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Article

Ursane Saponins from the Stems of *Firmiana simplex* and their Cytotoxic Activity

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Three new ursane triterpene saponins, together with twelve known ursane triterpenes were isolated from the stems of *Firmiana simplex*. The structures of the saponines were elucidated on the basis of spectroscopic and chemical methods. The cytotoxic activity of all compounds was evaluated *in vitro* against lung adenocarcinoma (A549), ovarian cancer (SK-OV-3), skin melanoma (SK-MEL-2), and colon cancer (HCT-15) human cell lines, using a sulforhodamine (SRB) assay. 23-Hydroxyursolic acid showed cytotoxicity against the tested cell lines with IC_{50} values ranging from 11.96 to 14.11 μM.

Keywords: *Firmiana simplex*, Sterculiaceae, ursane triterpene saponin, cytotoxicity

Introduction

Firmiana simplex W. F. Wight (synonym *Firmiana platanifolia* Schott et Endl), family of Sterculiaceae, known as "phoenix tree", is distributed throughout Korea and China.[1,2] Its seeds have been used as a folk medicine to treat symptoms of diarrhea and stomach disorders.[3] Previous chemical investigations on this plant reported the isolation of quinones,[2] flavonoids,[3] and an antipsychotic neolignan.[4]

In the course of our continuing search for potential lead compounds from Korean traditional medicinal plants, we investigated the MeOH extract of *F. simplex* stems and isolated three new ursane triterpene saponins (**1**-**3**), together with twelve known ursane triterpenes (**4**-**15**) (Figure 1). All the compounds (**1**-**15**) were tested for their cytotoxic activity against the cultured human tumor cell lines lung adenocarcinoma (A549), ovarian cancer (SK-OV-3), skin melanoma (SK-MEL-2), and colon cancer (HCT-15).

Experimental

General procedures

Optical rotations were obtained on a JASCO P-1020 polarimeter. Infrared (IR) spectra were recorded on a Bruker Vector 22 IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra including ^1H-^1H correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY), were recorded on a Varian UNITY INOVA 700 spectrometer operating at 700 MHz (^1H) and 175 MHz (^{13}C). High resolution fast atom bombardment mass spectrometry (HR-FABMS) was conducted using a JEOL JMS700 mass spectrometer. Preparative high performance liquid chromatography (HPLC) was performed using a Gilson 306 pump with a Shodex refractive index detector. Silica gel 60 (230-400 mesh, Merck) and reversed-phase (RP)-C_{18} silica gel (230-400 mesh, Merck) were used for column chromatography. A Hewlett-Packard gas chromatography (GC) system 6890 Series was equipped with a 5973 mass selective detector (MSD). The system was controlled by the Enhanced Chem Station version B.01.00 program. The capillary column used for GC was an Agilent J&W HP-5MSUI (30.0 m × 0.25 mm i.d., 0.25 μm film thickness, coated with 5% diphenyl and 95% dimethylpolysiloxane). Thin-layer chromatography (TLC) was performed using Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates. Spots were detected on TLC under ultraviolet (UV) light or by heating after spraying with 10% v/v H_2SO_4 in EtOH.

Plant material

F. simplex stems (7.0 kg) were collected at Jecheon in Chungcheongbuk-do, Korea, in June 2012, and

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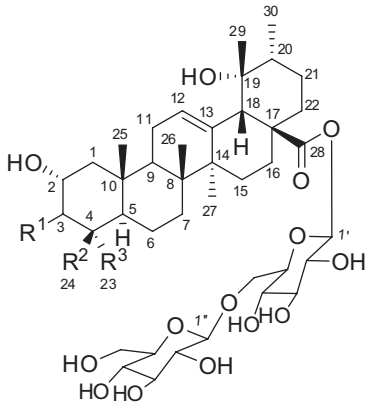
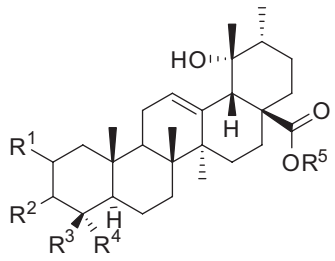
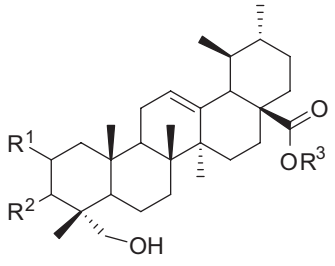


