Polyhydroxylated Spirostanol Saponins from the Tubers of *Dioscorea polygonoides*

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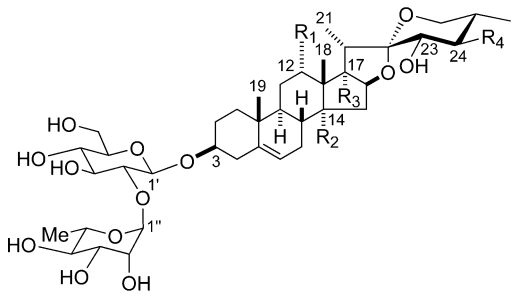
Three new polyhydroxylated spirostanol saponins (**1**−**3**) were isolated from the tubers of *Dioscorea polygonoides*. The structures of these new compounds were determined on the basis of extensive spectroscopic analysis and the results of acid or enzymatic hydrolysis as (23*S*,24*R*,25*S*)-23,24-dihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**1**), (23*S*,25*R*)-12α,17α,23-trihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**2**), and (23*S*,25*R*)-14α,17α,23-trihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**3**), respectively.

Some *Dioscorea* species are useful not only as sources for the preparation of steroidal saponins of commercial value, or as sources of steroidal hormones, but also as folk medicines.[1] *Dioscorea polygonoides* Humb. et Bonpl. (Dioscoreaceae) is distributed from Mexico to Brazil including Colombia. A phytochemical investigation of the tubers of *D. polygonoides* was carried out, with particular attention paid to the steroidal saponin constituents of the tubers, and has resulted in the isolation of three new polyhydroxylated spirostanol saponins (**1**−**3**). This paper deals with the structural determination of **1**−**3** on the basis of extensive spectroscopic analysis and the results of acid or enzymatic hydrolysis.



	R_1	R_2	R_3	R_4
1	H	H	H	OH
2	OH	H	OH	H
3	H	OH	OH	H

A MeOH extract of the tubers of *D. polygonoides* was partitioned between *n*-BuOH saturated with H_2O and H_2O. The *n*-BuOH-soluble phase was subjected to column chromatography on silica gel and octadecylsilanized (ODS) silica gel, as well as preparative HPLC, yielding **1** (12.6 mg), **2** (74.4 mg), and **3** (34.1 mg).

Compound **1**, isolated as an amorphous solid, showed an accurate $[M + H]^+$ ion at *m/z* 755.4213 in the positive-ion HRESIMS, corresponding to the empirical molecular formula $C_{39}H_{62}O_{14}$. The glycosidic nature of **1** was shown by strong IR absorptions at 3379 and 1044 cm^{-1}. The ^1H NMR spectrum of **1** in pyridine-d_5 displayed the following representative signals: four steroid methyl protons at δ 1.18 (d, $J = 7.0$ Hz), 1.09 (d, $J = 6.5$ Hz), 1.03 (s), and 0.98 (s); an olefinic proton at δ 5.28 (br d, $J = 4.9$ Hz); two anomeric protons at δ 6.33 (d, $J = 0.6$ Hz) and 5.00 (d, $J = 7.7$ Hz); and the methyl group of a 6-deoxyhexopyranose unit at δ 1.75 (d, $J = 6.2$ Hz). Enzymatic hydrolysis of **1** using naringinase failed to cleave the sugar moiety of **1** due to the insoluble nature of this glycoside in aqueous solution. Acid hydrolysis of **1** with 1.0 M HCl in dioxane−H_2O (1:1) gave D-glucose and L-rhamnose as the carbohydrate components, while the labile aglycon decomposed under acid conditions. The above data, along with two anomeric carbon signals observed at δ 102.0 and 100.3 and one distinctive quaternary carbon resonance appearance at δ 113.2, led to the realization that **1** is a spirostanol diglycoside.[2] Comparison of the ^1H and ^{13}C NMR assignments of the aglycon moiety, which were established by analysis of the $^1H-^1H$ COSY, HMQC, and HMBC spectra, with data of (25*R*)-spirost-5-en-3β-ol (diosgenin) 3-*O*-glycoside, isolated from several plants in the Liliaceae,[3−5] revealed that the structure of the ring A−E portion of the molecule (C-1−C-21) was identical to that of this reference compound, including the orientations of the C-3 oxygen atom (3β-equatorial) and Me-21 group (20α) and the ring junctions (B/C *trans*, C/D *trans*, D/E *cis*). However, significant differences were recognized in the signals from the ring F portion (C-22−C-27). The $^1H-^1H$ COSY spectrum was carefully inspected to assign the structure of the ring F residue, with the three-proton doublet at δ 1.09 ($J = 6.5$ Hz), attributable to Me-27, being used as the starting point for analysis. The Me-27 protons showed a spin-coupling correlation with the broad multiplet centered at δ 2.04, which was unambiguously assigned to H-25 and exhibited correlations with a pair of oxymethylene protons at δ 3.66 and 3.64 (H_2-26) and the oxymethine proton at δ 3.97 (H-24). The oxymethine proton, in turn, displayed a correlation with the terminal oxymethine proton at δ 3.88 (H-23). These subsequent correlations led the ring F fragment of **1** to be proposed as $-C_{(23)}H(O-)-C_{(24)}H(O-)-C_{(25)}H(C_{(27)}H_3)-C_{(26)}H_2-O-$. Thus, the presence of oxygen atoms at C-23 and C-24 was evident. The proton spin-coupling

