of the sugar chain at C-3 to be α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl.

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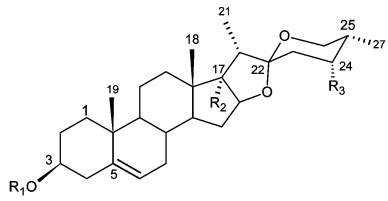

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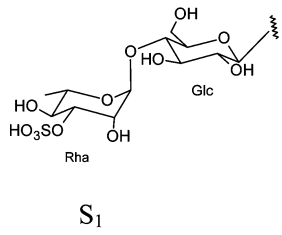
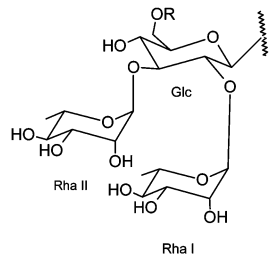
3	S_2	OH	H
4	S_3	OH	OH



S_1



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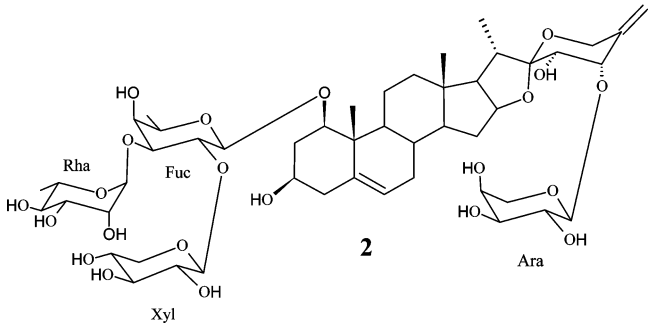
S_3 R= H



2

This sequence was confirmed by observation of NOESY cross-peaks between δ_H 5.74 (Rha H-1) and δ_H 4.27 (Glc H-4) and between δ_H 4.83 (Glc H-1) and δ_H 3.83 (Agly H-3). The deshielded values observed in the HSQC spectrum for the Rha C-3/Rha H-3 resonance at δ_C 79.2/δ_H 5.54 suggested acylation at C-3, but the substituent did not contain carbon atoms. Furthermore, a downfield shift of the Rha C-3 resonance (δ_C 79.2) and the upfield shifts of Rha C-2 and Rha C-4 at respectively δ_C 70.6 and 71.8 (α-effect) can be explained if the OH group at C-3 of Rha in **1** is acylated. This substituent was a SO_3H group, as confirmed by FABMS, which showed fragment ions at *m/z* 80 and 97, respectively. The COSY experiment unequivocally confirmed these conclusions, showing cross-peaks between Rha H-1 (δ_H 5.74) and Rha H-2 (δ_H 5.07), between Rha H-2 (δ_H 5.07) and Rha H-3 (δ_H 5.54), and finally between Rha H-3 (δ_H 5.54) and Rha H-4 (δ_H 4.48), respectively. Hence, the structure of deistelianoside A (**1**) was concluded to be diosgenin 3-*O*-[3-*O*-sulfate]-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside.

