Cytotoxic Furostanol Saponins and a Megastigmane Glucoside from *Tribulus parvispinus*

Angela Perrone,[†] Alberto Plaza,[†] Elena Bloise,[†] Patrizia Nigro,[†] Arafa I. Hamed,[‡] Maria Antonietta Belisario,[†] Cosimo Pizza,[†] and Sonia Piacente*,[†]

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy, and Faculty of Science, South Valley University, Aswan 81528, Egypt

Two new furostanol saponins, (25*R*)-26-*O*-β-D-glucopyranosyl-5α-furostan-2α,3β,22α,26-tetraol 3-*O*-{β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**1**) and (25*R*)-26-*O*-β-D-glucopyranosyl-5α-furostan-3β,22α,26-triol 3-*O*-{β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**2**), and their *O*-methyl derivatives (**3** and **4**), and a new megastigmane glucoside, (6*S*,7*E*,9ξ)-6,9,10-trihydroxy-4,7-megastigmadien-3-one 10-*O*-β-D-glucopyranoside (**6**), along with one known spirostanol saponin, gitonin (**5**), and four known megastigmane glucosides were isolated from the aerial parts of *Tribulus parvispinus*. Their structures were established by detailed spectroscopic analysis. The cytotoxic activities of **1**−**6** against U937, MCF7, and HepG2 cells were evaluated. Compounds **2** (IC$_{50}$ 0.5 μM) and **5** (IC$_{50}$ 0.1 μM) showed the highest activity against U937 cells.

Tribulus parvispinus Presl (Zygophyllaceae) is an annual prostrate herb that grows in the warm regions of Egypt, Iraq, Iran, and Pakistan.[1] Among the almost 25 species of *Tribulus*, only *T. terrestris*,[2−4] *T. cistoides*,[5,6] and *T. pentandrus*[7] have been chemically studied. *T. terrestris* is a well-known pharmaceutical herb that has long been used in the traditional Chinese and Indian systems of medicine for the treatment of various diseases. *Tribulus* species have been shown to be rich in furostane- and spirostane-type steroidal saponins that have displayed a wide range of biological activities including cytotoxic,[8−11] antiproliferative,[12] and antimicrobial effects.[13]

As a part of our ongoing search for new bioactive compounds from medicinal plants of the Egyptian desert, we have studied the aerial parts of *T. parvispinus* Presl. In the present paper, we describe the isolation of two new furostanol saponins, named parvispinosides A (**1**) and B (**2**), their corresponding 22-*O*-methyl derivatives (**3**, **4**), one new megastigmane glucoside, named parvispinoside C (**6**), one known spirostanol saponin, gitonin (**5**),[14] and four known megastigmane glucosides: alangionoside C,[15] sonchuionoside A,[16] citroside A,[17] and roseoside.[18] To the authors' knowledge, this is the first time that megastigmane glycosides have been isolated from a *Tribulus* species. The antiproliferative activity of **1**−**6** was evaluated against the U937 leukemia cell line and the solid tumor cell lines MCF7 (breast) and HepG2 (hepatoma).

The dried aerial parts of *T. parvispinus* were submitted to sequential extraction with petroleum ether, CHCl$_3$, and MeOH. Chromatography of the MeOH extract over Sephadex LH-20 followed by repeated column chromatography on reversed-phase HPLC yielded glycosides **1**−**4** and **6**.

Compound **1**, in the positive ESIMS, showed a major ion peak at *m/z* 1253 [M + Na]$^+$ and significant fragments at *m/z* 1121 [M + Na − 132]$^+$ and *m/z* 1091 [M + Na − 162]$^+$ attributable to the loss of a pentose or a hexose unit, respectively. The molecular formula of **1** was unequivocally

