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Phenolic Glycosides from Sugar Maple (*Acer saccharum*) Bark

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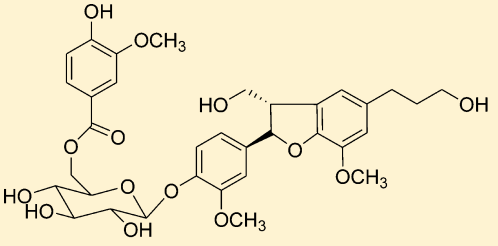
Supporting Information



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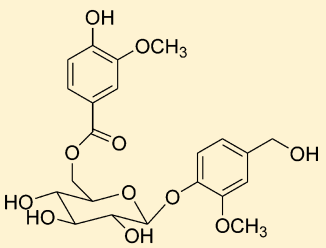

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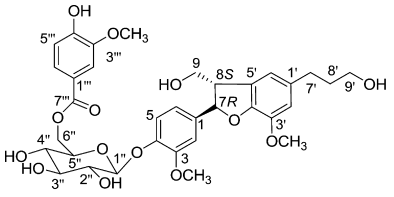
ABSTRACT: Four new phenolic glycosides, saccharumosides A–D (**1**–**4**), along with eight known phenolic glycosides, were isolated from the bark of sugar maple (*Acer saccharum*). The structures of **1**–**4** were elucidated on the basis of spectroscopic data analysis. All compounds isolated were evaluated for cytotoxicity effects against human colon tumorigenic (HCT-116 and Caco-2) and nontumorigenic (CCD-18Co) cell lines.

The genus *Acer* (Aceraceae), commonly known as maple, consists of more than 200 species distributed widely in temperate zones of the northern hemisphere.[1] The sugar maple species *Acer saccharum* Marsh. is native to northeastern North America and is widely regarded for its sap, which is concentrated to produce maple syrup, a natural sweetener. Previous investigations on the leaves and hardwood of this maple species has reported gallotannins, lignans, coumarins, and coumarinolignans therein.[2,3] Also, our group has recently isolated several antioxidant phenolic compounds from maple syrup.[4−6] However, to date, the chemical constituents of the bark of the sugar maple species have not been investigated. Herein, the isolation and structure elucidation of four new phenolic glycosides (**1**–**4**) are reported, along with the purification of eight known phenolic glycosides. These compounds were evaluated for cytotoxicity against several human colon tumorigenic and nontumorigenic cell lines.

Compound **1**, a colorless, amorphous solid, $[\alpha]^{20}_{D}$ −31 (*c* 0.5, MeOH), displayed a molecular formula of $C_{34}H_{40}O_{14}$, representing 15 degrees of unsaturation, as determined by HRESIMS at *m*/*z* 671.2314 $[M - H]^-$ (calcd for $C_{34}H_{39}O_{14}$, 671.2340). The IR absorptions revealed the presence of hydroxy (3344 cm^{-1}), ester carbonyl (1691 cm^{-1}), and aromatic (1608 and 1496 cm^{-1}) functionalities. In the ^1H NMR spectrum (Table 1), two ABX spin system signals at δ_H 7.04 (1H, d, *J* = 8.3 Hz, H-5), 7.00 (1H, d, *J* = 1.6 Hz, H-2), 6.68 (1H, dd, *J* = 8.3, 1.6 Hz, H-6) and δ_H 6.80 (1H, d, *J* = 8.2 Hz, H-5‴), 7.53 (1H, d, *J* = 1.9 Hz, H-2‴), 7.57 (1H, dd, *J* = 8.2, 1.9 Hz, H-6‴) were observed, as well as two aromatic proton signals at δ_H 6.72 (1H, brs, H-6′) and 6.73 (1H, brs, H-2′) and three methoxy group signals at δ_H 3.80, 3.82, and 3.85 (each 3H, s). The ^{13}C NMR (Table 1) and HSQC spectra revealed the presence of 34 carbon resonances, comprising three methyls, five methylenes, 15 methines (eight sp^2 and seven sp^3), and 11 quaternary carbons (of which one was characteristic of an ester carbonyl). The ^1H NMR data of **1** also showed the presence of a