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constant between H-23 and H-24 (J = 9.4 Hz), between H-24 and H-25 (J = 9.4 Hz), and between H-25 and H-26axial (J = 11.3 Hz), along with NOE correlations from H-23 to H-20, Me-21, and H-25 and from H-26axial to H-16 and H-24 in the PHNOSY spectrum of **1**, were consistent with the 22α, 23*S*, 24*R*, and 25*S* configurations. Treatment of **1** with Ac_2O in pyridine gave the corresponding octaacetate (**1a**). When the ^{1}H NMR spectrum of **1a** was compared with that of **1**, the H-23 and H-24 protons were moved downfield by 1.48 and 1.54 ppm and were observed at δ 5.36 and 5.51, respectively, whereas the chemical shift of H-3 was almost unaffected. This indicated that C-23 and C-24 have a free hydroxy group and that C-3 is glycosylated. Analysis of the ^{1}H and ^{13}C NMR spectra and the results of acid hydrolysis implied that the glycoside moiety of **1** was composed of a terminal α-L-rhamnopyranosyl unit (Rha) and a 2-substituted β-D-glucopyranosyl unit (Glc). In the HMBC spectrum, the anomeric proton of Rha at δ 6.33 showed a long-range correlation with C-2 of Glc at δ 77.8, for which the anomeric proton at δ 5.00 exhibited a correlation with C-3 of the aglycon at δ 78.0. All of these data were consistent with the structure (23*S*,24*R*,25*S*)-23,-24-dihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, which was given to **1**.