1 R$_1$ = OH R$_2$ = H

2 R$_1$ = H R$_2$ = H

3 R$_1$ = OH R$_2$ = CH$_3$

4 R$_1$ = H R$_2$ = CH$_3$

6

5
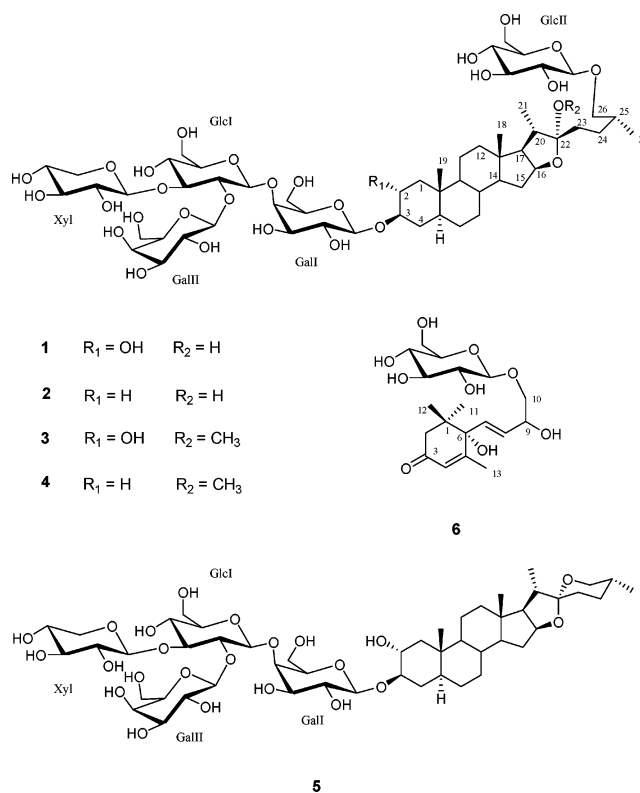
established to be C$_{56}$H$_{94}$O$_{29}$ by HR-MALDI-MS (*m/z* 1253.5792 [M + Na]$^+$). The ^{1}H NMR spectrum of **1** showed signals for four steroidal methyl groups at δ 0.84, 0.92, 0.97, and 1.03, two methine proton signals at δ 3.51 and 3.69 indicative of secondary alcoholic functions, two methylene proton signals at δ 3.36 and 3.82 ascribable to a primary alcoholic function, and five anomeric protons at δ 4.93, 4.65, 4.64, 4.41, and 4.27. The ^{13}C NMR spectrum displayed signals ascribable to a hemiacetal function at δ 111.9, three secondary alcoholic functions at δ 71.2, 82.2, and 84.4, and one primary alcoholic function at δ 75.6, suggesting the occurrence of a glycosidic furostanol skeleton. On the basis of the HSQC and HMBC correlations, the aglycon moiety

* To whom correspondence should be addressed. Tel: ++39089962616. Fax: ++39089962828. E-mail: piacente@unisa.it.
† Università degli Studi di Salerno.
‡ South Valley University.

Table 1. ^{13}C and ^{1}H NMR Data of the Sugar Portion of Compound **1** in CD$_3$OD^{a,b}

position	δ_C	δ_H (J in Hz)
	β-D-GalI	
1	102.6	4.41 d (7.9)
2	72.4	3.84 dd (8.5, 7.9)
3	75.5	3.58 dd (8.5, 2.9)
4	79.9	4.07 dd (2.9, 1.2)
5	75.5	3.60 m
6	61.2	3.89 dd (12.0, 2.0)
		3.70 dd (12.0, 4.5)
	β-D-GlcI	
1	104.7	4.65 d (7.9)
2	80.9	3.81 dd (9.0, 7.9)
3	87.5	3.76 dd (9.0, 9.0)
4	70.2	3.32 dd (9.0, 9.0)
5	77.6	3.31 m
6	62.9	3.94 dd (12.0, 2.5)
		3.62 dd (12.0, 4.5)
	β-D-GalII	
1	104.7	4.93 d (7.9)
2	73.1	3.61 dd (9.8, 7.9)
3	74.7	3.52 dd (9.8, 3.4)
4	70.5	3.89 dd (3.4, 1.1)
5	77.0	3.58 m
6	62.4	4.03 dd (11.8, 7.0)
		3.74 dd (11.8, 5.9)
	β-D-Xyl	
1	104.7	4.64 d (7.5)
2	75.1	3.28 dd (9.0, 7.5)
3	77.9	3.37 dd (9.0, 9.0)
4	70.9	3.56 m
5	67.1	3.95 dd (10.5, 4.5)
		3.30 t (10.5)
	β-D-GlcII	
1	104.4	4.27 d (7.9)
2	75.0	3.22 dd (9.0, 7.9)
3	77.8	3.37 dd (9.0, 9.0)
4	71.6	3.30 dd (9.0, 9.0)
5	77.8	3.29 m
6	62.7	3.90 dd (12.0, 2.5)
		3.71 dd (12.0, 4.5)