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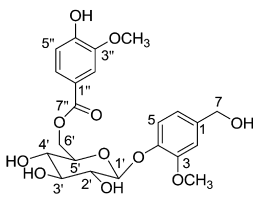

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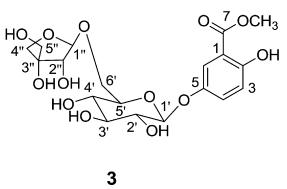

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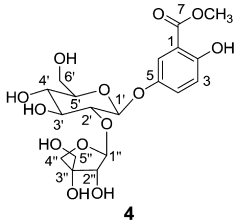

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Table 1. ^{1}H NMR and ^{13}C NMR Data of Compounds 1 and 2[a]

	1		2	
no.	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	136.9		136.3	
2	109.9	7.00 (d, 1.6)	111.2	6.98 (d, 1.8)
3	149.5		149.4	
4	146.0		145.6	
5	116.5	7.04 (d, 8.3)	116.5	7.02 (d, 8.3)
6	117.7	6.68 (dd, 8.3, 1.6)	119.1	6.62 (dd, 8.3, 1.8)
7	87.0	5.50 (d, 5.7)	63.5	4.49 (s, 2H)
8	54.1	3.37 (m)		
9	63.7	3.81 (m)		
		3.62 (m)		
1′	135.6			
2′	112.3	6.73 (brs)		
3′	143.9			
4′	146.0			
5′	128.1			
6′	116.4	6.72 (brs)		
7′	31.8	2.62 (t, 7.8)		
8′	34.3	1.81 (quint, 6.6)		
9′	60.8	3.56 (t, 6.3)		
1″ (1′)	101.1	4.89 (d, 7.1)	101.3	4.87 (d, 7.2)
2″ (2′)	73.4	3.52 (dd, 8.4, 7.1)	73.5	3.54 (dd, 8.9, 7.2)
3″ (3′)	76.3	3.49 (dd, 8.9, 8.4)	76.4	3.51 (dd, 8.9, 8.4)
4″ (4′)	70.6	3.41 (dd, 9.2, 8.9)	70.6	3.43 (dd, 9.5, 8.4)
5″ (5′)	74.2	3.74 (m)	74.2	3.73 (ddd, 9.5, 7.5, 2.1)
6″ (6′)	63.6	4.68 (dd, 12.0, 2.1)	63.6	4.66 (dd, 11.7, 2.1)
		4.34 (dd, 12.0, 7.4)		4.38 (dd, 11.7, 7.5)
1‴ (1″)	121.0		121.0	
2‴ (2″)	112.3	7.53 (d, 1.9)	112.4	7.52 (d, 1.8)
3‴ (3″)	147.8		147.4	
4‴ (4″)	151.9		151.6	
5‴ (5″)	114.5	6.80 (d, 8.2)	114.5	6.86 (d, 8.3)
6‴ (6″)	123.8	7.57 (dd, 8.2, 1.9)	123.8	7.56 (dd, 8.3, 1.8)
7‴ (7″)	166.4		166.4	
OCH_3	3-OCH_3: δ_C 55.2, δ_H 3.80 (s, 3H)		3-OCH_3: δ_C 55.2, δ_H 3.83 (s, 3H)	
	3′-OCH_3: δ_C 55.3, δ_H 3.85 (s, 3H)		3″-OCH_3: δ_C 55.1, δ_H 3.84 (s, 3H)	
	3‴-OCH_3: δ_C 55.1, δ_H 3.82 (s, 3H)			

[a]Data were measured in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C).

β-glucopyranose moiety, for which the anomeric proton resonated at δ_H 4.89 (1H, d, J = 7.1 Hz, H-1″).