Compound **2** was obtained as an amorphous solid with a molecular formula of $C_{39}H_{62}O_{15}$, as determined by the data of the positive-ion HRESIMS (*m/z* 771.4191 $[M + H]^+$). The ^{1}H NMR spectrum of **2** showed signals for two three-proton doublets at δ 1.36 (J = 7.2 Hz) and 0.69 (J = 7.3 Hz) and two three-proton singlets at δ 1.13 and 0.99, as well as two anomeric protons at δ 6.33 (d, J = 1.0 Hz) and 4.97 (d, J = 7.7 Hz), and the ^{13}C NMR spectrum exhibited an acetalic carbon signal at δ 112.0, suggesting that **2** is also a spirostanol diglycoside. Enzymatic hydrolysis of **2** with naringinase resulted in the production of a new steroidal sapogenin (**2a**: $C_{27}H_{42}O_6$) and D-glucose and L-rhamnose. The ^{1}H NMR spectrum of **2a** measured in DMSO-d_6 showed signals for four exchangeable protons at δ 5.98, 5.74, 5.29, and 4.52, which disappeared on the addition of HCl vapor, indicative of **2a** having four hydroxy groups. The gross structure of **2a** was established in the following spectroscopic data observation. The multiplet proton signal centered at δ 3.78 ($W_{1/2}$ = 21.3 Hz) was shown to be coupled with two methylene groups at δ 2.00 and 1.76 (H_2-2) and δ 2.60 and 2.59 (H_2-4) and was assigned to the H-3 axial proton geminally bearing an equatorial-oriented hydroxy group. The methyl singlet at δ 1.17 due to Me-18 exhibited $^3J_{C,H}$ correlations with the methine carbon at δ 74.1 and quaternary carbon at δ 93.4, each attached to a hydroxy group. The hydroxymethine carbon at δ 74.1 was associated with the one-bond coupled proton at δ 4.41, which showed proton spin-coupling correlations with the methylene protons at δ 1.81 and 1.74 (H_2-11) and was assigned to H-12. The H-20 and Me-21 protons were observed as an AM_3 spin system at δ 3.41 (q, J = 7.2 Hz) and 1.37 (d, J = 7.2 Hz) and showed a long-range correlations with the quaternary carbon at δ 93.4. Furthermore, the oxymethine proton at δ 4.63 due to H-16 also showed an HMBC correlation with δ 93.4. These data gave evidence for the presence of a hydroxy group at C-12 and C-17. The locus of the one remaining hydroxy group was determined to be at C-23 by a long-range correlation between the hydroxymethine proton at δ 3.92 and the C-22 acetal carbon at δ 112.1 and by proton spin-coupling correlations from δ 3.92 to the methylene protons at δ 2.09 and 1.84 (H_2-24). NOE correlations from H-9 to H-14, H-12 to H_2-11, Me-18, and Me-21, H-16 to H-14, H-15α, and H-26axial, Me-18 to H-8, H-15β, and H-20, Me-19 to H-8 and H-11axial, and H-23 to H-20, Me-21, and H-25, along with the proton spin-coupling constants of H-12 (br s), H-23 (dd, J = 11.2, 4.6 Hz), and H-26axial (dd, J = 11.2, 11.2 Hz), confirmed the B/C *trans*, C/D *trans*, and D/E *cis* ring junctions and the 12α, 17α, 20α, 22α, 23*S*, and 25*R* configurations. The diglycoside *O*-α-L-rhamnosyl-(1→2)-β-D-glucosyl group was ascertained to be linked to C-3 of the aglycon by comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** and by analysis of the HMBC spectrum of **2**. Thus, the structure of **2** was assigned as (23*S*,25*R*)-12α,17α,23-trihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **3** was analyzed for $C_{39}H_{62}O_{15}$ as determined by the positive-ion HRESIMS (*m/z* 793.3992 $[M + Na]^+$). The ^{1}H NMR spectrum of **3** showed signals for four steroid methyls at δ 1.34 (d, J = 7.3 Hz), 1.31 (s), 1.04 (s), and 0.68 (d, J = 5.9 Hz) and two anomeric protons at δ 6.33 (d, J = 1.0 Hz) and 4.98 (d, J = 7.7 Hz). Enzymatic hydrolysis of **3** with naringinase furnished a new steroidal sapogenin (**3a**: $C_{27}H_{42}O_6$) and D-glucose and L-rhamnose. The molecular formula of **3a** was the same as that of **2a**, and the ^{1}H NMR spectrum was essentially analogous to that of **2a**, showing signals for four exchangeable protons at δ 5.28, 4.94, 4.59, and 4.51, together with signals for four steroid methyls. These data implied that **3a** is an isomer of **2a** with regard to the linkage positions of the hydroxy groups to the aglycon. The structure of **3a** was determined by the following spectroscopic data. The multiplet proton signal centered at δ 3.80 ($W_{1/2}$ = 20.6 Hz) was assigned to the H-3 axial proton geminally bearing an equatorial-oriented hydroxy group. The hydroxymethine proton at δ 3.94 showed an HMBC correlation with the C-22 acetal carbon at δ 112.0 and proton spin-coupling correlations with the methylene protons at δ 2.10 and 1.84 (H_2-24) and was assigned to H-23. Consequently, the two quaternary carbons at δ 90.9 and 87.9 were concluded to each bear a hydroxy group. The H_2-15 and H-16 protons and the H-20 and Me-21 protons were observed as an ABX-spin system at δ 2.60 (dd, J = 12.8, 7.5 Hz) and 1.93 (dd, J = 12.8, 6.4 Hz) and 4.94 (dd, J = 7.5, 6.4 Hz) and an AM_3-spin system at δ 3.52 (q, J = 7.1 Hz) and 1.35 (d, J = 7.1 Hz). In the HMBC spectrum, long-range correlations were observed from H_2-15 and H-16 to δ 87.9, from H-16, H-20, and Me-21 to δ 90.9, and from the three-proton singlet at δ 1.35 due to Me-18 to both δ 87.9 and 90.9, allowing the δ 87.9 and 90.9 resonances to be assigned to C-14 and C-17, respectively. The above data led to placement of the hydroxy groups at C-3β, C-14, C-17, and C-23. NOE correlations from H-16 to H-15α and H-26axial, Me-18 to H-8, H-15β, and H-20, Me-19 to H-8 and H-11axial, and H-23 to H-20, Me-21, and H-25, along with the proton spin-coupling constants of H-23 (dd, J = 11.2, 4.7 Hz) and H-26axial (dd, J = 10.6, 10.6 Hz), confirmed the B/C *trans*, C/D *trans*, and D/E *cis* ring junctions and the 14α, 17α, 20α, 22α, 23*S*, and 25*R* configurations. The diglycoside attached at C-3 of the aglycon of **3** was identified as *O*-α-L-rhamnosyl-(1→2)-β-D-glucose, as in **1** and **2**, on the basis of the ^{1}H NMR, ^{13}C NMR, and HMBC spectra of **3**. Thus, the structure of **3** was formulated as (23*S*,25*R*)-14α,17α,-23-trihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Although several spirostanols have been reported to show potent cytotoxic activities for cultured tumor cell lines,[5−8] **1**−**3**, **2a**, and **3a** exhibited no apparent cytotoxic activities against HSC-2 human squamous cell carcinoma

