# Saponins from the Roots of *Nylandtia spinosa*

Cheikh Diome,[†] Anne-Claire Mitaine-Offer,[†] Tomofumi Miyamoto,[‡] Clément Delaude,[§] Jean-François Mirjolet,[⊥] Olivier Duchamp,[⊥] and Marie-Aleth Lacaille-Dubois*,[†]

*Laboratoire de Pharmacognosie, UMIB, UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7, Boulevard Jeanne D'Arc, BP 87900, 21079 Dijon Cedex, France, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan, Centre de Recherche Phytochimique, Université de Liège, Institut de Chimie-B6, Sart Tilman, B-4000-Liège I, Belgium, and Oncodesign, 20 Rue Jean Mazen, BP 27627, 21076 Dijon Cedex, France*

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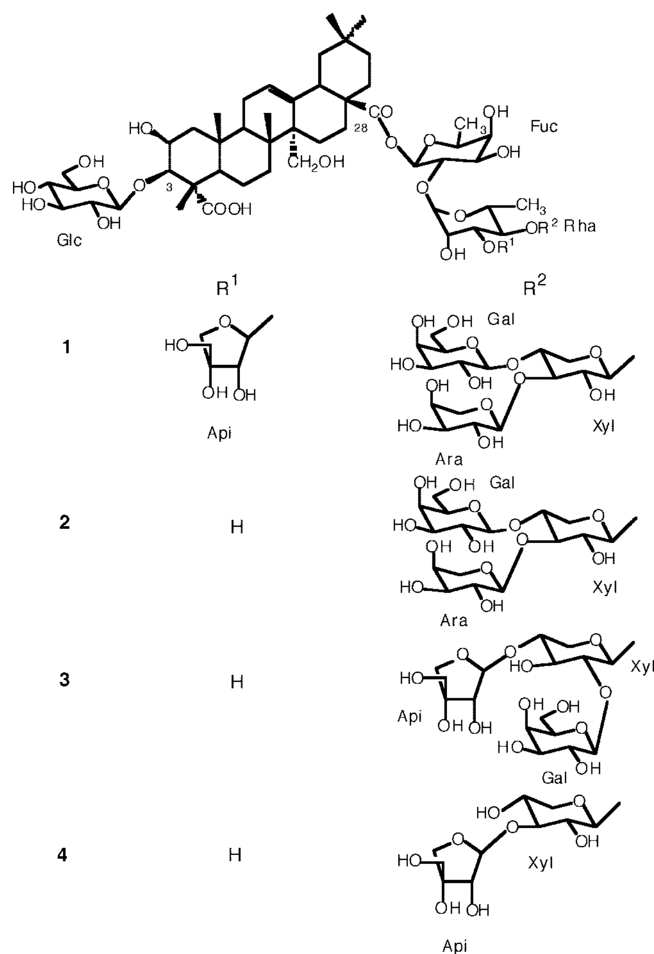
From the roots of *Nylandtia spinosa,* four new triterpene saponins, 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester (**1**), 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester (**2**), 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-apiofuranosyl-(1→4)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester (**3**), and 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester (**4**), were isolated, together with the known tenuifolin. Their structures were established mainly by 2D NMR techniques and mass spectrometry. Compounds **1**–**4** were evaluated for cytotoxicity against HCT 116 and HT-29 human colon cancer cells, but were inactive ($IC_{50}$ > 5 *μ*g/mL).

In a continuation of a study on saponin constituents of the family Polygalaceae,[1] we have examined the saponin fraction of the roots of *Nylandtia spinosa* (L.) Dumort. This small bush, very common in South Africa, is used in traditional medicine in the Cape region against hysteria, tuberculosis, insomnia, and stomachache.[2] The roots are also used to treat malaria in the traditional medicine of the Tswana.[2] A saponin was previously isolated from the roots, possessing presenegenin as aglycon and glucose, galactose, xylose, arabinose, fucose, and rhamnose as sugars, obtained by acid hydrolysis.[2] In this paper, we describe the isolation of four new triterpene saponins (**1**–**4**) and the known tenuifolin,[1,3] by successive chromatographic steps, and the elucidation of their structures mainly by NMR spectroscopic analysis, including 1D and 2D NMR methods ($^1$H–$^1$H COSY, TOCSY, NOESY, HSQC, HMBC), and by mass spectrometry.

