Furostane-Type Steroidal Saponins from the Roots of *Chlorophytum borivilianum*

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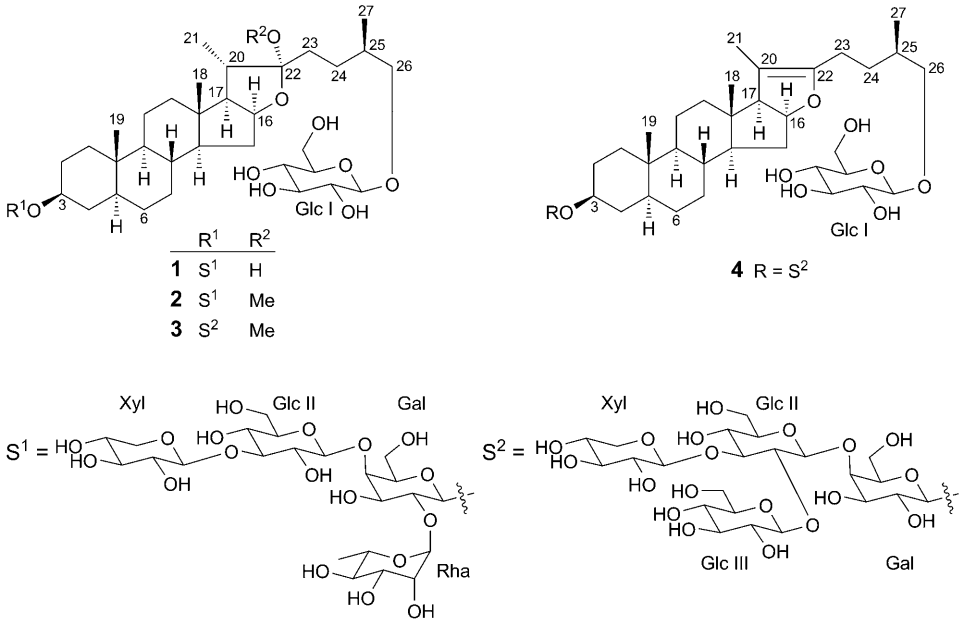
Four new furostanol steroid saponins, borivilianosides A–D (**1**–**4**, resp.), corresponding to (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside (**1**), (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside (**2**), (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**3**), and (3*β*,5*α*,25*R*)-26-(*β*-D-glucopyranosyloxy)furost-20(22)-en-3-yl *O*-*β*-D-xylopyranosyl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**4**), together with the known tribuluside A and (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside were isolated from the dried roots of *Chlorophytum borivilianum* SANT and FERN. Their structures were elucidated by 2D-NMR analyses (COSY, TOCSY, NOESY, HSQC, and HMBC) and mass spectrometry.

Introduction. – The genus *Chlorophytum* (Liliaceae) comprises *ca.* 215 species which grow as perennial rhizomatous herbs in pantropical regions [1]. They are mainly cultivated for their ornamental flowers. Traditionally, roots of these species are reputed to possess various pharmacological utilities having saponins as one of the important phytochemical constituents [2]. Steroid saponins have attracted the attention for their structural diversity and significant bioactivities such as cytotoxic, immunomodulating, antifungal, insecticidal, *etc.*, activities [3–5]. Previous phytochemical investigations of various *Chlorophytum* species have resulted in the isolation of steroidal saponins [1][6–8]. *Chlorophytum borivilianum* SANT. and FERN. commonly known as 'safed musli' in India is traditionally used as a general tonic in treating rheumatism and increasing general body immunity [9]. It is also reported to have spermatogenic and antidiabetic properties. A previous report reveals antidiabetic activity which was related to a polysaccharide fraction [9], but no systematic study on the secondary metabolites of this plant has been reported so far. In the present article, we describe the isolation and structure elucidation of four new steroid saponins from the roots of *C.*

borivilianum, borivilianosides A–D (**1**–**4**, resp.), together with two known compounds, tribuluside A (=(3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glactopyranoside) [10], and (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside [11].



Results and Discussion. – The BuOH fraction of the EtOH extract of the roots of *C. borivilianum* was submitted to multiple chromatographic steps involving vacuum-liquid chromatography (VLC; reversed-phase *RP-18* silica gel) and medium-pressure liquid chromatography (MPLC; silica gel and reversed-phase *RP-18* silica gel) yielding borivilianosides A–D (**1**–**4**), tribuluside A [10], and (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside [11]. Their structures were determined mainly by spectroscopic methods including 1D- and 2D-NMR experiments (COSY, TOCSY, HSQC, NOESY, and HMBC) in combination with HR-ESI- and FAB-MS.