Table 1. ^{1}H and ^{13}C NMR Data of **1**, **2a**, and **3a** in Pyridine-d_5

		1				**2a**				**3a**			
		^{1}H		*J* (Hz)	^{13}C	^{1}H		*J* (Hz)	^{13}C	^{1}H		*J* (Hz)	^{13}C
1	eq	1.71			37.4	1.77			37.7	1.83			38.0
	ax	0.95				1.13	ddd	13.3, 13.3, 3.4		1.13	ddd	13.5, 13.5, 3.5	
2	eq	2.11			30.1	2.00			32.4	2.05			32.5
	ax	1.85				1.76				1.78			
3		3.92	br m	$W_{1/2}$ = 15.8	78.0	3.78	br m	$W_{1/2}$ = 21.3	71.2	3.80	br m	$W_{1/2}$ = 20.6	71.2
4	eq	2.78	dd	11.3, 3.0	38.9	2.60	dd	12.3, 5.0	43.4	2.60			43.4
	ax	2.70	dd	11.3, 11.3		2.59	dd	12.3, 12.3					
5					140.7				142.0				141.3
6		5.28	br d	4.9	121.7	5.36	br d	4.8	121.1	5.43	br d	4.3	121.5
7	eq	1.82			32.2	2.00			32.4	1.88			26.1
	ax	1.47				1.71				2.57			
8		1.49			31.5	1.73			32.8	2.06			36.1
9		0.88			50.2	1.84			43.7	1.84			43.5
10					37.1				36.7				37.2
11	eq	1.40			21.0	1.81			29.6	1.65			20.1
	ax	1.35				1.74				1.60			
12	eq	1.72			40.1	4.41	br s		74.1	1.41			26.9
	ax	1.12								2.59			
13					40.9				47.3				49.0
14		1.09			56.6	2.89			46.7				87.9
15	α	2.04			32.1	2.36			31.1	2.60	dd	12.8, 7.5	40.1
	β	1.54				1.63	ddd	12.9, 12.7, 6.3		1.93	dd	12.8, 6.4	
16		4.64	q-like	8.5	81.9	4.63	dd	7.6, 6.3	90.4	4.94	dd	7.5, 6.4	91.3
17		1.86	dd	8.5, 7.1	61.8				93.4				90.9
18		1.03	s		16.5	1.17	s		18.1	1.35	s		20.9
19		0.98	s		19.3	0.98	s		19.3	1.04	s		19.4
20		3.05			36.4	3.41	q	7.2	38.9	3.52	q	7.1	39.0
21		1.18	d	7.0	14.5	1.37	d	7.2	9.7	1.35	d	7.1	9.4
22					113.2				112.1				112.0
23		3.88	d	9.4	73.6	3.92	dd	11.2, 4.6	68.0	3.94	dd	11.2, 4.7	67.9
24	eq	3.97	dd	9.4, 9.4	76.0	2.09			38.2	2.10			38.3
	ax					1.84				1.84			
25		2.04			39.1	1.82			31.5	1.83			31.6
26	eq	3.64			64.4	3.44			65.7	3.48			65.9
	ax	3.66	dd	11.3, 11.3		3.47	dd	11.2, 11.2		3.46	dd	10.6, 10.6	
27		1.09	d	6.5	13.6	0.69	d	5.8	16.8	0.68	d	5.8	16.8
1′		5.00	d	7.7	100.3								
2′		4.23	dd	9.1, 7.7	77.8								
3′		4.30	dd	9.1, 9.1	79.5								
4′		4.17	dd	9.3, 9.1	71.7								
5′		3.88	ddd	9.3, 5.0, 2.3	78.1								
6′	a	4.48	dd	12.0, 2.3	62.5								
	b	4.34	dd	12.0, 5.0									
1″		6.33	d	0.6	102.0								
2″		4.80	dd	3.3, 0.6	72.4								
3″		4.61	dd	9.3, 3.3	72.8								
4″		4.34	dd	9.3, 9.3	74.1								
5″		4.96	dq	9.3, 6.2	69.4								
6″		1.75	d	6.2	18.6								

cells and HL-60 human promyelocytic leukemia cells (IC_{50} > 200 μM), respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. ESIMS data were obtained on a Micromass LCT mass spectrometer (Manchester, UK). Silica gel (Fuji-Silysia Chemical, Aichi, Japan, or Merck, Darmstadt, Germany) and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) or a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C_{18} UG120 column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC.

Plant Material. *Dioscorea polygonoides* was collected at Aranzazu, Caldas, Colombia, in October 1998. The plant was identified by one of the authors (J.N.O.), and a voucher specimen has been deposited in the herbarium of the Universidad de Antioquia, Medellín, Colombia (voucher no. HUA 132745).

Extraction and Isolation. A dried powder (1.0 kg) of *D. polygonoides* tubers was extracted with MeOH (5 L) at 95 °C for 3 h twice. After removal of the solvent by evaporation, the viscous extract was partitioned between *n*-BuOH saturated with H_2O and H_2O. The *n*-BuOH-soluble portion (9.2 g) was subjected to vacuum-liquid chromatography on silica gel and elution with $CHCl_3$−MeOH (19:1), with increasing amounts of MeOH (5% to 100%), to give eight fractions (fractions I−VIII). Fraction V (1.5 g) was further separated by passage over a silica gel column, using $CHCl_3$−MeOH−H_2O (14:5:1) for elution, into five subfractions (fractions Va−Ve). Subfrac-

tion Vc (0.22 g) was subjected to ODS silica gel column chromatography, eluted with MeOH−H_2O (16:7) and MeCN−H_2O (2:5), and then to preparative HPLC using MeCN−H_2O (2:5), to give **1** (12.6 mg), **2** (74.4 mg), and **3** (34.1 mg).