Compound **1** exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at *m/z* 1553.6629 $[M + Na]^+$ (calcd 1553.6624), consistent with a molecular formula of $C_{69}H_{110}O_{37}Na$. Its FABMS (negative-ion mode) showed a quasimolecular ion peak at *m/z* 1529 $[M - H]^-$, indicating a molecular weight of 1530. Compound **1** and most of the saponins isolated from the Polygalaceae thus far[1] possess the chemotaxonomic marker 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester, with different substituents on the oligosaccharide chain. The $^1$H NMR spectrum of **1** displayed signals for this sequence represented by four anomeric protons at $\delta_H$ 6.43 (br s), 5.78 (d, $J$ = 8.1 Hz), 5.03 (d, $J$ = 6.9 Hz), and 4.91 (d, $J$ = 7.1 Hz), which gave correlations, in the HSQC spectrum, with anomeric carbon signals at $\delta_C$ 100.9, 94.4, 104.0, and 104.2, respectively. The ring protons of the four sugars were assigned starting from the readily identifiable anomeric protons by means of COSY, TOCSY, HSQC, and HMBC spectroscopic experiments (Table 1). Units of one *β*-D-glucopyranoside (Glc), one *β*-D-fucopyranoside (Fuc), one *β*-D-xylopyranoside (Xyl), and one α-L-rhamnopyranoside (Rha) were identified. The relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of the Glc, Fuc, and Xyl (7.1, 8.1, 6.9 Hz, respectively) moieties indicated a *β* anomeric proton for this group, and the multiplicity of the anomeric proton of the Rha unit as a broad singlet indicated an α-orientation.[4] The monosaccharides obtained by acid hydrolysis of the crude extract were identified as D-glucose, D-fucose, D-xylose, and L-rhamnose by TLC and by measurement of their optical rotations after purification. Correlations




* To whom correspondence should be addressed. Tel: 0033-3-80393229. Fax: 0033-3-80393300. E-mail: malacd@u-bourgogne.fr.
† Université de Bourgogne.
‡ Kyushu University.
§ Université de Liège.
⊥ Oncodesign.

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**Table 1.** $^{13}C$ NMR and $^{1}H$ NMR Data of the Sugar Moieties of Compounds **1**–**4** in Pyridine-$d_5$ ($\delta$ ppm)[a,b]

| | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 3-*O*-Glc-1 | *104.2* | *4.91 d (7.1)* | *104.0* | *4.91 d (7.4)* | *104.6* | *5.03 d (7.4)* | *104.7* | *4.95 d (7.6)* |
| 2 | 74.6 | 3.84 | 74.5 | 3.84 | 75.0 | 3.94 | 74.5 | 3.85 |
| 3 | 77.0 | 4.15 | 76.8 | 4.12 | 77.1 | 4.27 | 76.6 | 4.13 |
| 4 | 70.8 | 3.94 | 70.0 | 3.89 | 71.0 | 4.02 | 70.3 | 3.90 |
| 5 | 77.2 | 3.82 | 77.0 | 3.81 | 77.9 | 3.92 | 77.2 | 3.82 |
| 6 | 61.7 | 4.08, 4.28 | 61.5 | 4.03, 4.23 | 62.0 | 4.15, 4.36 | 61.3 | 4.05, 4.26 dd (10.9, 2.0) |
| 28-*O*-Fuc-1 | *94.4* | *5.78 d (8.1)* | *94.4* | *5.77 d (7.8)* | *94.7* | *5.93 d (7.9)* | *94.2* | *5.78 d (8.3)* |
| 2 | *72.5* | *4.65 t (8.1)* | *71.7* | *4.60* | *72.0* | *4.75* | *71.3* | *4.61* |
| 3 | 76.4 | 4.10 | 76.8 | 4.12 | 77.0 | 4.23 | 76.6 | 4.13 |
| 4 | 72.7 | 3.96 | 72.7 | 3.97 | 73.0 | 4.04 | 72.3 | 3.97 |
| 5 | 72.0 | 3.90 | 72.1 | 3.88 | 72.3 | 3.95 | 71.9 | 3.86 |
| 6 | 16.4 | 1.40 d (6.2) | 16.3 | 1.37 d (6.1) | 16.9 | 1.46 | 16.3 | 1.38 d (5.7) |
| Rha-1 | *100.9* | *6.43 br s* | *100.3* | *6.42 br s* | *100.6* | *6.56 br s* | *100.2* | *6.42 br s* |
| 2 | 71.3 | 4.79 br s | 71.0 | 4.60 | 71.5 | 4.72 br s | 71.1 | 4.61 |
| 3 | *81.0* | *4.37* | 72.0 | 4.41 | 72.2 | 4.57 | 71.8 | 4.44 dd (9.7, 2.5) |
| 4 | *78.5* | *4.26* | *85.6* | *4.09* | *85.0* | *4.18* | *84.5* | *4.09* |
| 5 | 67.0 | 4.40 | 67.4 | 4.31 | 67.8 | 4.44 | 67.2 | 4.32 dq (8.1, 5.5) |
| 6 | 18.1 | 1.52 d (5.7) | 18.3 | 1.53 d (5.7) | 18.2 | 1.60 | 18.0 | 1.56 d (5.5) |
| Xyl-1 | *104.0* | *5.03 d (6.9)* | *106.0* | *4.82 d (8.1)* | *107.0* | *4.79 d (7.4)* | *106.3* | *4.78 d (7.4)* |
| 2 | 74.6 | 3.84 | 75.6 | 3.93 | *76.2* | *4.04* | 74.7 | 3.96 |
| 3 | *84.5* | *4.01* | *84.8* | *4.04* | 72.3 | 4.20 | *85.8* | *3.78 t (9.0)* |
| 4 | *71.8* | *4.25* | *71.3* | *4.20* | *81.2* | *4.03* | 68.2 | 3.91 |
| 5 | 65.0 | 3.41t (10.3), 4.29 | 65.5 | 3.44 t (10.3), 4.28 | 64.6 | 3.33 t (10.5), 4.28 | 66.2 | 3.33 t (10.5), 4.03 |
| Ara-1 | *105.0* | *5.16 d (6.9)* | *105.1* | *4.91 d (7.1)* | | | | |
| 2 | 72.0 | 4.44 | 72.0 | 4.41 | | | | |
| 3 | 73.0 | 4.26 | 72.3 | 4.20 | | | | |
| 4 | 67.9 | 4.16 | 68.5 | 4.14 | | | | |
| 5 | 66.0 | 3.66 d (11.0), 4.22 | 67.0 | 3.56 d (12.1), 4.16 | | | | |
| Gal-1 | *102.2* | *4.78 d (8.3)* | *102.1* | *4.71 d (8.3)* | *102.4* | *4.70 d (7.6)* | | |
| 2 | 70.0 | 4.38 | 70.0 | 4.31 | 71.0 | 4.37 | | |
| 3 | 73.9 | 3.98 | 74.0 | 3.93 | 73.8 | 4.10 | | |
| 4 | 69.2 | 4.32 | 69.2 | 4.22 | 68.8 | 4.26 | | |
| 5 | 76.6 | 3.87 | 76.8 | 3.81 | 77.0 | 4.18 | | |
| 6 | 61.5 | 4.16, 4.20 | 61.4 | 4.04, 4.13 | 62.0 | 4.15, 4.28 | | |
| Api-1 | *111.0* | *5.88 br s* | | | *110.9* | *6.10 br s* | *110.4* | *5.86 d (3.7)* |
| 2 | 77.6 | 4.63 br s | | | 77.3 | 4.87 br s | 76.8 | 4.96 d (3.7) |
| 3 | 79.2 | | | | 80.0 | | 80.0 | |
| 4 | 74.0 | 4.13, 4.43 | | | 74.9 | 4.23, 4.78 | 74.0 | 4.12, 4.48 d (9.5) |
| 5 | 64.4 | 4.05 | | | 65.2 | 4.20, 4.37 | 63.4 | 4.03, 4.07 |