Compounds **1**–**4** were isolated as amorphous powders. The sugars obtained by aqueous acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as xylose, glucose, galactose, and rhamnose (=6-deoxymannose) in the cases of **1** and **2**, and as glucose, galactose, and xylose in the cases of **3** and **4**. The absolute configurations of the sugars were determined to be D for glucose, galactose, and xylose and L for rhamnose by GC analysis of their (trimethylsilyl)imidazole derivatives (see *Exper. Part*).

Borivilianoside A (**1**) exhibited in the HR-ESI-MS (pos.) the $[M+Na]^+$ peak at *m/z* 1221.5825 consistent with the molecular formula $C_{56}H_{94}O_{27}$. Negative-ion FAB-MS of **1** displayed a pseudomolecular-ion peak at *m/z* 1197 ($[M-H]^-$) in accordance with the $[M+Na]^+$ peak. Other fragment-ion peaks were observed at *m/z* 1065 ($[M-H-132]^-$), 903 ($[M-H-132-162]^-$), and 757 ($[M-H-132-162-146]^-$) suggesting the successive elimination of two pentosyl, one hexosyl, and one deoxyhexosyl moiety, respectively. The aglycone of **1** was identified as (3*β*,5*α*,22*R*,25*R*)-furostane-3,22,26-triol by comparison of its NMR spectral data based on correlations observed in the COSY, NOESY, HSQC, and HMBC spectra with those reported in [12–14]. On the basis of ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*) and 2D-NMR analyses, the structure of **1** was elucidated as (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-hydroxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-galactopyranoside.

The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited four characteristic Me signals at *δ*(H) 0.77 (*s*), 0.75 (*s*), 0.90 (*d*, *J* = 6.0 Hz), and 1.23 (*d*, *J* = 6.5 Hz), and a quaternary-C-atom resonance at *δ*(C) 110.2 (C(22)) indicating the presence of a steroidal skeleton. In addition, the *A*/*B trans* fusion was deduced by the signals at *δ*(C) 44.2 (C(5)), 54.0 (C(9)), and 12.1 (C(19)) suggesting that **1** was a (5*α*)-furostanol derivative [12]. This was confirmed by the NOESY correlation *δ*(H) 3.82 (H–C(3))/0.81–0.86 (*m*, H–C(5)). The (22*α*) configuration of **1** was deduced by considering the downfield shift of signals at *δ*(H) 1.23 (*d*, *J* = 6.5 Hz, Me(21)) and 4.86 (H–C(16)), which is due to the deshielding effect of the *cis*-oriented OH–C(22) [15]. The (25*R*) configuration was deduced by considering the chemical shifts at *δ*(C) 33.6 (C(25)), 74.9 (C(26)), and 16.8 (C(27)) [16][17]. The above was supported by the difference between the chemical shifts of H_a–C(26) and H_b–C(26) ($\delta_a - \delta_b = 0.27$) since the difference is usually > 0.57 for (25*S*) configurations and < 0.48 for (25*R*) configurations [16][17]. The ^{1}H-NMR spectrum of **1** displayed signals for five anomeric H-atoms at *δ*(H) 4.70 (*d*, *J* = 7.5 Hz), 4.72 (*d*, *J* = 7.5 Hz), 5.04 (*d*, *J* = 7.8 Hz), 4.83 (*d*, *J* = 7.5 Hz), and 5.96 (br. *s*), which showed HSQC cross-peaks with five anomeric C-atom signals at *δ*(C) 104.2, 104.1, 104.2, 104.5, and 101.2, resp. The evaluation of chemical shifts and spin–spin couplings obtained from the 2D-NMR data allowed the identification of two *β*-glucopyranosyl units (Glc I and Glc II), one *β*-galactopyranosyl unit (Gal), one *β*-xylopyranosyl unit (Xyl), and one *α*-rhamnopyranosyl unit (Rha) [18]. The relatively large ^3J(1,2) values (7.3–8.0 Hz) for the Glc, Gal, and Xyl indicated *β*-anomeric orientation for these sugars. The multiplicity of the anomeric ^{1}H-NMR signal of Rha (br. *s*) indicated *α*-anomeric orientation. The linkage of Glc I at the C(26) position was indicated by long-range coupling (^3J) in the HMBC spectrum between the anomeric H-atom at *δ*(H) 4.70 (Glc I H–C(1)) and C(26) at *δ*(C) 74.9 (Agly). The HMBC cross-peak *δ*(H) 4.72 (Gal H–C(1))/*δ*(C) 77.6 (Agly C(3)) established the linkage of Gal to C(3) of the aglycone. Furthermore, the HMBC cross-peaks *δ*(H) 4.30 (Gal H–C(2))/*δ*(C) 101.2 (Rha C(1)), *δ*(H) 4.83 (Glc II H–C(1))/*δ*(C) 81.0 (Gal C(4)), and *δ*(H) 5.04 (Xyl H–C(1)/*δ*(C) 87.1 (Glc II C(3)) established the sugar sequence in **1**.

