Cytotoxic Spirostane-Type Saponins from the Roots of *Chlorophytum borivilianum*

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Four new spirostane-type saponins named borivilianosides E−H (**1**−**4**) were isolated from an ethanol extract of the roots of *Chlorophytum borivilianum* together with two known steroid saponins (**5** and **6**). The structures of **1**−**4** were elucidated using mainly 2D NMR spectroscopic techniques and mass spectrometry. The cytotoxicity of borivilianosides F (**2**), G (**3**), and H (**4**) and three known compounds was evaluated using two human colon cancer cell lines (HT-29 and HCT 116).

Steroidal saponins have attracted much attention due to their structural diversity and significant bioactivities such as cytotoxic, immunomodulating, antifungal, and insecticidal effects.[1,2] Several *Chlorophytum* species have been studied chemically and biologically and were found to contain steroidal saponins.[3] Among them, chloromaloside A from *C. malayense* showed broad cytotoxicity against various human cancer cells.[4]

The dried roots of *Chlorophytum borivilianum* Sant. and Fern. (Liliaceae) are used under the name of "Safed musli" as an Indian herb for the treatment of rheumatism and increasing general body immunity.[5] This species is also reported to have antidiabetic and spermatogenic properties.[3] In a search for bioactive steroidal saponins, a previous phytochemical investigation on *C. borivilianum* roots led to the isolation and structure elucidation of four new furostane-type steroidal saponins called borivilianosides A−D.[6] In a continuing study on the roots of this species, we describe in this paper the isolation and structure elucidation of four additional new spirostane-type steroidal saponins, borivilianosides E−H (**1**−**4**), together with two known saponins. The cytotoxic activity of several of these compounds was evaluated against the HT-29 and HCT 116 colon cancer cell lines.

The *n*-BuOH-soluble portion of an ethanol-soluble extract of the roots of *C. borivilianum* was subjected to RP-18 vacuum-liquid chromatography (VLC), followed by successive medium-pressure liquid chromatography (MPLC) on normal-phase silica gel 60 and reversed-phase silica gel RP-18, yielding four new steroidal saponins, borivilianosides E−H (**1**−**4**), as well as two known saponins isolated for the first time from this genus. The known compounds were identified by extensive NMR and MS analyses and comparison with literature data as gitogenin 3-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside,[7] and 25(*R,S*)-5α-spirostan-3*β*-ol 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.[8]

Compounds **1**−**4** were isolated as white, amorphous powders. The sugars obtained by aqueous acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as glucose, galactose, xylose, arabinose, and rhamnose (in the case of **1** and **2**), glucose and galactose (in the case of **3**), and glucose, galactose, and xylose (in the case of **4**). The absolute configurations of the sugars were determined to be D for galactose, glucose, and xylose and L for arabinose and rhamnose by GC analysis of chiral derivatives of the sugars in the hydrolysate of each compound (see Experimental Section). In the ^{1}H NMR spectra of the compounds, the relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of the Glc, Xyl, and Ara (between 7.0 and 8.0 Hz) moieties indicated a *β* anomeric proton for Gal, Glc, and Xyl and an α anomeric proton for Ara. The broad singlet of the anomeric proton of the Rha unit indicated an α-orientation.[9]