The molecular formula of deistelianoside B (**2**) was determined as $C_{49}H_{76}O_{22}$ by HRESIMS, showing a pseudomolecular ion peak at *m/z* 1039.4721 $[M + Na]^+$ (calcd for 1039.4726). Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1015 $[M - H]^-$, indicating a molecular weight of 1016. Further fragment ion peaks were observed at *m/z* 883, 737, 591, and 459 corresponding to the loss of two pentosyl and two deoxyhexosyl moieties. Inspection of the aglycone part of the ^1H NMR spectrum of **2** led to the assignment of two tertiary methyl groups [δ_H 1.37 and 0.92 (each 3H, s)], a secondary methyl group [δ_H 1.05 (3H, d, $J = 6.9$ Hz)], an exomethylene group [δ_H 5.23 and 5.09 (each 1H, br s)], and one olefinic proton [δ_H 5.55 (1H, br d, $J = 5.2$ Hz)]. These proton signals together with a distinctive quaternary carbon signal at δ_C 111.7 showing long-range correlations with one of the methylene protons at δ_H 3.99 (Agly H-26a) along with the proton at δ_H 2.83 (Agly H-20) indicated that the aglycone of **2** was a spirosta-5,25(27)-diene type. On the other hand, HMBC correlations between the proton signal at δ_H 3.97 (1H, d, $J = 4.2$ Hz) and the carbon signal at δ_C 111.7 (C-22) and between the proton signal at δ_H 4.78 (1H, d, $J = 4.2$ Hz) and the carbon signals at δ_C 143.5 (C-25) and 113.8 (C-27), respectively, suggested the presence of adjacent OH groups at C-23 (δ_H/δ_C 3.97/70.0) and C-24 (δ_H/δ_C 4.78/82.2). This was confirmed by COSY correlations between the protons at δ_H 3.97 (1H, d, $J = 4.2$ Hz, Agly H-23) and δ_H 4.78 (1H, d, $J = 4.2$ Hz, Agly H-24). ROESY correlations between proton signals at δ_H 3.97 (Agly H-23), δ_H 2.83 (Agly H-20), and δ_H 1.05 (Agly H-21) and between δ_H 3.97 (Agly H-23) and δ_H 4.78 (Agly H-24) and the small coupling constant between H-23 and H-24 ($J = 4.2$ Hz) allowed assignment of the 23*S* and 24*S* configurations.[14] The aglycone of **2** was identified as (23*S*,24*S*)-spirosta-5,25(27)-diene-1*β*,3*β*,23α,24α-tetrol, by comparison of ^1H and ^{13}C NMR spectroscopic data obtained from 2D NMR spectra of **2** with those reported in the literature.[10,14] The presence of four sugar residues was confirmed from observation of four anomeric proton signals [δ_H 4.69 (d, $J = 7.3$ Hz), 4.95 (d, $J = 7.3$ Hz), 6.26 (br s), and 5.11 (d, $J = 8.1$ Hz)], giving HSQC correlations with four anomeric carbon signals at δ_C 100.5, 106.4, 101.7, and 106.3, respectively. Complete assignments of each sugar unit, and their sequence, were carried out by extensive 1D and 2D NMR analyses. One *β*-fucopyranosyl (Fuc), one *β*-xylopyranosyl (Xyl), one α-rhamnopyranosyl (Rha), and one α-arabinopyranosyl (Ara) unit were identified in **2**. The HMBC correlations between the anomeric ^1H NMR signal at δ_H 6.26 (br s, Rha H-1) and δ_C 84.4 (Fuc C-3), between δ_H 4.95 (d, $J = 7.3$ Hz, Xyl H-1) and δ_C 78.1 (Fuc C-2), and finally between δ_H 4.69 (d, $J = 7.3$ Hz, Fuc H-1) and δ_C 83.8 (Agly C-1) proved the sequence of the sugar chain at C-1 to be *β*-D-xylopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-*β*-D-fucopyranosyl. This sequence was confirmed by ROESY cross-peaks between δ_H 6.26 (Rha H-1) and δ_H 4.07 (Fuc H-3), between δ_H 4.95 (Xyl H-1) and δ_H 4.05 (Fuc H-2), and between δ_H 4.69 (Fuc H-1) and δ_H 3.77 (Agly H-1). Another HMBC correlation between δ_H 5.11 (d, $J = 8.1$ Hz, Ara H-1) and δ_C 82.2 (Agly C-24) and a ROESY cross-peak between δ_H 5.11 (Ara H-1) and 4.78 (1H, d, $J = 4.2$ Hz, Agly H-24) allowed the linkage of Ara at C-24. Thus, deistelianoside B (**2**) was determined to be 1-*O*-*β*-D-xylopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-*β*-D-fucopyranosyl(23*S*,24*S*)-spirosta-5,25(27)-diene-1*β*,3*β*,23α,24α-tetrol 24-*O*-α-L-arabinopyranoside.