Borivilianoside B (**2**) exhibited in the HR-ESI-MS (pos.) the $[M+Na]^+$ peak at *m/z* 1235.6043, consistent with the molecular formula $C_{57}H_{96}O_{27}$. Negative-ion FAB-MS of **2** revealed a pseudomolecular-ion peak at *m/z* 1211 ($[M-H]^-$) in accordance with the $[M+Na]^+$ peak. Other fragment-ion peaks were observed at *m/z* 1079 ($[M-H-132]^-$), 917 ($[M-H-132-162]^-$), and 771 ($[M-H-132-162-146]^-$) corresponding to the loss of one pentosyl, one hexosyl, and one desoxyhexosyl moiety, respectively. The ^{1}H- and ^{13}C-NMR and 2D-NMR data of **2** (*Tables 1* and *2*) led to the establishment of its structure as (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-galactopyranoside. Since 22-hydroxyfurostane de-

Table 1. *^{1}H- and ^{13}C-NMR Data* ((D_5)Pyridine) *of the Aglycone Part of* **1**–**4**. δ in ppm, J in Hz; n.d. = not determined.

	1		**2**		**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH_2(1)	36.8	0.73[a]), 1.45–1.50 (*m*)	36.8	0.73[a]), 1.45–1.50 (*m*)	36.5	0.68[a]), 1.40[a])	36.5	0.71[a]), 1.43[a])
CH_2(2)	29.6	1.17[a]), n.d.	29.2	1.17[a]), n.d.	29.0	1.58[a]), 1.98–2.02 (*m*)	29.0	1.59[a]), 1.98–2.02 (*m*)
H–C(3)	77.6	3.82[a])	77.6	3.82[a])	77.1	3.83[a])	77.1	3.84[a])
CH_2(4)	34.7	1.25[a]), n.d.	34.7	1.25[a]), n.d.	34.0	1.30–1.34 (*m*), 1.72–1.76 (*m*)	34.0	1.30–1.34 (*m*), 1.72–1.76 (*m*)
H–C(5)	44.2	0.81–0.86 (*m*)	44.2	0.82–0.85 (*m*)	43.5	0.78–0.84 (*m*)	43.5	0.78–0.84 (*m*)
CH_2(6)	28.6	1.11[a]), 1.13[a])	28.6	1.11[a]), 1.13[a])	28.3	1.00[a]), 1.06[a])	28.3	1.01[a]), 1.08[a])
CH_2(7)	31.9	1.23[a]), 1.40[a])	31.9	1.23[a]), 1.40[a])	31.5	1.26[a]), 1.38[a])	31.5	1.24[a]), 1.36[a])
H–C(8)	34.9	1.26–1.33 (*m*)	34.9	1.26–1.33 (*m*)	34.2	1.22–1.26 (*m*)	34.2	1.20–1.26 (*m*)
H–C(9)	54.0	0.39–0.43 (*m*)	54.0	0.39–0.43 (*m*)	53.5	0.36–0.42 (*m*)	53.5	0.36–0.42 (*m*)
C(10)	35.3		35.3		35.2		35.2	
CH_2(11)	21.2	1.31[a]), n.d.	21.2	1.31[a]), n.d.	20.4	1.06[a]), 1.28[a])	20.4	1.08[a]), 1.27[a])
CH_2(12)	39.5	0.97[a]), 1.63[a])	39.5	0.97[a]), 1.63[a])	39.2	0.92[a]), 1.56[a])	39.2	0.93[a]), 1.58[a])
C(13)	40.8		40.2		40.6		40.6	
H–C(14)	55.9	0.90[a])	55.9	0.90[a])	55.5	0.88[a])	55.5	0.89[a])
CH_2(15)	31.8	1.24[a]), 1.92[a])	31.8	1.24[a]), 1.92[a])	31.5	1.26[a]), 1.88–1.92 (*m*)	31.5	1.27[a]), 1.88–1.92 (*m*)
H–C(16)	81.0	4.86[a])	81.0	4.40[a])	80.7	4.37[a])	83.1	4.70–4.74 (*m*)
H–C(17)	62.9	1.85–1.89 (*m*)	64.0	1.66[a])	63.2	1.64–1.68 (*m*)	63.5	2.34–2.37 (*m*)
Me(18)	16.3	0.77 (*s*)	16.2	0.76 (*s*)	15.5	0.70 (*s*)	15.5	0.70 (*s*)
Me(19)	12.1	0.75 (*s*)	12.1	0.75 (*s*)	12.9	0.69 (*s*)	12.9	0.59 (*s*)
H–C(20) or C(20)	40.2	2.12–2.18 (*m*)	40.2	2.11–2.17 (*m*)	39.8	2.14–2.18 (*m*)	104.0	
Me(21)	15.9	1.23, (*d*, *J* = 6.5)	15.9	1.10 (*d*, *J* = 6.5)	15.5	1.09 (*d*, *J* = 6.5)	11.9	1.55 (*s*)
C(22)	110.2		112.1		112.0		152.0	
CH_2(23)	36.4	1.99[a]), n.d.	30.0	1.98[a]), 1.66[a])	30.8	1.95[a]), n.d.	30.8	1.94[a]), n.d.
CH_2(24)	27.8	1.57[a]), 1.95[a])	27.6	1.54[a]), 1.92[a])	27.5	n.d., n.d.	27.2	nd, n.d.
H–C(25)	33.6	1.80–1.85 (*m*)	33.6	1.80–1.85 (*m*)	33.8	1.78–1.84 (*m*)	33.8	1.78–1.84 (*m*)
CH_2(26)	74.9	3.55[a]), 3.82[a])	74.9	3.55[a]), 3.82[a])	74.2	3.54[a]), 3.89[a])	74.2	3.53[a]), 4.08[a])
Me(27)	16.8	0.90 (*d*, *J* = 6.0)	16.8	0.90 (*d*, *J* = 6.0)	15.9	0.92 (*d*, *J* = 6.4)	16.0	0.94 (*d*, *J* = 6.4)
*Me*O–C(22)			47.4	3.24 (*s*)	46.5	3.26 (*s*)		