Compound 1: amorphous solid; $[\alpha]_D^{26}$ −114.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3379 (OH), 2917 and 2849 (CH), 1044 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 755.4213 $[M + H]^+$ (calcd for $C_{39}H_{63}O_{14}$, 755.4218).

Acid Hydrolysis of 1. A solution of **1** (4.8 mg) in 1.0 M HCl (dioxane−H_2O, 1:1, 3 mL) was heated at 95 °C for 1 h under an Ar atmosphere. On cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93 ZU (Organo, Tokyo, Japan) column and then passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), eluted with 10% MeOH followed by MeOH. The 10% MeOH eluate fraction (1.2 mg) was analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN−H_2O (17:3); flow rate, 1.0 mL/min; detection, RI and OR. Identification of L-rhamnose and D-glucose was carried out by comparison of their retention times and optical rotations with those of authentic samples: t_R (min), 6.39 (L-rhamnose, negative optical rotation), 11.78 (D-glucose, positive optical rotation).

Acetylation of 1. Compound **1** (1.9 mg) was treated with Ac_2O (1 mL) in pyridine (1 mL) at room temperature for 20 h. After addition of H_2O, the reaction mixture was extracted with Et_2O and the Et_2O phase was chromatographed on silica gel, eluted with hexane−Me_2CO (3:1), to afford **1a** (1.6 mg).

Compound 1a: amorphous solid; $[\alpha]_D^{25}$ −56.0° (*c* 0.10, MeOH); IR ν_{max} (film) 2956, 2918 and 2849 (CH), 1748 (C=O) cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.83 (1H, dd, *J* = 9.5, 9.5 Hz, H-3′), 5.80 (1H, dd, *J* = 10.1, 3.4 Hz, H-3″), 5.68 (1H, dd, *J* = 10.1, 10.1 Hz, H-4″), 5.61 (1H, dd, *J* = 3.4, 1.5 Hz, H-2″), 5.51 (1H, dd, *J* = 10.3, 9.8 Hz, H-24), 5.45 (1H, d, *J* = 1.5 Hz, H-1″), 5.44 (1H, dd, *J* = 9.8, 9.5 Hz, H-4′), 5.43 (1H, br d, *J* = 5.0 Hz, H-6), 5.36 (1H, d, *J* = 9.8 Hz, H-23), 5.07 (1H, d, *J* = 7.8 Hz, H-1′), 4.93 (1H, dq, *J* = 10.1, 6.3 Hz, H-5″), 4.65 (1H, dd, *J* = 12.3, 4.5 Hz, H-6′a), 4.56 (1H, q-like, *J* = 8.5 Hz, H-16), 4.43 (1H, dd, *J* = 12.3, 2.2 Hz, H-6′b), 4.15 (1H, ddd, *J* = 9.8, 4.5, 2.2 Hz, H-5′), 4.12 (dd, *J* = 9.5, 7.8 Hz, H-2′), 3.94 (1H, br m, $W_{1/2}$ = 15.8 Hz, H-3), 3.60 (1H, dd, *J* = 11.3, 5.9 Hz, H-26eq), 3.57 (1H, dd, *J* = 11.3, 11.3 Hz, H-26ax), 2.21, 2.17, 2.15, 2.08, 2.05 × 2, 2.03, 2.02 (each 3H, s, Ac × 8), 1.51 (3H, d, *J* = 6.3 Hz, Me-6″), 1.20 (3H, d, *J* = 7.0 Hz, Me-21), 1.11 (3H, s, Me-18), 0.89 (3H, s, Me-19), 0.80 (3H, d, *J* = 6.6 Hz, Me-27).