^{a} Assignments were confirmed by 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. ^{b} NMR data for the sugar portion of compounds **2**–**4** were nearly identical to those of compound **1**.

of compound **1** was identified as (25*R*)-5α-furostan-2α,3β,-22α,26-tetraol. The configuration of the hydroxyl group at C-22 was established to be α from ROESY correlations between H-20 (δ 2.20) and the protons H-23a (δ 1.84) and H-23b (δ 1.63). The C-25 configuration was deduced to be *R* on the basis of the difference of chemical shifts ($\Delta_{ab} = \delta_a - \delta_b$) of the geminal protons at H$_2$-26 (Δ_{ab} = 0.46 ppm). It has been described that Δ_{ab} is usually >0.57 ppm in 25*S* compounds and <0.48 in 25*R* compounds.[19] It was evident from the ^{1}H and ^{13}C NMR data that the sugar chain of **1** consisted of four sugar units. The chemical shifts of all the individual protons of the four sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their relative attached carbons were assigned unambiguously from the HSQC spectrum (see Table 1). These data showed the presence of two *β*-galactopyranosyl units (δ 4.93 and 4.41), one *β*-glucopyranosyl unit (δ 4.65), and one *β*-xylopyranosyl unit (δ 4.64). Glycosidation shifts were observed for C-4$_{galI}$ (δ 79.9), C-2$_{glcI}$ (δ 80.9), and C-3$_{glcI}$ (δ 87.5). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signal at δ 4.41 (H-1$_{galI}$) and the carbon resonance at δ 84.4 (C-3), 4.65 (H-1$_{glcI}$) and 79.9 (C-4$_{galI}$), 4.93 (H-1$_{galII}$) and 80.9 (C-2$_{glcI}$), and the proton signal at δ 4.64 (H-1$_{xyl}$) and the carbon reso-

nance at δ 87.5 (C-3$_{glcI}$). The configurations of the sugar units were assigned after hydrolysis of **1** with 1 N HCl. The sugar units of **1** were determined to be D-galactose, D-glucose, and D-xylose in the ratio 2:2:1. Thus, the structure of **1** was established as (25*R*)-26-*O*-*β*-D-glucopyranosyl-5α-furostan-2α,3β,22α,26-tetraol 3-*O*-{*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside}, named parvispinoside A.

Compound **2** showed a quasimolecular ion peak at *m/z* 1237 [M + Na]$^+$ and significant fragments at *m/z* 1105 [M + Na − 132]$^+$ and 1075 [M + Na − 162]$^+$ in the positive ESIMS. The molecular formula of **2** was unequivocally established to be C$_{56}$H$_{94}$O$_{28}$ by HR-MALDI-MS (*m/z* 1237.5844 [M + Na]$^+$). The ^{1}H and ^{13}C NMR data of **2** in comparison to those of **1** clearly suggested that **2** differed from **1** only by the absence of the hydroxyl group at C-2 (see Experimental Section). Thus, compound **2** was identified as (25*R*)-26-*O*-*β*-D-glucopyranosyl-5α-furostan-3β,22α,-26-triol 3-*O*-{*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside}, named parvispinoside B.

The HR-MALDI mass spectra of **3** (*m/z* 1267.5946 [M + Na]$^+$) and **4** (*m/z* 1251.5998 [M + Na]$^+$) supported molecular formulas of C$_{57}$H$_{96}$O$_{29}$ and C$_{57}$H$_{96}$O$_{28}$, respectively. It was apparent from the NMR data (^{1}H, ^{13}C, 1D-TOCSY, DQF-COSY, HSQC, HMBC) of compounds **3** and **4** that these compounds only differed from **1** and **2**, respectively, by the presence of a OCH$_3$ group instead of a OH group at C-22. Therefore, **3** was deduced to be (25*R*)-26-*O*-*β*-D-glucopyranosyl-22α-methoxy-5α-furostan-2α,3β,26-triol 3-*O*-{*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside}, and **4** was established as (25*R*)-26-*O*-*β*-D-glucopyranosyl-22α-methoxy-5α-furostan-3β,26-diol 3-*O*-{*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside}. Thus, compounds **3** and **4** are 22-*O*-methyl derivatives of parvispinosides A (**1**) and B (**2**), respectively, and can be considered as secondary products formed from the corresponding 22-hydroxyfurostanols during the extraction of the plant material with methanol.