Borivilianoside E (**1**) exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at *m/z* 1643.7304 $[M + Na]^+$ (calcd for 1643.7310), consistent with the molecular formula, $C_{73}H_{120}O_{39}$. Its FABMS (negative-ion mode) showed a quasimolecular ion peak at *m/z* 1619 $[M - H]^-$, indicating a molecular weight of 1620. Further fragment ion peaks were observed at *m/z* 1457 $[(M - H) - 162]^-$, 1311 $[(M - H) - 162 - 146]^-$, 1149 $[(M - H) - 162 - 146 - 162]^-$, 1017 $[(M - H) - 162 - 146 - 162 - 132]^-$, 885 $[(M - H) - 162 - 146 - 162 - 132 - 132]^-$, and 723 $[(M - H) - 162 - 146 - 162 - 132 - 132 - 162]^-$, corresponding to the loss of three hexosyls, two pentosyls, and one desoxyhexosyl moiety. The ^{1}H and ^{13}C NMR spectra in combination with DEPT and HSQC spectra of **1** exhibited four characteristic methyls at δ_H 0.84 (s), 0.87 (s), 1.15 (d, $J = 6.8$ Hz), and 0.71 (d, $J = 4.9$ Hz) and a quaternary C atom resonance at δ_C 109.4 (C-22), indicating the presence of a steroidal spirostanol skeleton. Analysis of NMR data showed ^{13}C NMR signals at δ_C 44.6 (C-5), 54.4 (C-9), and 12.4 (C-19), characteristic of A/B *trans*-ring fusion, indicating that **1** is a 5α-steroidal spirostanol derivative. This was confirmed by the ROESY correlations observed between δ_H 3.92 (Agly H-3) and 0.92 (Agly H-5). The aglycon of **1** was identified as (25*R*)-3*β*,5α-spirostan-3-ol (tigogenin) by comparison of ^{13}C and ^{1}H NMR spectroscopic data of **1** obtained from the 2D-NMR spectra with those reported in the literature.[10] The 25*R* stereochemistry of the Me-27 group was deduced from the resonances of protons and carbons at C-25 (δ_C 30.6), C-26 (δ_C 66.8, δ_H 3.52, 3.59), and C-27 (δ_C 17.3, δ_H 0.71, d, $J = 4.9$ Hz).[11] The lower field resonance of C-27 (δ_C 17.3) as compared to the ^{13}C NMR chemical shift of (25*S*)-spirostanes at δ_C 16−16.5 and the ROESY correlations between H-3/H-5, H-11/H$_3$-19, H-11/H$_3$-18, H-9/H-14, H-16/H-17, and H-17/H$_3$-21 confirmed the relative configuration of **1** as having A/B *trans*, B/C *trans*, C/D *trans*, D/E *cis*, and C-20α stereochemistry.[11]

The ^{1}H NMR spectrum of **1** displayed signals for eight anomeric proton signals at δ_H 4.82 (d, $J = 7.8$ Hz), 4.94 (d, $J = 7.5$ Hz), 5.38 (d, $J = 8.0$ Hz), 5.26 (d, $J = 7.4$ Hz), 5.42 (brs), 6.14 (brs), 5.06 (d, $J = 7.9$ Hz), and 5.09 (d, $J = 7.0$ Hz), which gave HSQC correlations with eight anomeric carbon signals at δ_C 100.2, 105.4,

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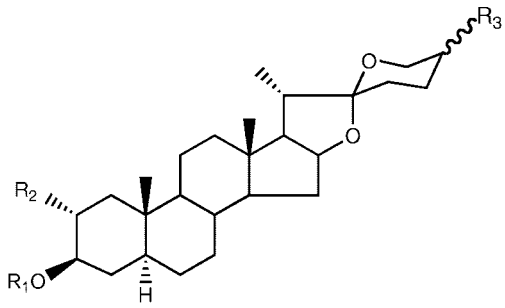

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3	S_2	OH	exo CH_2
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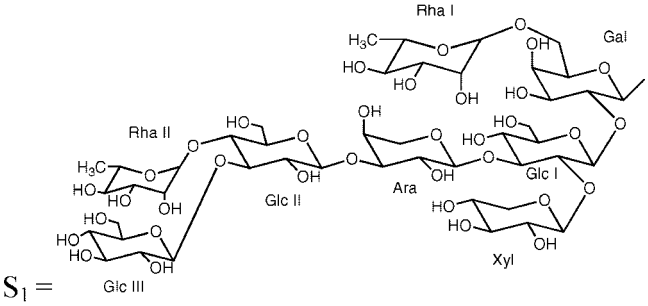

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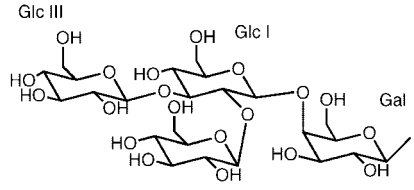

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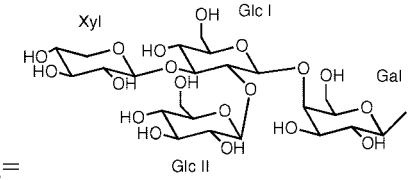

S_3=

105.4, 104.2, 102.9, 101.7, 104.6, and 104.9, respectively. The evaluation of chemical shifts and spin−spin couplings allowed the identification of one β-galactopyranosyl unit (Gal), three β-glucopyranosyl units (Glc I, Glc II, Glc III), two α-rhamnopyranosyl units (Rha I, Rha II), one β-xylopyranosyl unit, and one α-arabinopyranosyl unit in the molecule of **1**.[12] The absolute configuration of Glc, Gal, and Xyl was determined as D and those of Rha and Ara as L, as described above.