The molecular formula of arboreasaponin A (**3**) was $C_{47}H_{74}O_{18}$ (by HRESIMS). Its FABMS showed a quasimolecular ion peak at *m/z* 925 $[M - H]^-$, indicating a molecular weight of 926. Significant fragment ion peaks were observed at *m/z* 883, 737, and 429 corresponding to successive losses of one acetyl, two deoxyhexosyl, and one hexosyl moiety, respectively. The ^1H and ^{13}C NMR spectra of **3** were characteristic of a steroidal saponin having (25*R*)-3*β*,17α-spirost-5-ene-3,17-diol (pennogenin)[11] as the aglycone. The ^{13}C NMR signals of sugar moieties of **3** were identical to those of spiroconazole A, by comparison with literature data,[4] except for the primary alcoholic function at C-6 of a Glc unit, which was presumed to be acylated since the proton signals resonated at lower field [δ_H 4.62 (Glc H-6a) and 4.73 (Glc H-6b)]. This was confirmed by the deshielded value of the Glc C-6 signal at δ_C 63.9 and by long-range correlations in the HMBC spectrum between H-6a and H-6b (δ_H 4.62 and 4.73) and the carbonyl carbon signal of the acetyl

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data for the Aglycone Moieties of **14**[a]

	1		**2**		**3**		**4**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	37.1	0.92 t (10.7)	83.8	3.77 dd (4.0, 12.1)	37.2	0.92	37.0	0.83
		1.64				1.67		1.63
2	29.8	1.65	37.4	2.33 q (11.8)	29.7	1.52	31.2	1.72 dd (4.0, 14.5)
		2.03 br d (11.6)		2.70 m		2.04		2.07 t (14.5)
3	78.1	3.83 m	68.3	3.84 m	78.2	3.77	77.8	3.81 t (7.5)
4	38.4	2.41 br d (11.9)	43.8	2.54 dd (4.0, 12.1)	38.4	2.58 d (11.9)	38.1	2.56 d (11.9)
		2.70 br d (13.3)		2.66 br d (11.7)		2.65 d (11.9)		2.64 d (11.9)
5	140.6		139.6		140.4		140.3	
6	121.5	5.34 d (4.04)	124.6	5.55 d (5.2)	121.6	5.28 d (4.2)	121.5	5.28 d (4.2)
7	31.8	1.82 br d (10.9)	31.8	1.46	31.8	1.42	31.9	1.47
		1.99 m		1.74 m		1.86		1.86
8	31.3	1.44 m	32.9	1.48	32.0	1.50	32.1	1.49
9	49.9	0.82	50.3	1.51 d (2.0)	49.8	0.88	49.7	0.83
10	36.7		42.8		36.7		36.6	
11	20.3	nd[b]	23.9	1.54 d (5.3)	20.6	1.98	20.4	nd
		1.35		2.88 br d (12.6)		1.46		1.46
12	39.5	1.04 d (4.0)	40.6	1.27 m	32.0	1.85	31.7	1.86
		1.63		1.57		2.16 dd (3.9, 7.0)		2.16
13	40.1		40.4		44.8		44.8	
14	56.3	1.02	56.7	1.09 m	52.6	1.97	52.8	1.96
15	31.9	1.76	32.3	1.23 m	31.4	1.43	31.7	1.42 m
		1.96 m		1.56		2.14 m		1.45
16	80.9	4.46 t (7.5)	82.9	4.57	89.5	4.42	89.6	4.42 t (7.4)
17	62.4	1.76 t (7.5)	61.3	1.70 d (7.3)	89.9		89.9	
18	19.1	0.84 s	15.0	1.37 s	16.8	0.88	16.7	0.85 s
19	16.0	0.76 s	16.7	0.92 s	19.1	0.98 s	19.0	0.95 s
20	41.7	1.90 t (6.7)	37.4	2.83 t (6.9)	44.5	2.21 d (7.6)	44.6	2.26 d (7.3)
21	14.7	1.08 d (6.9)	14.6	1.05 d (6.9)	09.4	1.18 d (7.1)	09.2	1.16 d (7.4)
22	109.6		111.7		109.7		112.1	
23	31.4	1.62 d (3.3)	70.0	3.97 d (4.2)	31.4	nd	41.2	1.98
		1.64				nd		2.31 dd (12.4, 4.7)
24	28.9	1.52 m	82.2	4.78 d (4.2)	28.4	1.52	70.0	3.98 dd (5.0, 5.0)
		nd				nd		
25	29.8	1.25 m	143.5		30.0	1.50	39.0	1.78 m
26	66.6	3.43 br d (10.7)	61.4	3.99	66.4	3.43	64.8	3.50 dd (11.1, 11.6)
		3.53 d (8.5)		4.81 br d (11.7)		3.48		3.58 dd (4.5, 11.1)
27	17.0	0.63 d (5.0)	113.8	5.09 br s	16.9	0.62 (d, 5.1)	13.0	0.98 d (6.4)
				5.23 br s				