Compound **6**, named parvispinoside C, was isolated as an amorphous white powder. The molecular formula of **6** was established as C$_{19}$H$_{30}$O$_9$ by HR-MALDI-MS. The UV spectrum (λ_{max} 233) and IR absorption (ν_{max} 1663 cm^{-1}) suggested the occurrence of an α,β-unsaturated ketone.[20] The ^{1}H NMR spectrum showed signals for three olefinic protons at δ 5.95, 5.90, and 5.80, two methylene protons at δ 3.93 and 3.48 corresponding to a primary alcoholic function, one methine proton at δ 4.42 corresponding to a secondary alcoholic function, three methyl groups at δ 1.94, 1.06, and 1.04, and one anomeric proton at δ 4.31. The ^{13}C NMR spectrum of **6** showed 19 carbon signals, 13 of them corresponding to the aglycon moiety and the other six to a glucopyranosyl unit, suggesting the occurrence of a megastigmane glucoside. The NMR data (^{1}H, ^{13}C, 2D-HO-HAHA, DQF-COSY, HSQC, HMBC, ROESY) of **6** in comparison to those of the previously isolated corchoionoside B[18] revealed that **6** differed from corchoionoside B only by the occurrence of a hydroxyl group at C-9 instead of a ketone function. In particular, C-7 and C-9 were significantly shifted to upfield values of δ 132.6 and 71.8, while C-8 was shifted downfield to δ 131.4, when compared with corchoionoside B, in which they resonated at δ 148.5 (C-7), 127.5 (C-8), and 198.6 (C-9). The D configuration of the glucose unit was determined by acid hydrolysis of **6**

Table 2. Effect of Compounds **1**−**6** on the Growth of U937, MCF7, and HepG2 Cell Lines[a]

compound	IC$_{50}$ (μM)		
	U937	MCF7	HepG2
1	30 ± 3.5	>100	>100
2	0.5 ± 0.019	94 ± 11.0	>100
3	1 ± 0.13	50 ± 6.0	30 ± 3.1
4	2 ± 0.22	40 ± 4.3	30 ± 3.4
5	0.1 ± 0.015	3 ± 0.4	3 ± 0.41
6	15 ± 1.9	80 ± 8.0	>100
resveratrol[28]	17 ± 2.21	100 ± 11.2	50 ± 5.1

[a] Data represent the means $\pm$ SD from three independent experiments performed in quintuplicate wells.

Table 3. Effects of Compounds **1**−**5** on LDH Leakage and Apoptosis in U937 Cells[a,b]

compound	LDH leakage (% of total LDH activity)	apoptotic elements in the hypodiploid region (%)
control	5 ± 0.6	2 ± 0.2
1	28 ± 3.1	32 ± 3.7
2	27 ± 3.3	26 ± 3.2
3	38 ± 4.2	9 ± 1.1
4	50 ± 6.1	3 ± 0.4
5	25 ± 3.0	20 ± 2.3

[a] Data represent the means $\pm$ SD from three independent experiments performed in quintuplicate wells. [b] Each compound was tested at a concentration corresponding to its IC$_{50}$ value.

followed by GC analysis. The CD spectrum showed a positive Cotton effect at 240 nm (Δ_ϵ +12.7), suggesting C-6 to have the *S* configuration.[18,21] On the basis of this evidence, the structure of compound **6** was established as (6*S*,7*E*,9ξ)-6,9,10-trihydroxy-4,7-megastigmadien-3-one 10-*O*-β-D-glucopyranoside.

The antiproliferative activity of **1**−**6** was evaluated against the U937 leukemia cell line and the solid tumor cell lines MCF7 (breast) and HepG2 (hepatoma). As shown in Table 2, the U937 cell line was more sensitive to the cytotoxicity of **1**−**6** than HepG2 and MCF7 cells. Gitonin (**5**) exhibited the highest cytotoxic activity against the three cell lines, with IC$_{50}$ values of 0.1 μM (U937), 3.0 μM (MCF7), and 3.0 μM (HepG2). Compound **2** was cytotoxic against the U937 cell line (IC$_{50}$ = 0.5 μM), similarly to **5**. The antiproliferative activity of **2** and **5** was evaluated on the U937 cell line at doses of 0.5 and 0.1 μM, respectively. After 24 h of treatment with **2** and **5** a reduction of cell viability was observed. Nevertheless, after 48 h only **5** showed significant reduction of proliferation rate. These results suggested that the cell number reduction exhibited by **2** was mainly due to cell death rather than to impaired cell-cycle progression.