Analysis of the 1D and 2D NMR data (including ^{1}H−^{1}H COSY, HSQC, HMBC spectra) allowed for the establishment of the structure of **1**. The HSQC spectrum permitted the assignment of all the protons to their bonding carbons. The ^{1}H−^{1}H COSY and HMBC spectra (Figure 1) were then applied to construct the planar structure of **1**. From the ^{1}H−^{1}H COSY spectrum, a hexose moiety (C-1″ to C-6″) and two subunits (C-7 to C-9 and C-7′ to C-9′) (drawn with bold bonds in Figure 1) could be proposed. Analysis of the HMBC spectrum (Figure 1) then enabled the connectivity of the three spin coupling fragments and the other functional groups. The linkage between C-7 and C-1 was determined by the HMBC correlations from H-7 to C-1, C-2, and C-6, and the attachment between C-7′ and C-1′ was supported by similar HMBC correlations. The HMBC correlations from H-8, H-9 to C-5′, from H-7, H-8 to C-4′, and between two methoxy group signals and C-3, C-3′ were applied to determine the aglycone of **1** as dihydrodehydroconiferyl alcohol. The HMBC correlation between H-1″ and C-4 was used to place the sugar moiety at C-4. The HMBC correlations from H-5‴ to C-1‴ and C-3‴, from H-6‴ to C-2‴ and C-4‴, and from H-2‴ and H-6‴ to C-7‴ indicated the presence of a vanilloyl moiety, which was linked at C-6″ via oxygen, from the HMBC correlation between H_2-6″ and C-7‴. Thus, a planar structure of **1** was established.

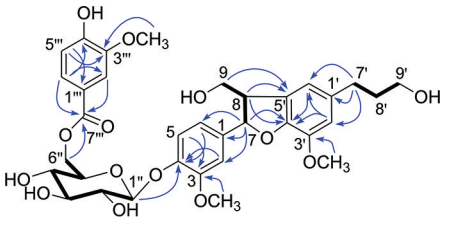

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Figure 1. Key ^{1}H−^{1}H COSY (−) and selected HMBC correlations (H→C) of **1**.

The *trans* configuration of the aglycone of **1** was determined on the basis of coupling constants of the vicinal protons ($J_{7,8}$ = 5.7 Hz).[7] Acid hydrolysis of **1** afforded D-glucose, which was identified by direct comparison with an authentic sample

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(see Experimental Section). Using Hudson's rules of isorotation,[8] the molecular rotation of the aglycone of **1** was calculated as a negative value from the measured specific rotations of **1**.[9] The absolute configurations of the aglycone were thus assigned as 7*R*, 8*S* by comparing its optical rotatory properties to reported values.[10] Therefore, the structure of compound **1** was determined as (7*R*,8*S*)- 4-*O*-(6-vanilloyl)-*β*-D-glucopyranosyl dihydrodehydroconiferyl alcohol, and this compound has been assigned the trivial name saccharumoside A.

Compound **2** (saccharumoside B) was obtained as a colorless, amorphous powder and gave a molecular formula of $C_{22}H_{26}O_{11}$, as determined by HRESIMS at *m*/*z* 465.1395 $[M - H]^-$ (calcd for $C_{22}H_{25}O_{11}$, 465.1397). The UV and IR spectra of **2** were similar to those of **1**. Detailed analysis of the 1D (Table 1) and 2D NMR data showed that compound **2** has the same substituted aromatic unit as **1**, in which a vanilloyl moiety is linked via oxygen to C-6′ of a glucopyranose unit. The major structural difference between **1** and **2** was found to be the aglycone. HMBC (Figure 2) correlations from H_2-7 to C-1,

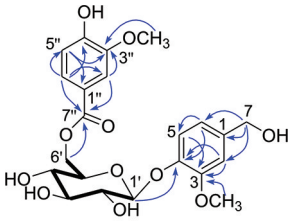

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Figure 2. Key $^1H-^1H$ COSY (−) and selected HMBC correlations (H→C) of **2**.

C-2, and C-6, from H-2 to C-3 and C-4, from H-6 to C-5 and C-4, and from H-5 to C-3 indicated that the aglycone of **1** is vanillyl alcohol. The sugar moiety is attached at C-4, as deduced by HMBC correlations between H-1′ and C-4. The resonance of the anomeric proton at δ_H 4.87 (1H, d, *J* = 7.2 Hz, H-1′) indicated a *β*-glycosidic linkage. The D-configuration of the glucopyranosyl moiety was determined by acid hydrolysis. Therefore, the structure of compound **2** was elucidated as 4-*O*-(6-vanilloyl)-*β*-D-glucopyranosyl vanillyl alcohol.