[a] 600 MHz (^{1}H), 125 MHz (^{13}C), pyridine-d_5, δ (ppm), coupling constants (J) in Hz are given in parentheses, overlapped signals are reported without designated multiplicity. [b] Not determined.

group at δ_C 170.9. Accordingly, the structure of **3** was determined to be pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-[6-*O*-acetyl]-β-D-glucopyranoside.

The molecular formula of arboreasaponin B (**4**) was determined to be $C_{45}H_{72}O_{18}$ by HRESIMS. Its FABMS showed a quasimolecular ion peak at *m/z* 899 $[M - H]^-$, indicating a molecular weight of 900, 16 mass units higher than that of **3**. Significant fragment ion peaks were observed at *m/z* 753 and 445, corresponding to the successive loss of two deoxyhexosyl and one hexosyl moiety. Comparison of the ^{1}H and ^{13}C NMR chemical shifts of **4** and **3** obtained from 2D NMR data (Tables 1 and 2) showed that most signals were superimposable, except for those of ring F in the aglycone of **4**. There were significant differences for the chemical shifts at δ_C 112.1, 41.2, 70.0, 39.0, 64.8, and 13.0, respectively, indicating the presence of one additional OH group. In the HMBC spectrum, the signals at δ_H 0.98 (d, $J = 6.4$ Hz, Me-27) showed longe-range correlations with carbons at δ_C 70.0 (C-24), 39.0 (C-25), and 64.8 (C-26), respectively. In the COSY spectrum, the Me-27 protons showed a correlation with δ_H 1.78 (m, H-25), which exhibited correlations with the pair of protons at C-26, respectively, at δ_H 3.50 (H-26ax, dd, $J = 11.1$, 11.6 Hz) and δ_H 3.58 (H-26eq, dd, $J = 4.5$ and 11.1 Hz) and with an oxymethine proton at δ_H 3.98 (H-24, dd, $J = 5.0$, 5.0 Hz). This proton, in turn, displayed correlations with methylene protons at δ_H 1.98 (H-23ax) and δ_H 2.31 (H-23eq). These correlations indicated an OH at C-24 in the F ring, which was confirmed by the deshielded signal of the C-27 methyl group (δ_C 13.0) instead of δ_C 9−9.5 in the case of CH_2-24.[15] The shift values of H_2-26 vicinal to β-axial H-25 and the coupling constant of the dd at δ_H 3.98 (H-24, dd, $J = 5.0$, 5.0 Hz) are understandable only if this oxymethine proton is located at C-24 in the β-equatorial configuration and is vicinal to the 25 β-axial and 23-methylene protons. These data revealed the α-axial orientation of the OH at C-24, which was confirmed by the absence of any NOESY correlation between δ_H 3.50 (H-26ax) and δ_H 3.98 (H-24). Therefore the aglycone of **4** was identified as 24α-hydroxypennogenin. This aglycone has been reported in only one plant species, *Paris axialis*,[16] but only partial NMR assignments were made. Scrutiny of the 2D NMR (COSY, NOESY, HMBC) data of **4** (Tables 1 and 2) showed that the oligosaccharidic moiety of **4** differed from that of **3** only by the absence of an acetyl group attached at C-6 of Glc. This was confirmed by the disappearance of the methyl signal at δ_C 20.4 and the carbonyl signal at δ_C 170.9 in the ^{13}C NMR spectrum of **4** and the presence of a shielded value of Glc C-6 at δ_C 61.6, with proton NMR signals H-6a and H-6b at δ_H 4.18 (d, $J = 5.2$ Hz) and δ_H 4.35 (dd, $J = 5.2$, 11.4 Hz), respectively. Thus, the structure of **4** was formulated as 24α-hydroxypennogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside.