The sequence of the oligosaccharide chain of **1** was determined by HMBC and ROESY experiments. The HMBC correlations between the ^{1}H NMR signal at δ_H 4.82 (d, J = 7.8 Hz, Gal H-1) and the ^{13}C NMR signal at δ_C 77.0 (Agly C-3) and in the ROESY spectrum between δ_H 4.82 (Gal H-1) and δ_H 3.92 (m, Agly H-3) suggested the Gal was linked at C-3 of the aglycon. The correlation in the HMBC spectrum between the ^{1}H NMR signal at δ_H 5.42 (brs, Rha-I H-1) and δ_C 68.6 (Gal C-6) and the reverse correlation between δ_H 3.95 (Gal H-6) and δ_C 102.9 (Rha-I H-1) showed that the terminal Rha-I was bound to Gal through a (1→6) linkage. Furthermore, the correlation in the HMBC spectrum between the ^{1}H NMR signal at δ_H 4.94 (d, J = 7.5 Hz, Glc-I H-1) and δ_C 81.3 (Gal C-2) and the ROESY cross-peak between δ_H 4.94 (d, J = 7.5 Hz, Glc-I H-1) and δ_H 4.48 (Gal H-2) showed the Glc-I unit to be linked to Gal at C-2. The linkage of Xyl to the 2-position of Glc-I was deduced by the HMBC correlation observed between δ_H 5.06 (Xyl H-1, d, J = 7.9 Hz) and δ_C 81.2 (Glc-I C-2) and the reverse correlation observed between δ_H 4.21 (Glc-I H-2) and δ_C 104.6 (Xyl C-1). Thus, the first part of the sugar sequence linked at C-3 was characterized as Xyl-(1→2)-Glc-I (1→ 2)-[Rha-I (1→6)]-Gal (1→Agly C-3). Then, the remaining part was established in the same way. The linkage of Glc-II to the C-3 position of Ara was deduced by the HMBC correlation between δ_H 5.38 (Glc II H-1, d, J = 8.0 Hz) and δ_C 81.2 (Ara C-3). The linkage of Glc-III to the C-3 position of Glc-II was deduced by the HMBC correlation between δ_H 5.26 (Glc III H-1, d, J = 7.4 Hz) and δ_C 87.5 (Glc-II, C-3) and the ROESY correlation between δ_H 5.26 (Glc III H-1, d, J = 7.4 Hz) and δ_H 4.05 (Glc II H-3). The linkage of Rha-II to the C-4 position of Glc-II was deduced by the HMBC correlation between δ_H 6.14 (Rha-II H-1, brs) and δ_C 81.1 (Glc-II C-4) and the ROESY correlation between δ_H 6.14 (Rha-II, H-1) and δ_H 4.46 (Glc-II, H-4), proving that Rha-II was bound to Glc-II through a (1→4) linkage. Thus, the sequence of this second part of the oligosaccharide was elucidated as Glc-III (1→3)-[Rha II-(1→4) Glc-II-(1→3)-Ara-. Finally the linkage between Ara and Glc-I was ascertained to be (1→3) by observation of the HMBC correlation between δ_H 5.09 (Ara H-1, d, J = 7.0 Hz) and δ_C 87.4 (Glc-I C-3) and the reverse correlation between δ_H 4.04 (Glc I H-3) and δ_C 104.9 (Ara C-1). On the basis of the above observations, the structure of borivilianoside E (**1**) was elucidated as 25(*R*)-5α-spirostan-3β-ol 3-*O*-α-L-rhamnopyranosyl(1→4)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside.