Compound **3** (saccharumoside C), a colorless, amorphous solid, exhibited a molecular formula of $C_{19}H_{26}O_{13}$, as determined by HRESIMS at *m*/*z* 507.1351 $[M + HCOO]^-$ (calcd for $C_{20}H_{27}O_{15}$, 507.1350). The IR spectrum exhibited absorption bands for hydroxy (3375 cm^{-1}) and conjugated ester (1701 cm^{-1}) functionalities. The ^1H NMR data (Table 2) exhibited an ABX spin system at δ_H 7.56 (1H, d, *J* = 1.9 Hz, H-6), 7.33 (1H, dd, *J* = 8.9, 1.6 Hz, H-4), and 6.91 (1H, d, *J* = 8.9 Hz, H-3), a methoxy group signal at δ_H 3.96, and sugar moiety resonances at δ_H 3.34−4.97. The ^{13}C NMR data showed six aromatic carbon signals, one ester carbonyl at δ_C 170.0, a methoxy group signal at δ_C 51.6, and 11 sugar-derived carbons. The HMBC correlations (Figure 3a) from H-3 to C-1 and C-5, from H-4 to C-2, from H-6 to C-2, C-4, and C-7, and from the methoxy protons to C-7 indicated that the aglycone of **3** is methyl gentisate. A glucopyranosyl unit in **3** was determined by the $^1H-^1H$ COSY correlations (Figure 3a). The $^1H-^1H$ COSY correlation of H-1″/H-2″ and the HMBC correlations from H-1″ to C-4″ and from H_2-5″ to C-2″, C-3″, and C-4″ indicated the presence of an apiofuranosyl moiety. On the basis of the HMBC correlation between H-1″ and C-6′, it

Table 2. ^1H NMR and ^{13}C NMR Data of Compounds 3 and 4[a]

	3		4	
no.	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	112.0		112.0	
2	156.9		156.8	
3	117.8	6.91 (d, 8.9)	117.7	6.88 (d, 8.9)
4	125.9	7.33 (dd, 8.9, 1.9)	125.7	7.29 (dd, 8.9, 2.1)
5	149.9		150.0	
6	117.4	7.56 (d, 1.9)	117.0	7.59 (d, 2.1)
7	170.0		170.0	
1′	102.2	4.74 (d, 6.2)	101.0	4.83 (overlap)
2′	73.5	3.41 (dd, 8.2, 6.2)	77.6	3.60 (m)
3′	76.4	3.43 (dd, 8.6, 8.2)	77.1	3.59 (m)
4′	70.1	3.34 (dd, 8.9, 8.6)	70.0	3.38 (m)
5′	75.6	3.53 (m)	76.7	3.38 (m)
6′	67.1	4.00 (brd, 11.1)	61.1	3.88 (brd, 12.3)
		3.62 (dd, 11.1, 6.4)		3.68 (dd, 12.3, 3.3)
1″	109.5	4.97 (d, 2.3)	109.5	5.45 (brs)
2″	76.6	3.90 (d, 2.3)	76.7	3.96 (brs)
3″	79.1		79.2	
4″	73.6	3.94 (d, 9.6)	74.0	4.05 (d, 9.6)
		3.74 (d, 9.6)		3.78 (d, 9.6)
5″	64.2	3.57 (s, 2H)	64.5	3.57 (s, 2H)
OCH_3	51.6	3.96 (s, 3H)	51.5	3.94 (s, 3H)

[a]Data were measured in CD_3OD at 500 MHz (^1H) and 125 MHz (^{13}C).

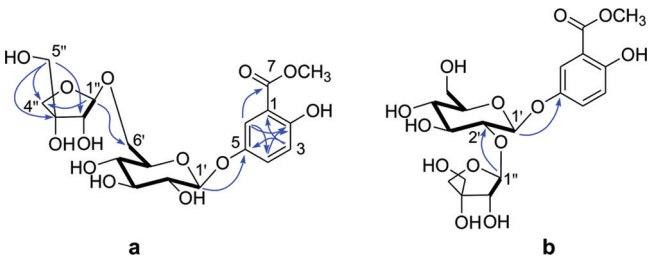

Figure 3. (a) Key $^1H-^1H$ COSY (−) and selected HMBC correlations (H→C) of **3**. (b) Key $^1H-^1H$ COSY (−) and selected HMBC correlations (H→C) of **4**.