[a] Overlapped proton signals are reported without designated multiplicity. [b] Chemical shifts of substituted residues are italicized.

observed in the HMBC spectrum between an anomeric signal at $\delta_H$ 4.91 (d, $J$ = 7.1 Hz) (Glc-1) and $\delta_C$ 86.6 (C-3), and in the NOESY spectrum between $\delta_H$ 4.91 (Glc-1) and $\delta_H$ 4.53 (H-3), confirmed the substitution at C-3 of the aglycon by a Glc unit. Moreover, a correlation in the HMBC spectrum between resonances at $\delta_H$ 5.78 (d, $J$ = 8.1 Hz) (Fuc-1) and $\delta_C$ 176.3 (C-28) proved the linkage between the aglycon and a Fuc sugar. In the NOESY spectrum, a correlation between $\delta_H$ 6.43 (br s) (Rha-1) and $\delta_H$ 4.65 (t, $J$ = 8.8 Hz) (Fuc-2) revealed the (1→2) linkage between these two sugars. This was confirmed by the HMBC correlation between the resonances at $\delta_H$ 4.65 (Fuc-2) and $\delta_C$ 100.9 (Rha-1). In this spectrum, cross-peaks between the signal at $\delta_H$ 5.03 (d, $J$ = 6.9 Hz) (Xyl-1) and $\delta_C$ 78.5 (Rha-4) and the reverse correlation between $\delta_H$ 4.26 (Rha-4) and $\delta_C$ 104.0 (Xyl-1) indicated that Xyl was linked to Rha by a (1→4) linkage. The downfield shifts observed in the HSQC spectrum for the Rha-3 resonances at $\delta_H$ 4.37 and $\delta_C$ 81.0 established that the Rha-3 position was substituted.[5] A correlation in the NOESY spectrum between $\delta_H$ 4.37 and an anomeric signal at $\delta_H$ 5.88 (brs) suggested a C-3 substitution of Rha by a terminal apiofuranosyl (Api) unit. A further examination of the HMBC spectrum showed cross-peaks between signals at $\delta_H$ 4.01 (Xyl-3) and $\delta_H$ 4.25 (Xyl-4) with $\delta_C$ 105.0 (Ara-1) and $\delta_C$ 102.2 (Gal-1), respectively, which suggested a (1→3) linkage between Ara-1 and Xyl-3 and a (1→4) linkage between Gal-1 and Xyl-4. The relatively large $^{3}J_{H-1,H-2}$ values of the Gal and Ara (8.3, 6.9 Hz, respectively) moieties indicated a $\beta$-anomeric proton for Gal and an α-anomeric proton for Ara, and the apiofuranosyl moiety was determined to be in the $\beta$-anomeric form by comparing its spectroscopic data with literature values.[6] After acid hydrolysis of the crude extract, D-galactose, L-arabinose, and D-apiose were identified by TLC and by their optical rotations. On the basis of the above results, the structure of **1** was elucidated as 3-*O*-$\beta$-D-glucopyranosylpresenegenin 28-*O*-$\beta$-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-$\beta$-D-xylopyranosyl-(1→4)-[$\beta$-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-fucopyranosyl ester. This molecule was described as arillatanoside D fom *Polygala arillata*,[7] but the saponins of this species were revised by correcting the arabinosyl moiety to a xylopyranosyl moiety.[8] Accordingly, the triterpene glycoside **1** may be considered to be a new natural product.