To investigate whether compounds **1**−**5** decreased cell viability by the induction of apoptosis or necrosis, lactate dehydrogenase (LDH) leakage, an indicator of necrosis, and hypoploidia induction, indicative of apoptosis, were measured in U937 cells. The results summarized in Table 3 clearly indicate that **1**, **2**, and **5** were able to induce apoptosis and cause necrosis, while compounds **3** and **4** only cause necrosis.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco DIP 1000 polarimeter. UV spectra were obtained on a Beckman DU 670 spectrometer. The CD spectrum was recorded using a JASCO J-810 spectropolarimeter with a cell of 1.0 mm path length. IR measurements were obtained on a Bruker IFS-48 spectrometer. Exact masses were measured by a Voyager DE mass spectrometer. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca

XP Max ion trap mass spectrometer equipped with Xcalibur software. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D-NMR spectra were acquired in CD$_3$OD. The standard pulse sequence and phase cycling were used for DQF-COSY, HSQC, HMBC, and ROESY spectra. Column chromatography was performed over Sephadex LH-20 (Pharmacia). HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC$_{18}$ column, and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

Plant Material. Fresh aerial parts of *Tribulus parvispinus* were collected in Aswan, Egypt, in August 2003 and identified by Dr. Mohamed G. Sheded. A voucher specimen (No. 10499) was deposited at the Botany Department Herbarium, Faculty of Science of Aswan, Egypt.

Extraction and Isolation. The dried and powdered aerial parts of *T. parvispinus* (285 g) were extracted at room temperature using solvents of increasing polarity (3 × 1.5 L), namely, petroleum ether (2.74 g), CHCl$_3$ (3.35 g), and MeOH (29.2 g). The MeOH extract was partitioned with *n*-BuOH−H$_2$O (1:1) to afford a dried *n*-BuOH extract (5.82 g). Part of the *n*-BuOH extract (3.3 g) was fractionated initially on a Sephadex LH-20 column (100 × 5.0 cm), using MeOH as mobile phase, and 47 fractions (8 mL each) were obtained. Fractions 22 and 23 (107 mg) were chromatographed by HPLC on a Waters (XTerra Prep MSC$_{18}$) column (300 × 7.8 mm i.d.) using MeOH−H$_2$O (3:2) as mobile phase to yield compounds **1** (1.7 mg), **2** (3.5 mg), **3** (15.7 mg), and **4** (7.8 mg). Fractions 24−26 (194.9 mg) were chromatographed under the same conditions to afford **5** (2.1 mg). Fractions 24−26 (50 mg) were chromatographed by RP-HPLC, using MeOH−H$_2$O (7:13) as mobile phase, to yield roseoside (1.0 mg) and alangionoside C (2.0 mg). Fractions 27 and 28 (93.7 mg) were chromatographed by RP-HPLC, using MeOH−H$_2$O (3:7) as mobile phase, to yield **6** (1.3 mg), citroside A (1.0 mg), and sonchuionoside A (1.1 mg).