was evident that the apiofuranosyl is linked to C-6′ of the glucopyranosyl moiety. The HMBC correlation from the anomeric proton, H-1′, to C-5 indicated that the diglycoside unit is attached to C-5. The *β*-glycosidic linkage of the glucopyranosyl and apiofuranosyl units was determined by the coupling constant of the anomeric protons H-1′ (*J* = 6.2 Hz) and H-2″ (*J* = 2.3 Hz), respectively.[11] Acid hydrolysis of **3** afforded D-glucose and D-apiose, which were identified by comparing their HPLC retention times and optical rotations with authentic samples. Thus, the structure of compound **3** was elucidated as 5-[*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranosyl] methyl gentisate.

Compound **4** (saccharumoside D) showed the same molecular formula as compound **3** ($C_{19}H_{26}O_{13}$) from the HRESIMS data, as well as similar UV and IR data. Comparison of the ^1H and ^{13}C NMR data (Table 2) of compounds **3** and **4** suggested that they are different only in their sugar portions, which was further established from the 2D NMR data. $^1H-^1H$ COSY and HMBC (Figure 3b) correlations revealed the presence of glucopyranosyl and apiofuranosyl units. The HMBC correlation from H-1″ to C-2′ indicated that the apiose moiety is attached to C-2′ of glucose, which was supported by the downfield shift of C-2′ ($\Delta\delta$ = 4.1 ppm) when compared

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with **3**. The β-glycosidic linkage and D-configurations of apiose and glucose were determined by the same methods as those of **3**. The structure of **4** was thus elucidated as 5-[*O*-β-D-apiofuranosyl-(1→2)-*O*-β-D-glucopyranosyl] methyl gentisate.

Eight known phenolic glycosides were also isolated and identified as 3,4,5-trimethoxyphenyl-1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside,[12] 4-hydroxymethyl-2-methoxyphenyl-1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside,[13] icariside E_4,[14] syringaresinol-β-D-glucopyranoside,[15] 3,5-dimethoxy-4-hydroxybenzyl alcohol-4-*O*-β-D-glucopyranoside,[16] koaburside,[17] vanilloloside,[18] and scopolin[19] on the basis of their spectroscopic data (^{1}H NMR, ^{13}C NMR, and ESIMS).

All compounds isolated were tested for their cytotoxicity effects against two human colon tumorigenic (HCT-116 and Caco-2) cell lines and a nontumorigenic (CCD-18Co) cell line. However, none of the compounds proved to be cytotoxic for any of these cell lines (IC_{50} < 5 μg/mL).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on an Auto Pol III automatic polarimeter (Rudolph Research, Flanders, NJ, USA) at room temperature. The IR spectra were recorded on a Nicolet 380 FT-IR spectrometer. The UV spectra were measured on a Shimadzu UV-2550 UV−visible spectrophotometer. 1D and 2D NMR data were recorded on a Varian 500 MHz instrument with TMS as internal standard. HRESIMS data were acquired using an LTQ Orbitrap XL mass spectrometer (Thermo Scientific). Semipreparative HPLC separations were performed on a Hitachi Elite LaChrom system consisting of an L2130 pump, an L-2200 autosampler, an L-2455 diode array detector, and a Phenomenex Luna C_{18} column (250 × 10 mm, S-5 μm), all operated by EZChrom Elite software. Medium-pressure liquid chromatography (MPLC) separations were carried out on prepacked C_{18} columns (4 × 37 cm) connected to a DLC-10/11 isocratic liquid chromatography pump (D-Star Instruments, Manassas, VA, USA) with a fixed-wavelength detector. All solvents were of ACS or HPLC grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA) through Wilkem Scientific (Pawcatuck, RI, USA). Silica gel (230−400 mesh, Sorbent Technologies), Sephadex LH-20 gel (Amersham Biosciences), and MCI gel (CHP20P, 63−150 μM, M & M Industries Inc.) were used for column chromatography, and precoated silica gel GF254 plates (Whatman Ltd., Maidstone, England) were used for TLC analysis. Samples of D-glucose, D-apiose, MTS salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium salt], and etoposide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material. The bark of *A. saccharum* was collected in the summer of 2009 by the Federation of Maple Syrup Producers of Quebec (Canada), shipped to our laboratory in August 2009, and identified by Mr. J. Peter Morgan (Senior Gardener, College of Pharmacy, University of Rhode Island). A voucher specimen (JPMCB1) has been deposited in the Heber-Youngken Herbarium and Greenhouse, College of Pharmacy, University of Rhode Island.