The cytotoxicity of **2**−**4**, pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (manioside A),[3] pennogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside (spiroconazole A),[4] and neoruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside[10] was evaluated against the HT-29 and HCT 116 human colon cancer cell lines using the MTT assay.[17] The most active compound on both cell lines was spiroconazole A[4] (penno-

Table 2. ^{13}C and ^{1}H NMR Spectroscopic Data for Sugar Moieties of **14**[a]

	1		**2**		**3**		**4**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1-O-								
Fuc-1			100.5	4.69 d (7.3)				
2			78.1	4.05 d (3.6)				
3			84.4	4.07				
4			73.4	4.59 t (8.9)				
5			69.4	4.39 t (8.5)				
6			17.1	1.46 d (6.5)				
Xyl-1			106.4	4.95 d (7.3)				
2			74.0	3.89 t (7.9)				
3			78.1	4.07				
4			69.5	4.39				
5			67.0	3.67 d (4.8)				
				4.25 m				
Rha-1			101.7	6.26 br s				
2			72.4	4.75				
3			72.4	4.57 m				
4			73.4	4.24 d (9.3)				
5			69.4	4.75 m				
6			19.0	1.68 d (6.1)				
3-O-								
Glc-1	101.8	4.83 d (7.6)			99.7	4.73 d (7.8)	99.3	4.73 d (7.8)
2	74.9	3.88 t (8.6)			78.3	3.90	78.4	3.83 dd (8.8, 7.8)
3	76.0	4.04 t (8.8)			85.7	4.05 t (9.0)	86.0	4.01 t (8.8)
4	77.8	4.27 t (9.2)			69.6	3.76	69.4	3.82 t (8.8)
5	76.3	3.41 t (9.0)			78.3	3.90	77.2	3.76 m
6	60.9	4.00 dd (3.5, 11.7)			63.9	4.62 dd (5.2, 11.9)	61.6	4.18 dd (5.2, 12.1)
		4.11 d (11.7)				4.75 d (11.9)		4.35 d (12.1)
$\underline{CH_3}$-CO					20.4	1.94 br s		
CH_3-$\underline{C}O$					170.9			
Rha-1	102.1	5.74 br s			103.3	5.70 br s	103.0	5.58 br s
2	70.6	5.07 br s			71.9	4.75	71.7	4.66 br s
3	79.2	5.54 dd (2.8, 9.3)			72.1	4.41	71.9	4.67
4	71.8	4.48 d (4.7)			72.9	4. 24	72.8	4.21
5	70.0	4.92 d (3.1)			70.2	4.65	70.0	4.64
6	18.1	1.58 (d, 6.1)			18.0	1.58 d (5.9)	17.8	1.56 d (5.9)
Rha-1					102.1	5.63 br s	102.1	5.63 br s
2					71.7	4.64	71.5	4.61 br s
3					71.9	4.72	71.9	4.71
4					72.9	4.24 t (9.2)	72.9	4.22
5					69.4	4.70 m	69.4	4.68
6					18.1	1.66 d (5.9)	18.1	1.66 d (5.9)
24-O-								
Ara-1			106.3	5.11 d (8.1)				
2			72.7	4.37 t (8.5)				
3			74.5	4.04 d (3.2)				
4			71.5	3.74 d (6.5)				
5			66.9	3.62				
				4.21				