Compound **2** exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at $m/z$ 1421.6197 $[M + Na]^+$ (calcd 1421.6201), consistent with a molecular formula of $C_{64}H_{102}O_{33}Na$. Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at $m/z$ 1397 $[M - H]^-$, indicating a molecular weight of 1398, differing from **1** by 132 amu. The $^{1}H$ and $^{13}C$ NMR signals of **2** assigned from the 2D NMR spectra were almost superimposable on those of **1** except for the Rha moiety (Table 1). In the HSQC spectrum, the values for the Rha-3 and Rha 4 resonances at $\delta_H$ 4.41 and $\delta_C$ 72.0, and $\delta_H$ 4.09 and $\delta_C$ 85.6, respectively, revealed that the Fuc-3 position was free instead of being substituted by an Api unit as in **1**. The structure of the new compound **2** was thus established as 3-*O*-$\beta$-D-glucopyranosylpresenegenin 28-*O*-$\beta$-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-$\beta$-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-$\beta$-D-fucopyranosyl ester.

Compound **3** exhibited the same pseudomolecular ion peak in the HRESIMS (positive-ion mode) and the same quasimolecular

ion peak in the FABMS (negative-ion mode) as **2**. The $^1$H and $^{13}$C NMR signals of **3** assigned from the 2D-NMR spectra were almost superimposable on those of **2** (Table 1), except for the Xyl substituents, especially at positions C-2 and C-4, which exhibited characteristic resonances at $\delta_C$ 76.2 and 81.2, respectively.[9] Signals of one Gal and one Api remained after subtraction of those of the common sequence. A correlation in the NOESY spectrum between $\delta_H$ 4.03 (Xyl-4) and an anomeric signal at $\delta_H$ 6.10 (br s) suggested a 4-substitution of the Xyl by an Api moiety and, therefore, a 2-substitution by a terminal Gal. These observations were used to assign the structure of the new saponin **3** as 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-apiofuranosyl-(1→4)-[*β*-D-galactopyranosyl-(1→2)]-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester.

Compound **4** exhibited in the HRESIMS (positive-ion mode) a pseudomolecular ion peak at *m/z* 1259.5677 [M + Na]$^+$ (calcd 1259.5673), consistent with a molecular formula of $C_{58}H_{92}O_{28}Na$. Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 1235 [M – H]$^-$, indicating a molecular weight of 1236, differing from **2** and **3** by 162 amu. Once again, changes were observed at the Xyl moiety, which showed signals of a typical C-3 substitution at $\delta_C$ 85.8/$\delta_H$ 3.78 (t, $J$ = 9.0 Hz) (Table 1).[10] In the NOESY spectrum, a correlation between the anomeric signal of an Api at $\delta_H$ 5.86 (d, $J$ = 3.7 Hz) and $\delta_H$ 3.78 (t, $J$ = 9.0 Hz) (Xyl-3) supported a (1→3) linkage. The structure of the new compound **4** was thus elucidated as 3-*O*-*β*-D-glucopyranosylpresenegenin 28-*O*-*β*-D-apiofuranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl ester.

As some saponins isolated from the family Polygalaceae have been found to exhibit cytotoxic activity,[11] compounds **1**–**4** were tested against the HCT 116 and HT-29 human colon cancer cell lines, using a MTT assay.[12] No significant activity was found for all compounds ($IC_{50}$ >5 μg/mL).