[a]) Overlapped with other signals.

rivatives are readily converted into 22-methoxyfurostane derivatives in solutions containing MeOH [21], the presence of **2** as a natural compound was confirmed by HPTLC after an extraction of 10 g of the roots of *C. borivilianum* in refluxing H_2O [22].

The comparison of the ^{1}H- and ^{13}C-NMR chemical shifts of **2**, assigned from 2D-NMR analysis, with those of **1** showed that **2** differed from **1** only by the presence of a MeO group instead of an OH group at C(22) of the aglycone (δ(C) 47.4 and δ(H) 3.24 (MeO–C(22)) and δ(C) 112.1 (C(22)) in **2** instead of δ(C) 110.2 (C(22)) in **1**). The α-orientation of MeO–C(22) was deduced by the observed NOESY correlation δ(H) 3.24 (*s*, MeO)/δ(H) 4.40 (H–C(16)) [19][20].

Table 2. *^{1}H- and ^{13}C-NMR Data* ((D_5)pyridine) *of the Sugar Moieties of* **1**–**4**. δ in ppm, J in Hz.

	1		**2**		**3**		**4**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Glc I:								
H–C(1)	104.2	4.70 (d, J = 7.5)	104.2	4.70 (d, J = 7.6)	103.6	4.72 (d, J = 7.5)	103.5	4.71 (d, J = 7.5)
H–C(2)	74.5	3.90[a])	74.5	3.90[a])	74.0	3.90[a])	74.1	3.92[a])
H–C(3)	77.6	4.14[a])	77.5	4.16[a])	77.0	4.16[a])	76.9	4.15[a])
H–C(4)	71.0	4.03[a])	71.0	4.02[a])	69.8	4.02[a])	69.9	4.01[a])
H–C(5)	77.6	3.83[a])	77.6	3.84[a])	77.0	3.84[a])	76.9	3.83[a])
CH_2(6)	62.1	4.17[a]), 4.36[a])	62.0	4.18[a]), 4.40[a])	61.7	4.18[a]), 4.37[a])	61.8	4.18[a]), 4.36[a])
Gal:								
H–C(1)	104.1	4.72 (d, J = 7.5)	104.2	4.72 (d, J = 7.5)	101.4	4.82 (d, J = 7.4)	101.3	4.83 (d, J = 7.4)
H–C(2)	77.0	4.30[a])	76.5	4.22[a])	71.8	4.30[a])	71.7	4.31[a])
H–C(3)	75.5	4.04[a])	75.5	4.04[a])	76.5	4.08[a])	76.6	4.09[a])
H–C(4)	81.0	4.40[a])	80.5	4.38[a])	78.8	4.49[a])	78.9	4.50[a])
H–C(5)	76.7	3.82[a])	76.8	3.83[a])	76.1	3.70–3.74 (m)	76.0	3.70–3.74 (m)
CH_2(6)	60.1	4.12[a]), 4.52–4.56 (m)	60.1	4.12[a]), 4.52–4.56 (m)	60.0	4.14[a]), 4.48[a])	60.1	4.14[a]), 4.46[a])
Glc II:								
H–C(1)	104.5	4.83 (d, J = 7.5)	104.5	4.81 (d, J = 7.5)	103.6	5.02 (d, J = 7.5)	103.5	5.03 (d, J = 7.5)
H–C(2)	75.5	4.02[a])	75.5	4.03[a])	79.7	4.19[a])	79.8	4.20[a])
H–C(3)	87.1	3.95[a])	87.1	3.93[a])	86.0	3.98[a])	86.1	3.99[a])