[a] 600 MHz (^{1}H), 125 MHz (^{13}C), pyridine-d_5, δ (ppm), coupling constants (J) in Hz are given in parentheses, overlapped signals are reported without designated multiplicity.

genin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucopyranoside), with IC_{50} values of 1.67 and 2.04 μM against HT-29 and HCT 116 cells, respectively. Compounds **2**, **3**, manioside A, and neoruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside exhibited moderate to low cytotoxicity on both cell lines, with IC_{50} values in the range 7.60−70.73 μM. Arboreasaponin B (**4**) was considered inactive, with an $IC_{50} > 100$ μM. Comparing the IC_{50} values of spiroconazole A with its acetylated derivative (**3**), it was observed that the acetyl group at Glc C-6 in **3** considerably decreased the cytotoxicity against HT-29 cells and had no effect against HCT116 cells.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on an AA-OR automatic polarimeter. 1D and 2D NMR spectra were recorded in pyridine-d_5 using a Varian INOVA-600 [600 MHz (^{1}H), 125 MHz (^{13}C)] NMR spectrometer. Solvent signals were used as internal standard (pyridine-d_5: δ_H 7.21, δ_C 123.5 ppm), and the coupling constants (J) are in Hz. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer and FABMS (negative-ion mode, glycerol matrix) on a Jeol-SX-102 mass spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph. TLC and HPTLC were carried out on precoated silica gel plates $60F_{254}$ (Merck) ($CHCl_3$−MeOH−H_2O−AcOH, 70:30:5:0.5 and 80:20:2:0.5). Saponins were detected with the Komarowsky reagent. Isolations were carried out using column chromatography (CC) on silica gel 60 (Merck, 70−200 μm), CC on Sephadex LH-20, vacuum-liquid chromatography (VLC) on reversed-phase RP-18 silica gel (Silicycle, 75−200 μm), medium-pressure liquid chromatography (MPLC) on silica gel 60 (Merck, 15−40 μm) (Gilson apparatus),[8] and flash chromatography on a Combiflash (Serlabo) [silica gel Redisep flash column (15−40 μm, 3.5 × 14 cm, 40 g)].

Plant Material. Stems of *D. deisteliana* and bark of *D. arborea* were collected in Yaoundé, village of Bangoua, near Bangangté, located in the Ndé Division of the western highlands of Cameroon in April 2007, and were identified by Dr. P. Nana, botanist of the National Herbarium of Cameroon (NHC), Yaoundé, where voucher specimens *D. deisteliana*: N1545 and *D. arborea*: 57326HNC were deposited.

Extraction and Isolation. Dried and finely powdered stems of *D. deisteliana* and bark of *D. arborea* (each 1.0 kg) were macerated with