Borivilanoside F (**2**) exhibited in the HRESIMS (positive-ion mode) a $[M + Na]^+$ peak at m/z 1641.7142 (calcd for 1641.7148), consistent with a molecular formula of $C_{73}H_{118}O_{39}$. The negative-ion FABMS displayed a quasimolecular ion peak at m/z 1617 $[M - H]^-$, two mass units lower than that of **1** and indicating a molecular weight of 1618. Further fragment ion peaks were observed at m/z 1455 $[(M - H) - 162]^-$, 1309 $[(M - H) - 162 - 146]^-$, 1147 $[(M - H) - 162 - 146 - 162]^-$, 1015 $[(M - H) - 162 - 146 - 162 - 132]^-$, 883 $[(M - H) - 162 - 146 - 162 - 132 - 132]^-$, 721 $[(M - H) - 162 - 146 - 162 - 132 - 132 - 162]^-$, corresponding respectively to the successive loss of four hexosyls, two pentosyls, and two desoxyhexosyl moieties. A detailed comparison of the ^{1}H NMR and ^{13}C NMR chemical shifts of **1** and **2** obtained from 2D-NMR data (Tables 1 and 2) showed that most signals were superimposable in both molecules except those of ring F in the aglycon part of **2**. The ^{1}H NMR spectrum of **2** allowed the assignment of two ethylenic protons at δ_H 4.79 brs and 4.82 brs (H_a-27 and H_b-27), with both giving a correlation with δ_C 108.8 (C-27) in the HSQC spectrum and long-range correlations in the HMBC spectrum respectively with δ_C 144.0 (C-25), δ_C 65.0 (C-26), and δ_C 29.0 (C-24), proving the presence of an exomethylene at C-25 in **2** instead a methyl group in **1**. The aglycon part of **2** was then identified as $\Delta^{25(27)}$-tigogenin.[13] Sugar analysis and sequencing was carried out using the same method as for **1**. Therefore, the structure of borivilianoside F (**2**) was elucidated as 5α-spirost-25(27)-ene-3β-ol 3-*O*-α-L-rhamnopyranosyl(1→4)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside.

The molecular formula of borivilianoside G (**3**) was deduced as $C_{51}H_{82}O_{24}$ on the basis of HRESIMS (positive-ion mode, $[M + Na]^+$ peak at m/z 1101.5110 (calcd for 1101.5094). The FABMS (negative-ion mode) showed a quasimolecular ion peak at m/z 1077 $[M - H]^-$, indicating a molecular weight of 1078. The NMR data of this compound revealed that its aglycon differs from the aglycon

Table 1. ^{1}H and ^{13}C NMR Data for the Aglycon Moieties of **1**−**4** in Pyridine-d_5 (δ in ppm, J in Hz)[a]

	1		**2**		**3**		**4**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	37.2	0.82, 1.58	37.2	0.82, 1.58	45.6	1.16, −[b]	45.6	1.17, 2.18
2	30.0	−[b], 2.04	30.0	−[b], 2.04	70.4	3.96	70.4	3.96
3	77.0	3.92 m	77.0	3.92 m	84.2	3.88 m	84.3	3.87 m
4	34.2	1.92, −[b]	34.4	1.92, −[b]	34.1	1.50, 1.86	34.1	1.42, 1.86
5	44.6	0.92	44.6	0.94	44.6	1.16	44.6	1.04
6	28.9	1.18, 2.26 m	28.9	1.18, 2.26	28.9	1.58, 2.70	28.9	1.16, −[b]
7	32.2	0.82, 1.54	32.2	0.82, 1.53	32.1	−[b], 1.53	32.1	−[b], 1.53
8	35.3	1.44	35.3	1.44	34.6	−[b]	34.6	−[b]
9	54.4	0.53	54.6	0.54	54.4	0.60	54.4	0.59
10	35.5		35.5		36.8		37.0	
11	21.4	−[b], 1.42	21.4	−[b], 1.45	21.4	−[b], 1.48	21.4	−[b], 1.48
12	40.2	1.06, 1.66	40.2	1.06, 1.66	40.0	1.63, −[b]	40.0	1.63, −[b]
13	40.8		40.9		40.4		40.4	
14	56.4	1.04	56.5	1.04	56.3	1.04	56.3	1.03
15	32.0	1.42, 2.02	32.0	1.42, 2.02	32.1	1.42, 2.02	32.1	1.42, 2.02
16	81.1	4.48	81.3	4.55	81.4	4.54	81.2	4.55
17	63.2	1.80 m	63.2	1.80 m	63.0	1.80	63.0	1.80
18	16.6	0.84 s	16.8	0.82 s	16.5	0.82 s	16.5	0.82 s
19	12.4	0.87 s	12.4	0.86 s	13.4	0.73 s	13.4	0.72 s
20	42.0	1.96 m	42.0	1.97 m	41.9	1.96	41.9	1.97 m
21	15.0	1.15 d (6.8)	15.0	1.10 d (6.9)	14.9	1.09 d (6.9)	14.9	1.09 d (6.9)
22	109.4		109.2		109.2		109.2	
23	31.8	1.66, 1.71	32.0	1.26, 1.54	31.9	1.40, 1.66	32.1	1.40, −[b]
24	29.2	1.58 m, −[b]	29.0	1.58 m, −[b]	29.2	1.36, −[b]	29.8	−[b], 2.25
25	30.6	−[b]	144.0		144.0		144.4	
26	66.8	3.52 m, 3.59 m	65.0	4.04, 4.47	65.3	4.04, 4.46	65.3	4.04, 4.45
27	17.3	0.71 d (4.9)	108.8	4.79, 4.82	108.6	4.79, 4.82	108.6	4.79, 4.82