H–C(4)	70.0	4.02[a])	70.0	4.02[a])	71.0	4.30[a])	71.1	4.31[a])
H–C(5)	76.7	3.70[a])	76.7	3.70[a])	76.9	3.96[a])	77.0	3.97[a])
CH_2(6)	61.9	3.91[a]), 4.40[a])	61.9	3.91[a]), 4.40[a])	61.5	4.26[a]), 4.37[a])	61.6	4.26[a]), 4.36[a])
Rha:								
H–C(1)	101.2	5.96 (br. s)	101.2	5.96 (br. s)				
H–C(2)	72.0	4.63–4.67 (m)	72.0	4.63–4.67 (m)				
H–C(3)	71.8	4.26–4.30 (m)	71.8	4.26–4.30 (m)				
H–C(4)	73.0	4.22[a])	73.0	4.22[a])				
H–C(5)	69.3	4.72–4.78 (m)	69.2	4.72–4.78 (m)				
Me(6)	17.9	1.62 (d, J = 6.0)	17.9	1.64 (d, J = 6.2)				
Xyl:								
H–C(1)	104.2	5.04 (d, J = 7.8)	104.2	5.07 (d, J = 8.0)	103.6	5.05 (d, J = 7.7)	103.5	5.04 (d, J = 7.7)
H–C(2)	74.4	3.83[a])	74.4	3.83[a])	73.9	3.82[a])	73.8	3.81[a])
H–C(3)	77.5	4.02[a])	77.5	4.02[a])	76.9	4.00[a])	77.0	4.02[a])
H–C(4)	71.0	4.03[a])	71.0	4.03[a])	71.8	4.26[a])	71.9	4.25[a])
CH_2(5)	66.5	3.54[a]), 4.14[a])	66.5	3.54[a]), 4.14[a])	66.0	3.58 (t, 10.3), 4.11[a])	66.1	3.56 (t, 10.3), 4.11[a])
Glc III:								
H–C(1)					103.2	5.44 (d, J = 7.7)	103.1	5.45 (d, J = 7.7)
H–C(2)					74.7	3.92[a])	74.6	3.93[a])
H–C(3)					76.5	4.08[a])	76.6	4.09[a])
H–C(4)					70.5	4.00[a])	70.6	4.02[a])
H–C(5)					77.0	3.83[a])	77.1	3.81[a])
CH_2(6)					61.4	4.29[a]), 4.50[a])	61.5	4.28[a]), 4.52[a])

[a]) Overlapped with other signals.

The molecular formula of Borivilianoside C (**3**) was determined as $C_{57}H_{96}O_{28}$ according to the $[M+Na]^+$ peak at *m/z* 1251.5991 in the HR-ESI-MS. Negative-ion FAB-MS showed the pseudomolecular-ion peak at *m/z* 1227 ($[M-H]^-$) in accordance with the $[M+Na]^+$ peak. Other fragment-ion peaks were observed at *m/z* 1065 ($[M-H-162]^-$), 933 ($[M-H-162-132]^-$), and 771 ($[M-H-162-132-162]^-$), corresponding to the loss of two hexosyl and one pentosyl moiety. On the basis of extensive ^{1}H- and ^{13}C-NMR and 2D-NMR analyses (*Tables 1* and *2*), the structure of borivilianoside C (**3**) was determined as (3*β*,5*α*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-22-methoxyfurostan-3-yl *O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-[*β*-D-glucopyranosyl-(1 → 2)]-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranoside.