## Experimental Section

**General Experimental Procedures.** Optical rotations were recorded on a AA-OR automatic polarimeter. 1D and 2D NMR spectra were performed using a Varian INOVA 600 NMR spectrometer equipped with a SUN 4 L-X computer system (600 MHz for $^1$H and 150 MHz for $^{13}$C spectra). $^1$H–$^1$H COSY, TOCSY, NOESY, HSQC, and HMBC spectra were determined as in ref 13. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer, and FABMS (negative-ion mode, glycerol matrix) on a JEOL SX 102 mass spectrometer. TLC precoated silica gel plates $60F_{254}$ (Merck) and the solvent systems $CHCl_3$–MeOH–AcOH–$H_2O$ (15:8:3:2) for saponins and $CHCl_3$–MeOH–$H_2O$ (8:5:1) for sugars were used. The spray reagent for both saponins and sugars was the Komarowsky reagent (2% 4-hydroxybenzaldehyde in MeOH–50% $H_2SO_4$, 5:1). Isolations were carried out using vacuum liquid chromatography (VLC) [reversed-phase RP-18 silica gel (25–40 μm)] and medium-pressure liquid chromatography (MPLC) [Gilson pump M 303, Büchi glass column (460 × 15 mm and 230 × 15 mm), Büchi precolumn (110 × 15 mm), silica gel 60 (Merck, 15–40 μm), RP-18 silica gel (25–40 μm)].

**Plant Material.** *Nylandtia spinosa* (L.) Dumort. was collected in March 1990, near the Western Cape University, Bellville, Republic of South Africa, and identified by Prof. C. Delaude, Université de Liège, Liège, Belgium. A voucher specimen (H. Breyne 5405) was deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium.

**Extraction and Isolation.** A crude saponin mixture (5 g) was obtained according to a previously described method,[14] and a 2 g aliquot was submitted to VLC on RP-18 silica gel (25–40 μm), with $H_2O$ containing increasing amounts of MeOH, to give three fractions. Fraction 2 (1.08 g), rich in saponins and eluted with MeOH–$H_2O$ (5:5, 500 mL), was fractionated by successive MPLC on silica gel, eluted with $CHCl_3$–MeOH–$H_2O$ (8:5:1 and 65:40:8), and purified on RP-18 silica gel, MeOH–$H_2O$ (5:5), yielding **1** (14 mg), **2** (7 mg), **3** (10 mg), **4** (3 mg), and tenuifolin (10 mg).

**Compound 1:** white, amorphous powder; $[\alpha]^{25}_D$ –9.2 (*c* 0.05, MeOH); $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Table 1; positive HRESIMS *m/z* 1553.6629 [M + Na]$^+$ (calcd 1553.6624); negative FABMS *m/z* 1529 [M – H]$^-$.

**Compound 2:** white, amorphous powder; $[\alpha]^{25}_D$ +10.4 (*c* 0.06, MeOH); $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Table 1; positive HRESIMS *m/z* 1421.6197 [M + Na]$^+$ (calcd 1421.6201); negative FABMS *m/z* 1397.

**Compound 3:** white, amorphous powder; $[\alpha]^{25}_D$ –5.2 (*c* 0.05, MeOH); $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Table 1; positive HRESIMS *m/z* 1421.6197 [M + Na]$^+$ (calcd 1421.6201); negative FABMS *m/z* 1397 [(M – H)]$^-$.

**Compound 4:** white, amorphous powder; $[\alpha]^{25}_D$ –4.5 (*c* 0.05, MeOH); $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Table 1; positive HRESIMS *m/z* 1259.5677 [M + Na]$^+$ (calcd 1259.5673); negative FABMS *m/z* 1235 [(M – H)]$^-$.

**Acid Hydrolysis.** A 200 mg aliquot of the crude saponin mixture was refluxed with 2 N $CF_3COOH$ for 2 h. After extraction with $CHCl_3$, the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral. Seven sugars were identified as glucose, fucose, rhamnose, xylose, galactose, apiose, and arabinose, by comparison with authentic samples on TLC in $CHCl_3$–MeOH-$H_2O$ (8:5:1). After preparative TLC of the sugar mixture in this solvent, the optical rotation of each purified sugar was measured.

**MTT Cytotoxicity Assay.** The bioassay was carried out according to the method described in ref 12 with two human colorectal cancer cell lines (HCT 116 and HT-29). Paclitaxel was used as a positive control and exhibited $IC_{50}$ values of 8.0 ± 6.0 and 5.3 ± 1.2 ng/mL against the HCT 116 and HT-29 cell lines, respectively.

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