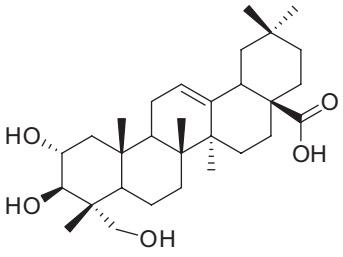
1 R^1 = α-OH, R^2 = CH_3, R^3 = CH_3
2 R^1 = α-OH, R^2 = CH_3, R^3 = CH_2OH
3 R^1 = β-OH, R^2 = COOH, R^3 = CH_3



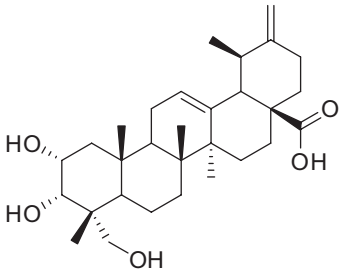
4 R^1 = α-OH, R^2 = α-OH, R^3 = CH_3, R^4 = CH_3, R^5 = β-D-Glc
5 R^1 = α-OH, R^2 = α-OH, R^3 = CH_3, R^4 = CH_2OH, R^5 = β-D-Glc
6 R^1 = α-OH, R^2 = α-OH, R^3 = CH_3, R^4 = CH_3, R^5 = H
7 R^1 = α-OH, R^2 = α-OH, R^3 = CH_3, R^4 = CH_2OH, R^5 = H
8 R^1 = H, R^2 = α-OH, R^3 = CH_2OH, R^4 = CH_3, R^5 = β-D-Glc
9 R^1 = α-OH, R^2 = β-OH, R^3 = COOH, R^4 = CH_3, R^5 = β-D-Glc
10 R^1 = H, R^2 = β-OH, R^3 = CH_3, R^4 = CH_3, R^5 = β-D-Glc
11 R^1 = α-OH, R^2 = β-OH, R^3 = CH_3, R^4 = CH_2OH, R^5 = β-D-Glc



12 R^1 = H, R^2 = β-OH, R^3 = H
13 R^1 = α-OH, R^2 = α-OH, R^3 = β-D-Glc



14



15

Figure 1. Structures of compounds **1**-**15** isolated from *Firmiana simplex*.

authenticated by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL-1209) was deposited at the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The stems of *F. simplex* (7.0 kg) were extracted with 80% MeOH under reflux. The filtered MeOH extract was concentrated under reduced pressure to afford a viscous concentrate (400 g), which was suspended in water (800 mL) and solvent-partitioned successively to yield hexane (24 g), $CHCl_3$ (14 g), EtOAc (50 g), and BuOH (270 g) extracts. The $CHCl_3$ extract (14 g) was separated over a silica gel column (230-400 mesh, 500 g) with hexane:EtOAc:MeOH (5:1:0.5, v/v) to give five fractions (C1-C5). Fraction C3 (6.5 g) was separated on a RP-C_{18} silica gel column (230-400 mesh, 150 g) with a gradient solvent system of MeOH:H_2O (2:3, 3:2, 4:1, and 1:0, v/v) to give sixteen subfractions (C3-1-C3-16). Fraction C3-12 (110 mg) was further separated over a silica gel column with $CHCl_3$:MeOH (20:1, v/v) elution, and further purified through RP-C_{18} silica gel semi-preparative HPLC with 40% CH_3CN elution, at a flow rate of 2.0 mL min^{-1} (Econosil RP-18 column; 250 × 10 mm; 10 μm particle size; Shodex refractive index detector) to yield **7** (3 mg, t_R = 14.3 min). Fraction C3-13 (470 mg) was further separated over a silica gel column with $CHCl_3$:MeOH (20:1, v/v) elution, and purified through a RP-C_{18} silica gel semi-preparative HPLC with 60% CH_3CN elution, to yield **6** (6 mg, t_R = 17.6 min), **14** (12 mg, t_R = 19.1 min), and **15** (7 mg, t_R = 21.7 min). Fraction C3-16 (120 mg) was purified through RP-C_{18} silica gel semi-preparative HPLC with 70% CH_3CN elution, to yield **12** (6 mg, t_R = 12.1 min). The EtOAc layer (18 g) was chromatographed on a RP-C_{18} silica gel (230-400 mesh, 300 g), eluting with a gradient solvent system of MeOH:H_2O (2:3, 3:2, 4:1, and 1:0, v/v) to yield eight subfractions (E1-E8). Fraction E3 (1.9 g) was separated over a Sephadex LH-20 column with MeOH:H_2O (4:1, v/v), and purified through RP-C_{18} silica gel semi-preparative HPLC with 30 and 40% CH_3CN elution, to yield **5** (12 mg, t_R = 12.1 min), **8** (3 mg, t_R = 14.5 min), **10** (14 mg, t_R = 16.2 min), and **11** (12 mg, t_R = 19.4 min). Fraction E4 (1.0 g) was separated over a Sephadex LH-20 column with MeOH:H_2O (4:1, v/v)