Most of the ^{1}H- and ^{13}C-NMR signals corresponding to the aglycone part of **3**, assigned from 2D-NMR experiments, were superimposable with those described above for compounds **1** and **2**. The ^{1}H-NMR spectrum of **3** displayed signals for five anomeric H-atoms at *δ*(H) 4.72 (*d*, *J* = 7.5 Hz), 4.82 (*d*, *J* = 7.4 Hz), 5.02 (*d*, *J* = 7.5 Hz), 5.05 (*d*, *J* = 7.7 Hz), and 5.44 (*d*, *J* = 7.7 Hz) giving cross-peaks in the HSQC spectrum with five anomeric C-atoms at *δ*(C) 103.6, 101.4, 103.6, 103.6, and 103.2, resp. The complete assignment of the glycosidic NMR signals was achieved by analyses of COSY, TOCSY, NOESY, HSQC, and HMBC data. Evaluation of spin–spin couplings and chemical shifts allowed the identification of three *β*-glucopyranosyl (Glc I, Glc II, and Glc III), one *β*-galactopyranosyl (Gal), and one *β*-xylopyranosyl (Xyl) unit. The linkage of Glc I at C(26) of the aglycone was deduced by the HMBC cross-peak *δ*(H) 4.72 (Glc I H–C(1))/*δ*(C) 74.2 (Agly C(26)). The HMBC cross-peaks *δ*(H) 5.05 (Xyl H–C(1))/*δ*(C) 86.0 (Glc II C(3)), *δ*(H) 4.19 (Glc II H–C(2))/*δ*(C) 103.2 (Glc III C(1)), and *δ*(H) 5.02 (Glc II H–C(1))/*δ*(C) 78.8 (Gal C(4)), and the NOESY correlations *δ*(H) 5.44 (Glc III H–C(1))/*δ*(H) 4.19 (Glc II H–C(2)) and *δ*(H) 4.82 (Gal H–C(1))/*δ*(H) 3.83 (Agly H–C(3)) established the linkages within the oligosaccharide chain at C(3).

The positive-ion HR-ESI-MS of borivilianoside D (**4**) exhibited the $[M+Na]^+$ peak at *m/z* 1219.5730 consistent with the molecular formula $C_{56}H_{92}O_{27}$. Negative-ion FAB-MS showed a pseudomolecular-ion peak at *m/z* 1195 ($[M-H]^-$) in accordance with the $[M+Na]^+$ peak. Other fragment-ion peaks were observed at *m/z* 1033 ($[M-H-162]^-$), 901 ($[M-H-162-132]^-$), 739 ($[M-H-162-132-162]^-$), corresponding to the loss of two hexosyl and one pentosyl moiety. Extensive study of the ^{1}H- and ^{13}C-NMR (*Tables 1* and *2*) and 2D-NMR spectra of **4** led to the establishment of its structure as (3*β*,5*α*,25*R*)-26-(*β*-D-glucopyranosyloxy)furost-20(22)-en-3-yl *O*-*β*-D-xylopyranosyl-(1 → 3)-*O*-[*β*-D-glucopyranosyl-(1 → 2)]-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranoside.

The structure elucidation of **4** was supported by comparison of its MS and NMR data with those of **3**. The molecular formula $C_{56}H_{92}O_{27}$ of **4** indicated a loss of 32 amu as compared to **3**. The ^{1}H- and ^{13}C-NMR signals of the aglycone of **4**, assigned by extensive 2D-NMR analyses, were almost superimposable with those of **3**, except for the signals at *δ*(C) 39.8 (C(20)) and *δ*(C) 112.0 (C(22)) which were replaced by two quaternary-C-atom signals at *δ*(C) 104.0 and 152.0, resp. This feature is a characteristic of a tetra-substituted C(20)=C(22) bond and was supported by signals at *δ*(H) 1.55 (*s*, Me(21)) and 2.34–2.37 (*m*, H–C(17)) which were downfield shifted in comparison with the corresponding signals of **3** at *δ*(H) 1.09 (*d*, *J* = 6.5 Hz, Me(21)) and *δ*(H) 1.64–1.68 (*m*, H–C(17)). The presence of five monosaccharide units was suggested by the five anomeric H-atom resonances at *δ*(H) 4.71 (*d*, *J* = 7.5 Hz), 4.83 (*d*, *J* = 7.4 Hz), 5.03 (*d*, *J* = 7.5 Hz), 5.04 (*d*, *J* = 7.7 Hz), and 5.45 (*d*, *J* = 7.7 Hz), which exhibited HSQC cross-peaks with *δ*(C) 103.5, 101.3, 103.5, 103.5, and 103.1, resp. The ^{1}H- and ^{13}C-NMR data of the oligosaccharide moiety of **4**, assigned from the 2D-NMR analyses, were about the same as those of **3**, suggesting that **4** possessed the same sugar chain at C(3), *i.e.*, an *O*-*β*-D-

xylopyranosyl-(1 → 3)-*O*-[*β*-D-glucopyranosyl-(1 → 2)]-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranosyloxy moiety, and the same sugar unit at C(26), *i.e.*, a *β*-D-glucopyranosyloxy group.