MeOH (5 L) for 48 h. After evaporation of the solvent under vacuum, dark residues of 9.5 and 90 g, respectively, were obtained. The MeOH extracts were partitioned between *n*-BuOH and water. The *n*-BuOH-soluble portions were evaporated to dryness to afford yellowish powders (2.7 and 7.0 g, respectively). The *n*-BuOH extract (2.5 g) of *D. deisteliana* was then fractionated by flash chromatography on silica gel using $CHCl_3-MeOH-H_2O$ (70:30:2) as eluent, affording five fractions: F-1 (82 mg), F-2 (55 mg), F-3 (53 mg), F-4 (55 mg), and F-5 (157 mg). F-2 was purified by successive MPLC on RP-18 silica gel eluted with $MeOH-H_2O$ (60% → 100%), yielding **2** (18 mg). F-5 was purified under the same conditions to yield **1** (5.3 mg) and neoruscogenin 1-*O*-α-L-arabinopyranoside (10.3 mg),[9] neoruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside (7.6 mg),[10] and neoruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (7.4 mg).[10] Using the same procedure, an aliquot (2 g) of the *n*-BuOH extract of *D. arborea* afforded nine fractions. F-5 (71 mg) was a pure compound identified as spiroconazole A by comparison with literature data.[4] By successive MPLC on RP-18 silica gel, F-2 (21 mg) and F-3 yielded floribundasaponin A (3.2 mg), previously isolated from *Dioscorea floribunda*,[11] namogenin A (3.0 mg) isolated from *Dracaena augustifolia*,[12] and manioside A (10.6 mg) isolated from *Dracaena mannii*,[3] respectively. F-4 (55.3 mg) and F-6 (44 mg) were purified by repeated MPLC (silica gel, 15−40 μm) using a gradient system of solvent, $CHCl_3-MeOH-H_2O$ (80:20:2; 70:30:5), followed by MPLC on RP-18 silica gel eluted with a $MeOH-H_2O$ gradient system to yield **3** (4.1 mg) and **4** (12.2 mg), respectively.

Deistelianoside A (1): white, amorphous powder; $[\alpha]^{21}_D$ −60.1 (*c* 0.2, MeOH); IR (KBr) ν_{max}3385 (OH), 2925 and 2905 (CH), 1320 (C=C), 1255 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 801 $[M - H]^-$, 97 $[OSO_3H]^-$, 80 $[SO_3]^-$; HRESIMS (positive-ion mode) *m/z* 825.9560 $[M + Na]^+$ (calcd for 825.9553).

Deistelianoside B (2): white, amorphous powder; $[\alpha]^{21}_D$ −55.1 (*c* 0.15, MeOH); IR (KBr) ν_{max}3380 (OH), 2925 and 2905 (CH), 1405, 1320 (C=C), 1085 (OH), 1030, 965 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion mode) *m/z* 1015 $[M - H]^-$, 883 $[(M - H) - 132]^-$, 737 $[(M - H) - 132 - 146]^-$, 591 $[(M - H) - 132 - 146 - 146]^-$, 461 $[(M - H) - 132 - 146 - 146 - 132]^-$; HRESIMS (positive-ion mode) *m/z* 1039.4721 $[M + Na]^+$ (calcd for 1039.4726).

Arboreasaponin A (3): white, amorphous powder; $[\alpha]^{22}_D$ −48.2 (*c* 0.22, MeOH); IR (KBr) ν_{max}3420 (OH), 2910 (CH), 1320 (C=C), 1740 (C=O), 1255 cm-^{1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion) *m/z* 925 $[M - H]^-$, 883 $[(M - H) - 42]^-$, 737 $[(M - H) - 42 - 146]^-$, 429 $[(M - H) - 42 - 146 - 146 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 949.4769 $[M + Na]^+$ (calcd 949.4773).

Arboreasaponin B (4): white, amorphous powder; $[\alpha]^{22}_D$ −59.0 (*c* 0.22, MeOH); IR (KBr) ν_{max} 3420 (OH), 2910 (CH), 1320 (C=C), 1255 cm^{-1}; ^1H NMR and ^{13}C NMR (pyridine-d_5), see Tables 1 and 2; FABMS (negative-ion) *m/z* 899 $[M - H]^-$, 753 $[(M - H) - 146]^-$, 445 $[(M - H) - 146 - 146 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 923.4621 $[M + Na]^+$ (calcd 923.4616).