and purified by RP-C_{18} silica gel semi-preparative HPLC with 40% CH_3CN elution, to yield **9** (15 mg, t_R = 11.8 min). Fraction E5 (250 mg) was separated over a Sephadex LH-20 column with MeOH:H_2O (4:1, v/v) and purified by RP-C_{18} silica gel semi-preparative HPLC with 50% CH_3CN elution, to yield **4** (5 mg, t_R = 15.5 min). Fraction E6 (230 mg) was separated over a Sephadex LH-20 column with MeOH:H_2O (4:1, v/v), and purified by RP-C_{18} silica gel semi-preparative HPLC with 60% CH_3CN elution, to yield **13** (5 mg, t_R = 14.7 min). The BuOH extract (30 g) was separated over a silica gel column (230-400 mesh, 500 g) with $CHCl_3$:MeOH (5:1, v/v), to give six fractions (B1-B6). Fraction B4 (8.7 g) was chromatographed on a RP-C_{18} silica gel eluting with a gradient solvent system of MeOH:H_2O (3:7, 5:5, 7:3, and 1:0, v/v) to yield eight subfractions (B41-B48). Fraction B45 (200 mg) was purified by RP-C_{18} silica gel semi-preparative HPLC with 25% CH_3CN elution, to yield **2** (15 mg, t_R = 16.1 min). Fraction B47 (700 mg) was separated over a Sephadex LH-20 column with MeOH:H_2O (4:1, v/v), and purified by RP-C_{18} silica gel semi-preparative HPLC with 30% CH_3CN elution, to yield **1** (19 mg, t_R = 22.7 min). Fraction B5 (16.4 g) was chromatographed on a RP-C_{18} silica gel eluting with a gradient solvent system of MeOH:H_2O (2:3, 3:2, 4:1, and 1:0, v/v) to yield seven subfractions (B51-B57). Fraction B54 (700 mg) was separated over a Sephadex LH-20 column with MeOH:H_2O (4:1, v/v) and purified by RP-C_{18} silica gel semi-preparative HPLC with 25% CH_3CN elution, to yield **3** (90 mg, t_R = 18.7 min).

28-*O*-[β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3α,19α-trihydroxy-12-en-28-ursolic acid (**1**)

White gum; $[\alpha]_D^{25}$ –2.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} / cm^{-1} 3385, 2938, 2879, 2843, 1732, 1651, 1454, 1390, 1228, 1205, 1166, 1062, 637; ^{1}H NMR (700 MHz, CD_3OD), see Table 1; ^{13}C NMR (175 MHz, CD_3OD), see Table 2; HRMS-FAB $[M–H]^-$ calcd. for $C_{42}H_{67}O_{15}$: 811.4474; found: 811.4474.

28-*O*-[β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3α,19α,23-tetrahydroxy-12-en-28-ursolic acid (**2**)

White gum; $[\alpha]_D^{25}$ –1.0 (*c* 0.6, MeOH); IR (KBr) ν_{max} / cm^{-1} 3376, 2939, 2835, 1731, 1600, 1453, 1382, 1265, 1164, 1032, 637; ^{1}H NMR (700 MHz, CD_3OD), see Table 1; ^{13}C NMR (175 MHz, CD_3OD), see Table 2; HRMS-FAB $[M–H]^-$ calcd. for $C_{42}H_{67}O_{16}$: 827.4424; found: 827.4423.

28-*O*-[β-D-Glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3β,19α-trihydroxyurs-12-ene-24,28-dioic acid (**3**)

White gum; $[\alpha]_D^{25}$ + 0.2 (*c* 0.9, MeOH); IR (KBr) ν_{max} / cm^{-1} 3366, 2935, 2839, 1732, 1695, 1454, 1380, 1263, 1228, 1167, 1032, 646; ^{1}H NMR (700 MHz, CD_3OD), see Table 1; ^{13}C NMR (175 MHz, CD_3OD), see Table 2; HRMS-FAB $[M–H]^-$ calcd. for $C_{42}H_{65}O_{17}$: 841.4216; found: 841.4216.