[a] Overlapping ^{1}H NMR signals are reported without designated multiplicity. [b] Not determined.

of compound **2** in the NMR signals of ring A (Table 1). The main difference was the observation of a downfield ^{13}C NMR chemical shift at δ_C 70.4 (C-2), showing a secondary alcoholic function at this position and the downfield ^{13}C NMR chemical shift of C-3 at δ_C 84.2 instead of 77.0 in compound **2**. The 2α orientation of the hydroxyl unit was evident from the ROESY cross-peak between δ_H 0.73 (s, ax H_3-19) and δ_H 3.96 (m, H-2), proving the axial orientation of this proton. The comparison of NMR data of **3** with literature values allowed the identification of the aglycon as the previously reported 2α,3β-5α-spirostene-25(27)-2,3-diol ($\Delta^{25(27)}$-gitogenin),[13] which has ben encountered for the first time in the genus *Chlorophytum*.

The ^{1}H NMR spectrum of **3** displayed four anomeric proton signals at δ_H 4.91(d, J = 7.6 Hz), 5.17 (d, J = 7.5 Hz), 5.59 (d, J = 7.6 Hz), and 5.29 (d, J = 7.5 Hz), which gave correlations with four anomeric carbon signals at δ_C 103.2, 104.6, 104.9, and 104.5, respectively, in the HSQC spectrum. The complete assignment of the glycosidic NMR signals was achieved by analysis of COSY, TOCSY, ROESY, HSQC, and HMBC experiments (Table 2). Evaluation of spin−spin couplings and chemical shifts allowed the identification of three β-glucopyranosyl (Glc I, Glc II, Glc III) and one β-galactopyranosyl (Gal) unit. The D configuration was determined as previously described for compounds **1** and **2**. The cross-peaks in the HMBC spectrum between δ_H 5.29 (Glc III H-1) and δ_C 88.6 (Glc I C-3) and between δ_H 5.59 (Glc II H-1) and δ_C 81.2 (Glc I C-2), the reverse correlation between δ_H 4.32 (Glc I H-2) and δ_C 104.9 (Glc II C-1), further HMBC correlations between δ_H 5.17 (Glc I H-1) and δ_C 79.6 (Gal C-4), the reverse correlation between δ_H 4.58 (Gal H-4) and 104.6 (Glc I C-1) and between δ_H 4.91 (Gal H-1) and δ_C 84.2 (Agly C-3), and the ROESY correlations between δ_H 5.29 (Glc III H-1) and δ_H 4.17 (Glc I H-3), between δ_H 5.59 (Glc II H-1) and δ_H 4.32 (Glc I H-2), between δ_H 5.17 (Glc I H-1) and δ_H 4.58 (Gal H-4), and between δ_H 4.91 (Gal H-1) and δ_H 3.88 (Agly H-3) provided evidence for the nature of the oligosaccharide chain at C-3. Therefore, the structure of borivilianoside C (**3**) was established as 5α-spirost-25(27)-ene-2α,3β-diol 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Borivilianoside H (**4**) exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at m/z 1071.4992 $[M + Na]^+$ (calcd for 1071.4988), consistent with a molecular formula of $C_{50}H_{80}O_{23}$. Its FABMS (negative-ion mode) showed a quasimolecular ion peak at m/z 1047 $[M - H]^-$, indicating a molecular weight of 1048. The ^{1}H and ^{13}C NMR signals assigned from the 2D NMR spectroscopic data, corresponding to the aglycon part of **4** (Table 1), were superimposable with those described for **3**, showing the presence of the same aglycon characterized as 5α-spirost-25(27)-ene-2α,3β-diol ($\Delta^{25(27)}$gitogenin).[13]