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Experimental Part

General. Medium-pressure liquid chromatography (MPLC): silica gel *60* (SiO_2, 15–40 μm; *Merck*), *Gilson M 305* pump; *Büchi* glass column (460 × 25 mm, 460 × 15 mm, and 230 × 15 mm), *Büchi* precolumn (110 × 15 mm). Vacuum-liquid chromatography (VLC): reversed-phase SiO_2 *RP-18* (25–40 μm; *Merck*). TLC and HPTLC: SiO_2 *60* F_{254} (*Merck*); solvent systems: for saponins, $CHCl_3$/MeOH/H_2O 13:7:2, lower phase (*A*); for sapogenins, $CHCl_3$/MeOH 19:1 (*B*); spray reagent: for the saponins, *Komarowsky* reagent, a 5:1 mixture of 2% 4-hydroxybenzaldehyde/MeOH and 50% H_2SO_4/EtOH. GC: *Thermoquest* gas chromatograph with a *DB-1701* capillary column (30 m × 0.25 mm, i.d.; *J & W Scientific*), FID detection; initial temp. 80° for 5 min, then temp. increase to 270° at the rate of 15°/min; carrier gas He. Optical rotation: *AA-OR* automatic polarimeter. 1D- and 2D-NMR Spectra: *Varian-Inova-600* spectrometer equipped with a *Sun-4-L-X* computer system; at 600 (^1H) and 150 MHz (^{13}C) in (D_5)pyridine; *δ* in ppm, *J* in Hz; conventional pulse sequences for COSY, HSQC, and HMBC; TOCSY with the standard MLEV17 spin-locking sequence and a 90-ms mixing time; mixing time in the NOESY experiment 500 ms; C-atom type (Me, CH_2, CH) by DEPT experiments. Fast-atom-bombardment (FAB) MS (neg.): *Jeol-SX-102* spectrometer; glycerol as matrix; in *m*/*z*. HR-ESI-MS (pos.): *Micromass Q-TOF-1* apparatus; in *m*/*z*.

Plant Material. The roots of *C. borivilianum* SANT. and FERN. were provided from *Jeevan Herbs* in 2006 (New Delhi, India) and identified by Dr. *M. Ahmedullah*, Botanic Garden of Indian Republic, BGIR (Noida, India). A voucher specimen (No. 6625) was deposited with the herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, Burgundy University, France.

Extraction and Isolation. Powdered roots (500 g) of *C. borivilianum* were refluxed in EtOH (3 × 2 l) for 50 min. After evaporation, the resulting EtOH extract (19 g) was suspended in H_2O (200 ml) and partitioned successively with CH_2Cl_2 (3 × 300 ml) and BuOH (sat. with H_2O; 3 × 200 ml), yielding after evaporation the corresponding CH_2Cl_2 (3.2 g) and BuOH (4.1g) fractions. An aliquot (4 g) of the BuOH residue was submitted to VLC (*RP-18*; MeOH/H_2O (0:100, 50:50, and 100:0)): Fractions *VLC (1)*, *VLC (2)*, and *VLC (3)* after evaporation. *VLC (3)* (1.1 g) was submitted to MPLC (system *C:* SiO_2 (15–40 μm), $CHCl_3$/MeOH/H_2O 13:7:2, lower phase): *Fractions 1–17. Fr. 10* was subjected to MPLC (reversed phase *RP-18*, MeOH/H_2O 40 → 100%): *Fr. 10.1–10.6. Fr. 10.4* (92.3 mg) was submitted to MPLC (system *C*): **1** (4.7 mg) and **2** (4.3 mg). *Fr. 10.8* (41.6 mg) and *Fr. 10.6* (38.4 mg) of *VLC (3)* were purified by MPLC (system *C*): **3** (3.4 mg) and **4** (3.9 mg), resp.

Ten grams of powdered roots of *C. borivilianum* were refluxed in H_2O (2 × 100 ml) for 1 h. The solvent was evaporated yielding an aq. extract which was analyzed by HPTLC (SiO_2 *60* F_{254}, system *A*) showing the presence of compounds **2** and **3**, which means they represent true natural products and not artefacts of isolation.