Compound 2: amorphous solid; $[\alpha]_D^{27}$ −98.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3392 (OH), 2954, 2932 and 2905 (CH), 1052 cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.33 (1H, d, *J* = 1.0 Hz, H-1″), 5.28 (1H, br d, *J* = 4.8 Hz, H-6), 4.97 (1H, d, *J* = 7.7 Hz, H-1′), 4.63 (1H, dd, *J* = 7.5, 6.3 Hz, H-16), 4.38 (1H, br s, H-12), 3.89 (1H, br m, $W_{1/2}$ = 17.8 Hz, H-3), 3.91 (1H, m, H-23), 3.46 (2H, m, H_2-26), 1.74 (3H, d, *J* = 6.2 Hz, Me-6″), 1.36 (3H, d, *J* = 7.2 Hz, Me-27), 1.13 (3H, s, Me-18), 0.99 (3H, s, Me-19), 0.69 (3H, d, *J* = 7.3 Hz, Me-21); ^{13}C NMR (pyridine-d_5) δ 37.3 (C-1), 30.1 (C-2), 77.8 (C-3), 38.9 (C-4), 140.8 (C-5), 121.8 (C-6), 32.4 (C-7), 32.7 (C-8), 43.6 (C-9), 36.8 (C-10), 29.5 (C-11), 74.0 (C-12), 47.3 (C-13), 46.5 (C-14), 31.1 (C-15), 90.3 (C-16), 93.3 (C-17), 18.0 (C-18), 19.1 (C-19), 38.9 (C-20), 9.7 (C-21), 112.0 (C-22), 68.0 (C-23), 38.2 (C-24), 31.5 (C-25), 65.7 (C-26), 16.8 (C-27), 100.2 (C-1′), 77.8 (C-2′), 79.5 (C-3′), 71.7 (C-4′), 78.1 (C-5′), 62.5 (C-6′), 102.0 (C-1″), 72.4 (C-2″), 72.8 (C-3″), 74.1 (C-4″), 69.4 (C-5″), 18.6 (C-6″); HRESIMS *m/z* 771.4191 $[M + H]^+$ (calcd for $C_{39}H_{63}O_{15}$, 771.4167).

Enzymatic Hydrolysis of 2. Compound **2** (20.9 mg) was treated with naringinase (EC 232-962-4; Sigma, St. Louis, MO) (80 mg) in AcOH−AcOK buffer (pH 4.3, 10 mL) at room temperature for 72 h. The reaction mixture was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA), eluted with 20% MeOH followed by MeOH. The MeOH eluate fraction was purified by silica gel column chromatography, eluted with $CHCl_3$−MeOH (20:1), to afford **2a** (12.4 mg). HPLC analysis of the 20% MeOH eluate fraction under the same conditions as used for **1** showed the presence of L-rhamnose and D-glucose: t_R (min), 6.41 (L-rhamnose, negative optical rotation), 11.87 (D-glucose, positive optical rotation).

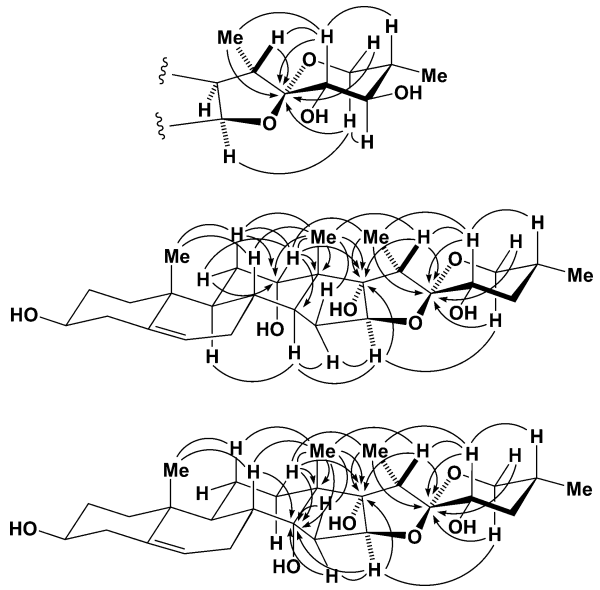

Figure 1. Partial HMBC (arrows) and NOE (curved lines) correlations of **1**, **2a**, and **3a**.