The ^{1}H NMR spectrum of **4** showed four anomeric proton signals at δ_H 4.91 (d, J = 7.4 Hz), 5.21 (d, J = 7.6 Hz), 5.59 (d, J = 7.6 Hz), and 5.25 (d, J = 7.7 Hz), which gave correlations in the HSQC spectrum with four anomeric carbon signals at δ_C 103.3, 104.8, 104.5, and 104.8, respectively. 2D-NMR experiments indicated that two β-D-glucopyranosyls, one β-D-galactopyranosyl, and one β-D-xylopyranosyl unit was present (Table 2). The D configuration was determined as for previously described compounds (**1**−**3**). The cross-peak in the HMBC spectrum between δ_H 5.25 (Xyl H-1) and δ_C 87.2 (Glc I C-3), between δ_H 5.59 (Glc II H-1) and δ_C 81.1 (Glc I C-2), between δ_H 5.21 (Glc I H-1) and δ_C 79.5 (Gal C-4), and between δ_H 4.91 (Gal H-1) and δ_C 84.3 (Agly C-3), together with the reverse correlations between δ_H 4.14 (Glc I H-3) and δ_C 104.8 (Xyl C-1), between δ_H 4.36 (Glc I H-2) and δ_C 104.5 (Glc II H-1), between δ_H 4.61 (Gal H-4) and δ_C 104.8 (Glc I C-1), and the ROESY correlations between δ_H 5.25 (Xyl H-1) and δ_H 4.14 (Glc I H-3), between δ_H 5.59 (Glc II H-1) and δ_H 4.36 (Glc I H-2), between δ_H 5.21 (Glc I H-1) and δ_H 4.61 (Gal H-4), and between δ_H 4.91 (Gal H-1) and δ_H 3.87 (Agly H-3) provided evidence for the oligosaccharide chain at C-3. Therefore, the structure of borivilianoside H (**4**) was established as 5α-spirost-25(27)-ene-2α,3β-diol 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(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] we have tested borivilianosides F (**2**), G (**3**), and H (**4**), 25(*R,S*)-5α-spirostan-3β-ol 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, and the

Table 2. ^{1}H and ^{13}C NMR Data for the Sugar Moities of **1**−**4** in Pyridine-d_5 (δ in ppm, J in Hz)[a]

	1		**2**		**3**		**4**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Gal-1	100.2	4.82 d (7.8)	100.2	4.83 d (7.8)	103.2	4.91 d (7.6)	103.3	4.91 d (7.4)
2	81.3	4.48	81.3	4.48	72.5	4.54	72.5	4.54
3	75.8	4.13	75.8	4.13	75.3	4.11	75.6	4.13
4	69.6	4.11	69.6	4.11	79.6	4.58	79.5	4.61
5	75.6	3.95	75.6	3.95	76.0	4.04	75.5	4.04
6	68.6	3.95, 4.62	68.6	3.96, 4.62	60.5	4.18, 4.60	60.5	4.18, 4.60
Glc-I-1	105.4	4.94 d (7.5)	105.4	4.94 d (7.5)	104.6	5.17 d (7.5)	104.8	5.21 d (7.6)
2	81.2	4.21	81.2	4.21	81.2	4.32 t (9.0)	81.1	4.36 t (9.0)
3	87.4	4.04	87.4	4.04	88.6	4.17	87.2	4.14
4	72.4	4.50	72.5	4.50	70.4	3.82	70.4	3.81
5	77.8	3.78	77.8	3.78	77.5	3.83	77.5	3.83
6	62.9	4.00, 4.49	62.9	4.00, 4.50	62.9	4.02, 4.47	62.9	4.06, 4.49
Glc-II-1	105.4	5.38 d (8.0)	105.4	5.38 d (8.0)	104.9	5.59 d (7.6)	104.5	5.59 d (7.6)
2	74.8	3.95	74.8	3.95	75.4	4.03	75.5	4.05
3	87.5	4.05	87.5	4.05	78.3	4.13	78.2	4.13
4	81.1	4.46	81.1	4.47	70.4	3.96	70.4	4.09
5	78.0	3.94	78.0	3.95	78.2	3.88	78.2	3.92
6	61.8	4.36, 4.44	61.8	4.38, 4.44	62.3	4.25, 4.53	62.7	4.40, dd (11.2, 2.1), 4.54
Glc-III-1	104.2	5.26 d (7.4)	104.3	5.23 d (7.4)	104.5	5.29 d (7.5)		
2	75.0	3.97	74.9	3.98	75.4	4.03		
3	77.4	4.14	77.6	4.15	78.4	4.18		
4	70.0	4.10	70.0	4.10	71.5	4.11		
5	77.6	3.82	77.6	3.94	78.6	4.01		
6	60.4	4.20, 4.68	60.4	4.22, 4.66	62.4	4.25, 4.42		
Rha-I-1	102.9	5.42 brs	102.9	5.42 brs				
2	71.8	4.72	71.8	4.72				
3	72.5	4.53	72.5	4.53				
4	73.8	4.19	74.0	4.22				
5	69.8	4.89	69.6	4.89				
6	18.5	1.59 d (6.2)	18.5	1.60 d (6.2)				
Rha-II-1	101.7	6.14 brs	101.7	6.25 brs				
2	72.4	4.74	72.4	4.78				
3	72.6	4.52	72.6	4.52				
4	74.0	4.22	74.0	4.26				
5	69.5	4.90	69.5	4.89				
6	18.4	1.71 d (6.3)	18.4	1.70 d (6.3)				
Xyl-1	104.6	5.06 d (7.9)	104.6	5.06 d (7.9)			104.8	5.25 d (7.7)
2	74.8	4.00	74.7	3.99			75.2	3.97
3	76.7	4.02	76.7	4.02			78.2	4.08
4	71.7	3.94	69.1	4.11			70.4	4.13
5	66.4	3.60 t (9.9), 4.16	66.4	3.60 t (9.8), 4.16			67.2	3.66 t (9.5), 4.22
Ara-1	104.9	5.09 d (7.0)	104.9	5.09 d (7.0)				
2	74.0	4.00	74.5	4.00				
3	81.2	4.15	81.2	4.15				
4	70.7	4.42	70.7	4.42				
5	63.0	4.45, −[b]	63.0	4.45, 4.50				