Borivilianoside A (=*(3β,5α,22*R,*25*R*)-26-(β-*D*-Glucopyranosyloxy)-22-hydroxyfurostan-3-yl* O-*β*-D-*xylopyranosyl-(1 → 3)*-O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*[6-deoxy-α-*L*-mannopyranosyl-(1 → 2)]-β-*D-*galactopyranoside*; (**1**): White amorphous powder. $[\alpha]_D^{20} = -46.0$ (*c* = 0.10, MeOH). ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 1197 ($[M-H]^-$), 1065 ($[M-H-132]^-$), 903 ($[M-H-132-162]^-$), 757 ($[M-H-132-162-146]^-$). HR-ESI-MS (pos.): 1221.5825 ($[M+Na]^+$, $C_{56}H_{94}O_{27}Na^+$; calc. 1221.5880).

Borivilianoside B (=*(3β,5α,22*R,*25*R*)-26-(β-*D*-Glucopyranosyloxy)-22-methoxyfurostan-3-yl* O-*β*-D-*xylopyranosyl-(1 → 3)*-O-*β*-D-*glucopyranosyl-(1 → 4)*-O-*[6-deoxy-α-*L*-mannopyranosyl-(1 → 2)]-β-*D-*galactopyranoside*; (**2**): White amorphous powder. $[\alpha]_D^{20} = -43.0$ (*c* = 0.10, MeOH). ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 1211 ($[M-H]^-$), 1079 ($[M-H-132]^-$), 917 ($[M-H-132-162]^-$), 771 ($[M-H-132-162-146]^-$). HR-ESI-MS (pos.): 1235.6043 ($[M+Na]^+$, $C_{57}H_{96}O_{27}Na^+$; calc. 1235.6037).

*Borivilianoside C (=(3β,5α,22*R*,25*R*)-26-(β-*D*-Glucopyranosyloxy)-22-methoxyfurostan-3-yl* O-*β*-*D*-*xylopyranosyl-(1 → 3)*-O-*[β*-*D*-*glucopyranosyl-(1 → 2)]*-O-*β*-*D*-*glucopyranosyl-(1 → 4)-β*-*D*-*galactopyranoside* (**3**): White amorphous powder. $[\alpha]_D^{20} = -45.6$ ($c = 0.08$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 1227 ($[M-H]^-$), 1065 ($[M-H-162]^-$), 933 ($[M-H-162-132]^-$), 771 ($[M-H-162-132-162]^-$). HR-ESI-MS (pos.): 1251.5991 ($[M+Na]^+$, $C_{57}H_{96}O_{28}Na^+$; calc. 1251.5986).

*Borivilianoside D (=(3β,5α,25*R*)-26-(β-*D*-Glucopyranosyloxy)furost-20(22)-en-3-yl* O-*β*-*D*-*xylopyranosyl-(1 → 3)*-O-*[β*-*D*-*glucopyranosyl-(1 → 2)]*-O-*β*-*D*-*glucopyranosyl-(1 → 4)-β*-*D*-*galactopyranoside* (**4**): White amorphous powder. $[\alpha]_D^{20} = -32.4$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 1195 ($[M-H]^-$), 1033 ($[M-H-162]^-$), 901 ($[M-H-162-132]^-$), 739 ($[M-H-162-132-162]^-$). HR-ESI-MS (pos.): 1219.5730 ($[M+Na]^+$, $C_{56}H_{92}O_{27}Na^+$; calc. 1219.5724).

Acid Hydrolysis. A soln. of each saponin **1**–**4** (2 mg) in H_2O (2 ml) was treated with 2N aq. CF_3COOH (5 ml) and refluxed for 3 h. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly concentrated with MeOH until neutral, and then analyzed by TLC (SiO_2, $CHCl_3$/MeOH/H_2O 8:5:1) with authentic samples: galactose (R_f 0.21), glucose (R_f 0.23), xylose (R_f 0.47), and rhamnose (R_f 0.51) for **1** and **2**, and galactose (R_f 0.21), glucose (R_f 0.23), and xylose (R_f 0.47) for **3** and **4**. Furthermore, a silylated derivative of the sugars was prepared according to the procedure described previously [23]. L-Cysteine methyl ester hydrochloride (*Sigma*, U.S.A.; 0.06 mol/l) and HMDS/TMCS (=hexamethyldisilazane/trimethylchlorosilane) 3:1 (*Fluka*, U.S.A.; 150 µl) were added to the aq. residue. After centrifugation of the precipitate, the supernatant was concentrated and partitioned between hexane and H_2O (0.1 ml each), and the hexane layer (1 µl) was analyzed by GC. D-Glucose, D-galactose, D-xylose, and L-rhamnose were detected for **1** and **2**, and D-glucose, D-galactose, and D-xylose for **3** and **4**.

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