Parvispinoside A (1): amorphous white powder (MeOH); [α]$^{22}_D$ +7.1° (*c* 0.1 MeOH); IR (KBr) ν_{max} 3409, 2927, 1373, 1287, 1245, 1166, 1236, 785, 675 cm^{-1}; ^{1}H NMR (CD$_3$OD, 600 MHz) aglycon moiety δ 4.57 (1H, dd, *J* = 14.5, 7.5 Hz, H-16), 3.82 (1H, m, H-26a), 3.69 (1H, m, H-2), 3.51 (1H, m, H-3), 3.36 (1H, m, H-26b), 1.20 (1H, m, H-5), 1.03 (3H, d, *J* = 6.6 Hz, Me-21), 0.97 (3H, s, Me-27), 0.92 (3H, s, Me-19), 0.84 (3H, s, Me-18); ^{13}C NMR (CD$_3$OD, 150 MHz) aglycon moiety δ 45.7 (C-1), 71.2 (C-2), 84.4 (C-3), 33.5 (C-4), 45.7 (C-5), 28.9 (C-6), 33.1 (C-7), 35.8 (C-8), 55.6 (C-9), 37.6 (C-10), 40.9 (C-11), 40.9 (C-12), 42.1 (C-13), 57.3 (C-14), 32.5 (C-15), 82.2 (C-16), 65.1 (C-17), 16.7 (C-18), 13.4 (C-19), 40.7 (C-20), 16.2 (C-21), 111.9 (C-22), 37.4 (C-23), 28.6 (C-24), 34.7 (C-25), 75.6 (C-26), 17.2 (C-27); ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data of the sugar portion are reported in Table 1; ESIMS *m/z* 1253 [M + Na]$^+$, ESIMS/MS *m/z* 1121 [M + Na − 132]$^+$, 1091 [M + Na − 162]$^+$; HR-MALDI-MS *m/z* [M + H]$^+$ calcd for C$_{56}$H$_{94}$O$_{29}$Na 1253.5778, found 1253.5792.

Parvispinoside B (2): amorphous white powder (MeOH); [α]$^{22}_D$ −29.1° (*c* 0.1 MeOH); IR (KBr) ν_{max} 3419, 2917, 1378, 1277, 1246, 1168, 1236, 793, 672 cm^{-1}; ^{1}H NMR (CD$_3$OD, 600 MHz) aglycon moiety δ 4.57 (1H, dd, *J* = 14.5, 7.5 Hz, H-16), 3.82 (1H, m, H-26a), 3.68 (1H, m, H-3), 3.36 (1H, m, H-26b), 1.20 (1H, m, H-5), 1.03 (3H, d, *J* = 6.6 Hz, Me-21), 0.96 (3H, s, Me-27), 0.89 (3H, s, Me-19), 0.83 (3H, s, Me-18); ^{13}C NMR (CD$_3$OD, 150 MHz) aglycon moiety δ 37.9 (C-1), 27.3 (C-2), 79.1 (C-3), 33.9 (C-4), 45.8 (C-5), 28.9 (C-6), 33.1 (C-7), 35.8 (C-8), 55.6 (C-9), 36.4 (C-10), 22.0 (C-11), 40.9 (C-12), 42.1 (C-13), 57.3 (C-14), 32.5 (C-15), 82.2 (C-16), 65.1 (C-17), 16.7 (C-18), 12.4 (C-19), 40.8 (C-20), 16.2 (C-21), 112.0 (C-22), 37.0 (C-23), 28.6 (C-24), 34.3 (C-25), 75.3 (C-26), 17.1 (C-27); ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data of the sugar portion were almost superimposable on those reported for **1** in Table 1; ESIMS *m/z* 1237 [M + Na]$^+$, ESIMS/MS *m/z* 1105 [M + Na − 132]$^+$, 1075 [M + Na − 162]$^+$; HR-MALDI-MS *m/z* [M + Na]$^+$ calcd for C$_{56}$H$_{94}$O$_{28}$Na 1237.5829, found 1237.5844.

22-*O*-Methylparvispinoside A (3): amorphous white powder (MeOH); $[\alpha]^{22}_D$ −11.9° (*c* 1.9 MeOH); IR (KBr) ν_{max} 3406, 2920, 1375, 1290, 1243, 1164, 1235, 780, 675 cm^{-1}; ^{1}H NMR (CD$_3$OD, 600 MHz) aglycon moiety δ 4.39 (1H, dd, *J* = 14.5, 7.5 Hz, H-16), 3.76 (1H, m, H-26a), 3.69 (1H, m, H-2), 3.51 (1H, m, H-3), 3.42 (1H, m, H-26b), 3.17 (3H, s, OMe), 1.23 (1H, m, H-5), 1.03 (3H, d, *J* = 6.6 Hz, Me-21), 0.99 (3H, s, Me-27), 0.92 (3H, s, Me-19), 0.85 (3H, s, Me-18); ^{13}C NMR (CD$_3$OD, 150 MHz) aglycon moiety δ 45.7 (C-1), 71.3 (C-2), 84.6 (C-3), 33.9 (C-4), 45.7 (C-5), 28.9 (C-6), 33.1 (C-7), 35.8 (C-8), 55.6 (C-9), 37.6 (C-10), 22.1 (C-11), 40.9 (C-12), 41.9 (C-13), 57.3 (C-14), 32.5 (C-15), 82.4 (C-16), 65.1 (C-17), 16.7 (C-18), 13.7 (C-19), 40.5 (C-20), 16.3 (C-21), 114.0 (C-22), 31.3 (C-23), 28.3 (C-24), 34.3 (C-25), 75.1 (C-26), 17.3 (C-27), 47.3 (OMe); ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data of the sugar portion were almost superimposable on those reported for **1** in Table 1; ESIMS *m/z* 1267 [M + Na]$^+$, ESIMS/MS *m/z* 1235 [M + Na − 32]$^+$, 1135 [M + Na − 132]$^+$, 1105 [M + Na − 162]$^+$; HR-MALDI-MS *m/z* [M + Na]$^+$ calcd for C$_{57}$H$_{96}$O$_{29}$Na 1267.5935, found 1267.5946.