[a] Overlapping ^{1}H NMR signals are reported without designated multiplicity. [b] Not determined.

previously reported borivilianosides A[6] and C[6] for cytotoxicity against the HCT 116 and HT-29 human colon tumor cell lines, using paclitaxel as a standard anticancer drug. As determined by a MTT assay,[14] the only active substance was compound **4**, which showed the best cytotoxicity (0.38 μM in HCT 116 cells and 2.6 μM in HT-29 cells).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on an AA-OR automatic polarimeter. NMR experiments were measured on a Varian VNMR-S 600 MHz spectrometer equipped with 3 mm triple resonance inverse and 3 mm dual broadband probe-heads. Spectra were recorded in 150 μL of pyridine-d_5. Solvent signals were used as internal standard (pyridine-d_5: δ_H = 7.21, δ_C = 123.5 ppm), and all spectra were recorded at T = 35 °C. Pulse sequences were taken from the Varian pulse sequence library (gCOSY, gHSQCAD, and gHMBCAD with adiabatic pulses). TOCSY spectra are acquired using DIPSI spin-lock and 150 ms mixing time. Mixing time in ROESY experiments: 300 ms. Carbon type (CH_3,CH_2, CH): DEPT experiments. The 1D and 2D NMR spectra of certain compounds were recorded in pyridine-d_5 on a Varian INOVA-600 (600 MHz).[15] HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FABMS (negative-ion mode, glycerol matrix) was conducted on a JEOL SX 102 spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph. TLC and HPTLC were achieved on precoated silica gel plates 60 F254 (Merck).[15] Isolations were carried out using vacuum-liquid chromatography (VLC) on reversed-phase RP-18 (Merck, 25−40 μm) and medium-pressure liquid chromatography (MPLC) on silica gel 60 (Merck, 15−40 μm) and reversed-phase RP-18 (Merck, 25−40 μm).[15]

Plant Material. The roots of *C. borivilianum* were provided from Jeevan Herbs in 2006 (New Delhi, India) and identified by Dr. M. Ahmedullah (Botanic Garden of Indian Republic, BGIR, New Delhi, India). A voucher specimen (no. 6628) is deposited in the herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Burgundy, France.

Extraction and Isolation. The dried, powdered roots of *C. borivilianum* (500 g) were refluxed three times with EtOH (3 × 2 L) for 1 h. After evaporation of the solvent in vacuo, the resulting EtOH extract (19 g) was suspended in water (200 mL) and partitioned successively with CH_2Cl_2 (3 × 300 mL) and *n*-BuOH (3 × 200 mL), yielding after evaporation of solvents the corresponding CH_2Cl_2 (3.2 g) and *n*-BuOH (4.1 g) fractions. A 4 g aliquot of the *n*-BuOH residue was submitted to VLC on C_{18} reversed-phase silica gel using H_2O (3 × 100 mL), MeOH−H_2O mixtures (5:5, 3 × 100 mL), and finally MeOH (100 mL)