Extraction and Isolation. The air-dried powder of the bark (4.2 kg) of *A. saccharum* was extracted by maceration with methanol (10 L × 3 times for 7 days per time period) at room temperature to afford 258.1 g of crude extract. The extract was suspended in distilled water (1 L) and then extracted successively with ethyl acetate (1 L × 3 times) and *n*-butanol (1 L × 3 times). The ethyl acetate fraction (33.4 g) was chromatographed over a column (5 × 50 cm) of MCI gel (MeOH−H_2O, 50:50 to 90:10) to yield four fractions (A−D). Fraction A (23.5 g) was subjected to silica gel chromatography (CC) eluted with chloroform−methanol (20:1 to 2:1) in a gradient to obtain three fractions (A1−A3). Fraction A3 was chromatographed over a column (3 × 70 cm) of Sephadex LH-20 eluted with MeOH to give four subfractions (A3a−A3d). Fraction A3a was separated by semipreparative HPLC eluted with MeOH−H_2O (20:80 to 80:20 in 30 min, 3 mL/min) to yield koaburside (10 mg), **1** (11 mg), and icariside E_4 (63 mg). Further purification of fraction A3a by semipreparative HPLC (MeOH−H_2O, 40:60, 3 mL/min) yielded compound **2** (6 mg).

The *n*-butanol extract (117.0 g) was subjected to an XAD-16 Amberlite resin column (8 × 35 cm) eluting with MeOH−H_2O (20:80 to 100:0) to give four fractions (1−4). Fraction 2 was subjected to CC eluted with chloroform−methanol (10:1 to 1:1) in gradient to obtain three fractions (2A−2C). Purification of fraction 2A by semipreparative HPLC (MeOH−H_2O, 30:70 to 50:50, in 25 min, 3 mL/min) gave syringaresinol-β-D-glucopyranoside (15 mg). Fraction 2C was purified on a column of Sephadex LH-20 eluted with MeOH to give scopolin (20 mg) and subfraction 2C1. Subfraction 2C1 was separated over MPLC, by elution with MeOH−H_2O (10:90 to 70:30, 3 mL/min), to obtain five fractions (2C1a−2C1e). Fraction 2C1b was subjected to semipreparative HPLC (MeOH−H_2O, 10:90 to 35:65 in 14 min, 3 mL/min), to yield 3,5-dimethoxy-4-hydroxybenzyl alcohol 4-*O*-β-D-glucopyranoside (8 mg) and 4-hydroxymethyl-2-methoxyphenyl 1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (3 mg). Purification of 2C1c by semipreparative HPLC (MeOH−H_2O, 23:77, 3 mL/min) gave 3,4,5-trimethoxyphenyl 1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (10 mg). Fraction 2C1d was separated using semipreparative HPLC (MeOH−H_2O, 10:90 to 50:50 in 18 min, 3 mL/min) and yielded **3** (8 mg) and **4** (4 mg). Fraction 2C1e was purified by semipreparative HPLC (MeOH−H_2O, 10:90 to 40:60 in 12 min, 3 mL/min) to yield vanilloloside (8 mg).

*Saccharumoside A (**1**):* colorless, amorphous solid; $[\alpha]^{20}_D$ −31 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 293 (3.45), 267 (3.73), 223 (3.91) nm; IR ν_{max} 3344, 2968, 1691, 1608, 1496, 1043 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 671.2314 $[M - H]^-$ (calcd for $C_{34}H_{39}O_{14}$, 671.2340).