22-*O*-Methylparvispinoside B (4): amorphous white powder (MeOH); $[\alpha]^{22}_D$ −14.3° (*c* 0.1 MeOH); IR (KBr) ν_{max} 3408, 2925, 1374, 1287, 1245, 1164, 1235, 783, 677 cm^{-1}; ^{1}H NMR (CD$_3$OD, 600 MHz) aglycon moiety δ 4.39 (1H, dd, *J* = 14.5, 7.5 Hz, H-16), 3.77 (1H, m, H-26a), 3.71 (1H, m, H-3), 3.42 (1H, m, H-26b), 3.18 (3H, s, OMe), 1.14 (1H, m, H-5), 1.03 (3H, d, *J* = 6.6 Hz, Me-21), 0.99 (3H, s, Me-27), 0.90 (3H, s, Me-19), 0.85 (3H, s, Me-18); ^{13}C NMR (CD$_3$OD, 150 MHz) aglycon moiety δ 37.9 (C-1), 27.3 (C-2), 79.1 (C-3), 33.9 (C-4), 45.8 (C-5), 28.9 (C-6), 33.1 (C-7), 35.8 (C-8), 55.6 (C-9), 36.4 (C-10), 22.0 (C-11), 40.9 (C-12), 42.1 (C-13), 57.3 (C-14), 32.5 (C-15), 82.2 (C-16), 65.1 (C-17), 16.7 (C-18), 12.4 (C-19), 40.6 (C-20), 16.3 (C-21), 114.0 (C-22), 31.3 (C-23), 28.2 (C-24), 34.3 (C-25), 75.1 (C-26), 17.2 (C-27), 47.4 (OMe); ^{1}H NMR (CD$_3$OD, 600 MHz) and ^{13}C NMR (CD$_3$OD, 150 MHz) data of the sugar portion were almost superimposable on those reported for **1** in Table 1; ESIMS *m/z* 1251 [M + Na]$^+$, ESIMS/MS *m/z* 1219 [M + Na − 32]$^+$, 1119 [M + Na − 132]$^+$, 1089 [M + Na − 162]$^+$; HR-MALDI-MS *m/z* [M + Na]$^+$ calcd for C$_{57}$H$_{96}$O$_{28}$Na, 1251.5986, found 1251.5998.