as eluents. After evaporation of the solvents, three fractions were obtained: Fr-1 (H_2O), Fr-2 (MeOH−H_2O, 5:5) (300 mg), and Fr-3 (MeOH) (1.1 g). Fr-3 (1.1 g) was submitted to MPLC [system A: silica gel (15−40 μm), $CHCl_3$−MeOH−H_2O (65:35:10, lower phase)] to give 17 subfractions (1−17). Subfraction 7 (38 mg) was rechromatographed by MPLC [system B: reversed-phase silica gel RP-18 eluted with MeOH−H_2O (40 → 100%)], giving compounds **1** (4.5 mg) and **2** (4.1 mg). Subfraction 3 (90 mg) was submitted to MPLC (system A), giving compounds **3** (3.4 mg) and **5** (3.6 mg). Subfraction 2 (97.6 mg) was submitted to MPLC (System B) yielding **4** (3.9 mg) and **6** (6 mg).

Borivilianoside E (1): white, amorphous powder; $[\alpha]^{20}_D$ −66.7 (*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* 1619 $[M - H]^-$, 1457 $[(M - H) - 162]^-$, 1311 $[(M - H) - 162 - 146]^-$, 1149 $[(M - H) - 162 - 146 - 162]^-$, 1017 $[(M - H) - 162 - 146 - 162 - 132]^-$, 885 $[(M - H) - 162 - 146 - 162 - 132 - 132]^-$, 723 $[(M - H) - 162 - 146 - 162 - 132 - 132 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1643.7304 $[M + Na]^+$ (calcd for $C_{73}H_{120}O_{39}Na$ 1643.7310).

Borivilianoside F (2): white, amorphous powder; $[\alpha]^{20}_D$ −70.8 (*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* 1617 $[M - H]^-$, 1455 $[(M - H) - 162]^-$, 1309 $[(M - H) - 162 - 146]^-$, 1147 $[(M - H) - 162 - 146 - 162]^-$, 1015 $[(M - H) - 162 - 146 - 162 - 132]^-$, 883 $[(M - H) - 162 - 146 - 162 - 132 - 132]^-$, 721 $[(M - H) - 162 - 146 - 162 - 132 - 132 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1641.7142 $[M + Na]^+$ (calcd for $C_{73}H_{118}O_{39}Na$ 1641.7148).

Borivilianoside G (3): white, amorphous powder; $[\alpha]^{20}_D$ −57.9 (*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* 1077 $[M - H]^-$, 915 $[(M - H) - 162]^-$, 753 $[(M - H) - 162 - 162]^-$, 591 $[(M - H) - 162 - 162 - 162]^-$, 429 $[(M - H) - 162 - 162 - 162 - 162)^-$; HRESIMS (positive-ion mode) *m/z* 1101.5110 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{24}Na$ 1101.5094).

Borivilianoside H (4): white, amorphous powder; $[\alpha]^{20}_D$ −65.1 (*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* 1047 $[M - H]^-$, 915 $[(M - H) - 132]^-$, 753 $[(M - H) - 132 - 162]^-$, 591 $[(M - H) - 132 - 162 - 162]^-$; HRESIMS (positive-ion mode) *m/z* 1071.4992 $[M + Na]^+$ (calcd for $C_{50}H_{80}O_{24}Na$ 1071.4988).

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: galactose (R_f 0.21), glucose (R_f 0.23), xylose (R_f 0.47), arabinose (R_f 0.44), and rhamnose (R_f 0.51) for **1** and **2** and galactose (R_f 0.21) and glucose (R_f 0.23) for **3**, and galactose (R_f 0.21), glucose (R_f 0.23), and xylose (R_f 0.47) for **4**. The trimethylsilyl thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC.[16] The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich). This, D-glucose, D-galactose, D-xylose, L-arabinose, and L-rhamnose were detected for **1** and **2**, giving single peaks at 18.5, 21.9, 14.0, 12.0, and 13.5 min, respectively. In the same manner, D-glucose and D-galactose were identified for **3** and D-glucose, D-galactose, and D-xylose for **4**.

MTT Cytotoxicity Assay. The bioassay was carried out according to the method described in ref 14 with two human colorectal cancer cell lines (HCT 116 and HT-29). Paclitaxel was used as positive control and exhibited IC_{50} values of 1.1 nM (HCT 116) and 3.6 nM (HT-29).

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Supporting Information Available: Figure S1 showing FABMS fragments and main HMBC correlations of **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

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