*Saccharumoside B (**2**):* colorless, amorphous powder; $[\alpha]^{20}_D$ +75 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 299 (3.56), 265 (3.78), 220 (3.99) nm; IR ν_{max} 3415, 2935, 1699, 1598, 1500, 1064 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 465.1395 $[M - H]^-$ (calcd for $C_{22}H_{25}O_{11}$, 465.1397).

*Saccharumoside C (**3**):* colorless, amorphous solid; $[\alpha]^{20}_D$ −86 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 320 (3.69), 234 (3.92) nm; IR ν_{max} 3375, 1701, 1601, 1501 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 507.1351 $[M + HCOO]^-$ (calcd for $C_{20}H_{27}O_{15}$, 507.1350).

*Saccharumoside D (**4**):* colorless, amorphous solid; $[\alpha]^{20}_D$ −13 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 321 (3.69), 236 (3.87) nm; IR ν_{max} 3430, 1698, 1603, 1495 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 507.1352 $[M + HCOO]^-$ (calcd for $C_{20}H_{27}O_{15}$, 507.1350).

Acid Hydrolysis of Compounds 1−4 and Sugar Analysis. Each compound (2 mg) was added to a mixture of concentrated HCl (0.5 mL), H_2O (1.5 mL), and dioxane (3 mL) and refluxed for 2 h. After completion of the reaction (monitored by TLC), H_2O was added to the reaction mixture, which was then extracted with $CHCl_3$ (3 × 5 mL). The aqueous layer was neutralized with $NaHCO_3$ and then concentrated to dryness under reduced pressure and purified by Sephadex LH-20 chromatography to give a sugar fraction. The sugar fraction was analyzed by HPLC under the following conditions: column, Waters Xbridge Amide (100 × 4.6 mm, 3.5 μm); column temperature, 35 °C; mobile phase, acetonitrile−water (75/25, v/v) with 0.2% TEA; flow rate, 1.5 mL/min; detector, refractive index (35 °C). Identification of D-glucose and D-apiose was carried out by comparison of these retention times and optical rotations with those of authentic samples. D-Glucose: t_R 2.4 min, positive optical rotation; D-apiose: t_R 1.5 min, positive optical rotation.

Cytotoxicity Assay. Two human colon cancer cell lines, Caco-2 (adenocarcinoma) and HCT-116 (carcinoma), and the nontumorigenic colon cell line CCD-18Co were obtained from American Type Culture Collection (Rockville, MD, USA). Caco-2 and CCD-18Co cells were grown in EMEM medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v L-glutamine, and 1% v/v antibiotic solution (Sigma). HCT-116 cells were grown in McCoy's 5A medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 2% v/v HEPES, and 1% v/v

antibiotic solution. For each cell line, passage numbers between 26 and 35 were used. Cells were maintained at 37 °C in an incubator under a 5% CO_2/95% air atmosphere at constant humidity.

The cytotoxicity studies were carried out using an MTS assay, as described previously, with suitable modifications.[20] Briefly, test samples and a positive control, etoposide (4 μg/mL), were solubilized in DMSO by sonication. All samples were diluted with medium to the desired treatment concentration, and the final DMSO concentration per well did not exceed 0.5%. Control wells were also included on all plates. Following a 72 h drug-incubation period at 37 °C with serially diluted test compounds, MTS, in combination with the electron coupling agent phenazine methosulfate, was added to the wells, and cells were incubated at 37 °C in a humidified incubator for 3 h. Absorbance at 490 nm (OD 490) was monitored with a spectrophotometer (SpectraMax M2, Molecular Devices Corp., operated by SoftmaxPro v.4.6 software, Sunnyvale, CA, USA) to obtain the number of surviving cells relative to control populations. The results were determined as the median cytotoxic concentrations (IC_{50} values) and were calculated from six-point dose–response curves using 4-fold serial dilutions. Each point on the curve was included. Data are presented as means ± SD of three separate experiments on each cell line. Etoposide was used as a positive control and exhibited IC_{50} values of 9.9 ± 0.9 (HCT-116), 9.4 ± 0.7 (Caco-2), and 43.6 (CCD-18Co) μg/mL, respectively.

■ ASSOCIATED CONTENT

Supporting Information

The 1D and 2D NMR spectra of compounds **1**–**4** are available free of charge via the Internet at http://pubs.acs.org.

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