Parvispinoside C (6): amorphous yellow powder (MeOH); $[\alpha]^{22}_D$ −8.9° (*c* 0.1 MeOH); UV (MeOH) λ_{max} (log $\in$) 233 (4.22); CD (MeOH) $\Delta_\in$ (nm) +12.7 (240), −1.0 (319); IR (KBr) ν_{max} 3407, 2927, 1663, 1075, 1045 cm^{-1}; ^{1}H NMR (CD$_3$OD, 600 MHz) δ 5.95 (1H, dd, *J* = 15.8, 1.3 Hz, H-7), 5.90 (1H, t, *J* = 1.3 Hz, H-4), 5.80 (1H, dd, *J* = 15.8, 5.8 Hz, H-8), 4.42 (1H, m, H-9), 3.93 (1H, dd, *J* = 10.0, 3.8 Hz, H-10a), 4.31 (1H, d, *J* = 7.9 Hz, H-1′), 3.88 (1H, dd, *J* = 12.0, 2.5 Hz, H-6′a), 3.68 (1H, dd, *J* = 12.0, 4.5 Hz, H-6′b), 3.48 (1H, dd, *J* = 10.0, 7.8 Hz, H-10b), 3.21 (1H, dd, *J* = 9.0, 7.8 Hz, H-2′), 3.37 (1H, dd, *J* = 9.0, 9.0 Hz, H-3′), 3.31 (1H, dd, *J* = 9.0, 9.0 Hz, H-4′), 3.29 (1H, m, H-5′), 2.55 (1H, d, *J* = 16.0 Hz, H-2a), 2.19 (1H, d, *J* = 16.0 Hz, H-2b), 1.94 (3H, d, *J* = 1.3 Hz, Me-13), 1.06 (3H, s, Me-12), 1.04 (3H, s, Me-11); ^{13}C NMR (CD$_3$OD, 150 MHz) δ 42.0 (C-1), 50.7 (C-2), 201.2 (C-3), 126.9 (C-4), 167.0 (C-5), 80.0 (C-6), 132.6 (C-7), 131.4 (C-8), 71.8 (C-9), 74.5 (C-10), 23.1 (C-11), 24.2 (C-12), 19.0 (C-13), 104.5 (C-1′), 74.9 (C-2′), 77.8 (C-3′), 71.5 (C-4′), 77.7 (C-5′), 62.3 (C-6′); ESIMS *m/z* 425 [M + Na]$^+$, ESIMS/MS *m/z* 263 [M + Na − 162]$^+$; HR-MALDI-MS *m/z* [M + Na]$^+$ calcd for C$_{19}$H$_{30}$O$_9$Na 425.1788, found 425.1779.

Acid Hydrolysis. A solution (0.8 mg each) of **1** and **6** in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N$_2$. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N$_2$, the residue was partitioned between H$_2$O and CH$_2$Cl$_2$ (1 mL, 1:1 v/v). The CH$_2$-Cl$_2$ layer was analyzed by GC using an L-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate of **1** were detected at 10.96 and 11.98 min (D-xylose), 13.98 and 14.95 min (D-galactose), and 14.72 min (D-glucose). Peak of the hydrolysate of **6** was detected at 14.74 min (D-glucose).

Retention times for authentic samples after being treated simultaneously with 1-(trimethylsilyl)imidazole in pyridine were detected at 10.95 and 11.99 min (D-xylose), 13.97 and 14.96 min (D-galactose), 14.73 min (D-glucose), 11.02 and 12.08 min (L-xylose), 13.73 and 14.75 min (L-galactose), and 14.64 min (L-glucose).

Cell Culture. Human hepatoblastoma HepG2 cells and breast cancer MCF7 cells were maintained in DMEM medium supplemented with 10% (v/v) fetal bovine serum at 37 °C in humidified atmosphere with 5% CO$_2$. Human monocytic leukemia U937 cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum at the same conditions as described above. Compounds **1**−**6** were dissolved in DMSO (37 mM) and stored as stock solutions at −20 °C. Further dilutions were made in culture medium immediately prior to use. In all experiments, the final concentration of DMSO did not exceed 0.15% (v/v), a concentration that was not toxic to the cells. To ensure logarithmic growth, cells were subcultured every 2 days.

Cell Survival Test (growth assay and viability). Cytotoxic assays were performed using an acid phosphatase method for HepG2 and MCF7[22] and WST1 test for U937 cells.[23] Cells were continuously exposed to different concentrations of compounds **1**−**6** in 96-well plates at a cell density of 0.75 × 10^4/well for 48 h. Compounds were applied in DMSO, and controls were always treated with the same amount of vehicle. U937 cell injury was quantitatively assessed by measurement of LDH release,[24] from damaged or destroyed cells, in the extracellular fluid 24 h after the experiment. An aliquot of medium was removed from culture dishes after 24 h of exposure to compounds **1**−**5** at the same concentrations of their respective IC$_{50}$ values and was analyzed for LDH leakage into the culture medium by spectrophotometric method. The total LDH activity was determined after cells were disrupted by sonication. LDH leakage was expressed by percentage of total activity. The potential pro-apoptotic effect of compounds **1**−**5** was evaluated by propidium iodide (PI) incorporation in permealized cells and flow cytometry according to the method previously reported.[25,26] Data from 10^4 cells were collected for each data file, and the percentage of the elements in the hypoploid region was calculated.

Resveratrol, a cell cycle arrest-inducing compound, was included in the assays as a positive control.[27]

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