Compound 2a: amorphous solid; $[\alpha]_D^{24}$ −102.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3352 (OH), 2953, 2927 and 2861 (CH), 1091, 1057 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.98 (s), 5.74 (s), 5.29 (d, *J* = 5.0 Hz), 4.52 (d, *J* = 7.7 Hz); ^1H (pyridine-d_5) and ^{13}C NMR, see Table 1; HRESIMS *m/z* 463.3069 $[M + H]^+$ (calcd for $C_{27}H_{43}O_6$, 463.3060).

Compound 3: amorphous solid; $[\alpha]_D^{26}$ −84.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3393 (OH), 2957, 2932 and 2874 (CH), 1055 cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.33 (1H, d, *J* = 1.0 Hz, H-1″), 5.33 (1H, br d, *J* = 4.8 Hz, H-6), 4.98 (1H, d, *J* = 7.7 Hz, H-1′), 4.94 (1H, m, H-16), 3.94 (1H, dd, *J* = 11.2, 4.7 Hz, H-23), 3.87 (1H, br m, $W_{1/2}$ = 18.0 Hz, H-3), 3.47 (2H, m, H_2-26), 1.75 (3H, d, *J* = 6.2 Hz, Me-6″), 1.34 (3H, d, *J* = 7.3 Hz, Me-21), 1.31 (3H, s, Me-18), 1.04 (3H, s, Me-19), 0.68 (3H, d, *J* = 5.9 Hz, Me-27); ^{13}C NMR (pyridine-d_5) δ 37.7 (C-1), 30.1 (C-2), 77.8 (C-3), 38.9 (C-4), 140.1 (C-5), 122.2 (C-6), 26.1 (C-7), 36.0 (C-8), 43.4 (C-9), 37.3 (C-10), 20.0 (C-11), 26.8 (C-12), 48.9 (C-13), 87.9 (C-14), 40.1 (C-15), 91.2 (C-16), 90.8 (C-17), 20.8 (C-18), 19.3 (C-19), 38.9 (C-20), 9.4 (C-21), 112.0 (C-22), 67.9 (C-23), 38.3 (C-24), 31.5 (C-25), 65.9 (C-26), 16.8 (C-27), 100.2 (C-1′), 77.8 (C-2′), 79.5 (C-3′), 71.7 (C-4′), 78.2 (C-5′), 62.5 (C-6′), 102.0 (C-1″), 72.4 (C-2″), 72.8 (C-3″), 74.1 (C-4″), 69.4 (C-5″), 18.6 (C-6″); HRESIMS *m/z* 793.3992 $[M + Na]^+$ (calcd for $C_{39}H_{62}O_{15}Na$, 793.3986).

Enzymatic Hydrolysis of 3. Compound **3** (15.2 mg) was subjected to enzymatic hydrolysis using naringinase as described for **2** to give an aglycon (**3a**) (9.1 mg) and a sugar fraction. HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-rhamnose and D-glucose: t_R (min), 6.45 (L-rhamnose, negative optical rotation), 11.89 (D-glucose, positive optical rotation).

Compound 3a: amorphous solid; $[\alpha]_D^{24}$ −72.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3357 (OH), 2927 and 2871 (CH), 1058 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.28 (d, *J* = 4.8 Hz), 4.94 (s), 4.59 (s), 4.51 (d, *J* = 7.9 Hz); ^1H (pyridine-d_5) and ^{13}C NMR, see Table 1; HRESIMS *m/z* 485.2824 $[M + Na]^+$ (calcd for $C_{27}H_{42}O_6Na$, 485.2879).

HSC-2 and HL-60 Cell Culture Assay. The cell growth was measured with an MTT reduction assay procedure as described in previous papers.[7,8]

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