Polyhydroxylated Spirostanol Saponins from the Tubers of *Dioscorea polygonoides*

Jaime Niño Osorio,[†] Oscar M. Mosquera Martinez,[†] Yaned M. Correa Navarro,[†] Kazuki Watanabe,[‡] Hiroshi Sakagami,[§] and Yoshihiro Mimaki*,[‡]

Grupo de Biotecnología-Productos Naturales, Escuela de Tecnología Química, Universidad Tecnológica de Pereira, La Julita, A. A. 97 Pereira, Colombia, Laboratory of Medicinal Pharmacognosy, Tokyo University of Pharmacy and Life Science, School of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan, and Department of Dental Pharmacology, Meikai University School of Dentistry, 1-1, Keyaki-dai, Sakado, Saitama 350-0283, Japan

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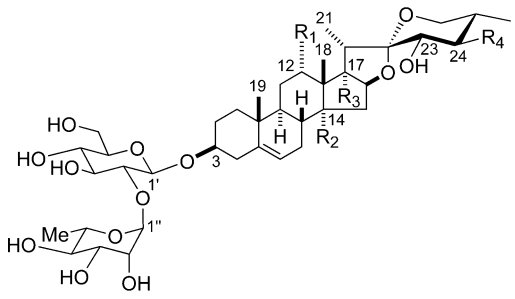
Three new polyhydroxylated spirostanol saponins (**1**−**3**) were isolated from the tubers of *Dioscorea polygonoides*. The structures of these new compounds were determined on the basis of extensive spectroscopic analysis and the results of acid or enzymatic hydrolysis as (23*S*,24*R*,25*S*)-23,24-dihydroxyspirost-5-en-3β-yl *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

* To whom correspondence should be addressed. Tel: +81-426-76-4577. Fax: +81-426-76-4579. E-mail: mimakiy@ps.toyaku.ac.jp.
† Universidad Tecnológica de Pereira.
‡ Tokyo University of Pharmacy and Life Science.
§ Meikai University School of Dentistry.

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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).

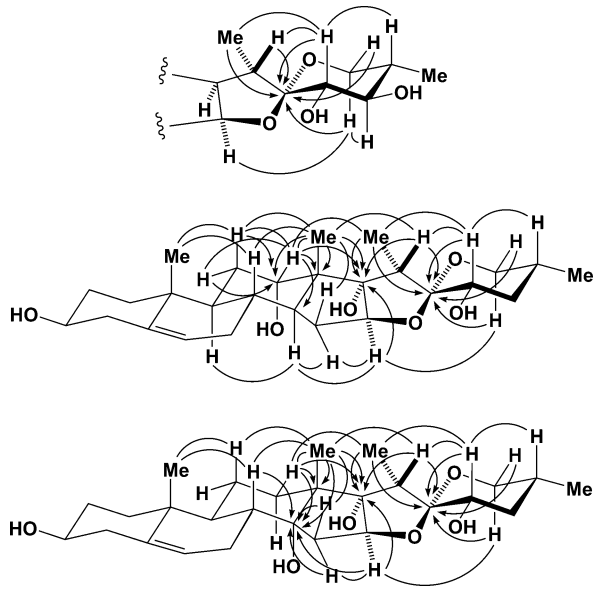

Figure 1. Partial HMBC (arrows) and NOE (curved lines) correlations of **1**, **2a**, and **3a**.

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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