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.[18] D-Glucose, D-fucose, D-xylose, L-rhamnose, and L-arabinose were detected by co-injection of the hydrolysate with standard silylated samples. Identification of D-glucose and L-rhamnose was carried out for **1**, **3**, and **4**, giving peaks at 18.30 and 13.00 min for **1**, 18.32 and 12.99 min for **3**, and 18.32 and 13.02 min for **4**, respectively. Identification of D-fucose, D-xylose, L-rhamnose, and L-arabinose was carried out, giving peaks at 18.38, 13.50, 13.05, and 11.95 min, respectively, for **2**.

MTT Cytotoxicity Assay. The bioassay was carried out according to the method described by Carmichael et al.[17] with two human colon cancer cell lines (HCT 116 and HT-29). Paclitaxel was used as a positive control and exhibited IC_{50} values of 3.21 and 1.40 *n*M against HCT 116 and HT-29, respectively.

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Supporting Information Available: ^1H NMR and ^{13}C NMR spectra of compounds **1**−**4** and the results of the cytotoxicity of some compounds (Table 3) are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Mimaki, Y.; Kuroda, M.; Takaashi, Y.; Sashida, Y. *Phytochemistry* **1998**, *47*, 1351–1356.
(2) Noumi, E.; Amvam, Z. P.H.; Lontsi, D. *Fitoterapia* **1998**, *69*, 125–134.
(3) Tapondjou, L. A.; Ponou, K. B.; Teponno, R. B.; Mbiantcha, M.; Djoukeng, J. D.; Nguelefack, T. B.; Watcho, P.; Cadenas, A. G.; Park, H.-J. *Arch. Pharm. Res.* **2008**, *31*, 653–658.
(4) Okunji, C. O.; Iwu, M. M.; Hostettmann, K. *Int. J. Crude Drug Res.* **1991**, *29*, 66–70.
(5) Moharram, F. A.; El-Shenawy, S. M. *Planta Med.* **2007**, *73*, 1101–1106.
(6) Himmelreich, U.; Masaoud, M.; Adam, G.; Ripperger, H. *Phytochemistry* **1995**, *39*, 949–951.
(7) González, A. G.; León, F.; Hernández, J. C.; Padrón, J. I.; Sánchez-Pinto, L.; Barrera, J. B. *Biochem. Syst. Ecol.* **2004**, *32*, 179–184.
(8) Kougan, G. B.; Miyamoto, T.; Mirjolet, J. F.; Duchamp, O.; Sondengam, B. L.; Lacaille-Dubois, M.-A. *J. Nat. Prod.* **2009**, *72*, 1081–1086.
(9) De Combarieu, E.; Falzoni, M.; Fuzzati, N.; Gattesco, F.; Giori, A.; Lovati, M.; Pace, R. *Fitoterapia* **2002**, *73*, 583–596.
(10) Mimaki, Y.; Takaashi, Y.; Sashida, Y.; Nikaido, T. *Phytochemistry* **1996**, *42*, 1609–1615.
(11) Shashi, B.; Mahato, S. B.; Sahu, N. P; Ganguly, A. N. *Phytochemistry* **1981**, *20*, 1943–1946.
(12) Tran, L. Q.; Tran, K. Q.; Shigetoshi, K. *J. Chem.* **2004**, *42*, 122–124.
(13) Nakano, K.; Murakami, K.; Takaishi, Y.; Tomimatsu, T.; Nohara, T. *Chem. Pharm. Bull.* **1989**, *37*, 116–118.
(14) Mimaki, Y.; Watanabe, K.; Sakuma, C.; Sakagami, H.; Sashida, Y. *Helv. Chim. Acta* **2003**, *86*, 398–407.
(15) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479–2496.
(16) Changxiang, C.; Zhou, J. *Yunnan Zhiwu Yanjiu* **1984**, *6*, 111–117.
(17) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–942.
(18) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501–506.

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