Table 1. ^{1}H NMR data in CD_3OD for compounds **1**-**3** (δ in ppm (*J* in Hz))

Position	**1**	**2**	**3**
1	1.60 m, 1.29 m	1.63 m, 1.34 m	2.05 m, 0.93 m
2	3.95 m	3.91 m	4.09 m
3	3.38 m	3.63 d (2.0)	2.91 d (9.0)
4	–	–	–
5	1.28 m	1.58 m	1.05 m
6	1.46 m, 1.40 m	1.42 m, 1.37 m	1.79 m
7	1.59 m, 1.34 m	1.65 m, 1.31 m	1.53 m, 1.40 m
8	–	–	–
9	1.87 m	1.90 m	1.72 m
10	–	–	–
11	2.05 m, 1.99 m	2.07 m, 2.02 m	2.07 m, 2.01 m
12	5.33 t (3.5)	5.34 t (3.5)	5.33 t (3.5)
13	–	–	–
14	–	–	–
15	1.84 m, 1.04 m	1.84 m, 1.03 m	1.84 m, 1.05 m
16	2.64 td (13.0, 4.5), 1.64 m	2.64 td (13.0, 4.0), 1.66 m	2.63 td (13.0, 3.0), 1.66 m
17	–	–	–
18	2.54 brs	2.56 brs	2.54 brs
19	–	–	–
20	1.40 m	1.40 m	1.39 m
21	1.77 m, 1.28 m	1.76 m, 1.28 m	1.76 m, 1.27 m
22	1.79 m, 1.64 m	1.81 m, 1.62 m	1.80 m, 1.63 m
23	1.01 s	3.56 d (11.0), 3.41 m	1.45 s
24	0.89 s	0.81 s	-
25	1.02 s	1.05 s	0.98 s
26	0.79 s	0.80 s	0.81 s
27	1.36 s	1.37 s	1.35 s
28	–	–	–
29	1.22 s	1.23 s	1.22 s
30	0.95 d (7.0)	0.96 d (6.5)	0.95 d (7.0)
1’	5.31 d (8.0)	5.31 d (8.0)	5.32 d (8.0)
2’	3.36 m	3.35 m	3.34 m
3’	3.42 m	3.42 m	3.44 m
4’	3.44 m	3.44 m	3.44 m
5’	3.52 m	3.52 m	3.52 m
6’	4.17 dd (12.0, 2.0), 3.78 dd (12.0, 5.0)	4.13 dd (12.0, 2.0), 3.78 dd (12.0, 5.0)	4.13 m, 3.78 dd (12.0, 5.0)
1”	4.37 d (8.0)	4.37 d(8.0)	4.37 d (8.0)
2”	3.23 dd (9.0, 8.0)	3.23 dd (9.0, 8.0)	3.23 t (8.5)
3”	3.38 m	3.38 m	3.38 m
4”	3.32 m	3.31 m	3.32 m
5”	3.26 m	3.27 m	3.26 m
6”	3.87 dd (12.0, 2.0), 3.69 dd (12.0, 5.0)	3.87 dd (12.0, 2.0), 3.69 dd (12.0, 5.0)	3.87 dd (12.0, 2.0), 3.70 dd (12.0, 5.0)

Table 2. ^{13}CNMR data in CD_3OD for compounds **1**-**3** (δ in ppm)

Position	1	2	3
1	42.6	42.0	48.5
2	67.3	67.3	69.2
3	80.2	78.4	84.3
4	39.5	42.3	49.9
5	49.7	43.9	57.6
6	19.4	19.2	21.5
7	34.1	33.7	34.3
8	41.5	41.1	41.2
9	48.3	48.1	48.0
10	39.5	38.9	39.7
11	24.9	24.8	25.0
12	129.7	129.2	129.6
13	139.8	139.5	139.7
14	42.9	42.6	42.9
15	29.8	29.5	29.7
16	26.3	26.6	26.6
17	49.3	49.3	49.2
18	55.0	54.9	55.0
19	73.8	73.4	73.7
20	43.0	42.9	42.8
21	27.3	27.2	27.1
22	38.5	38.1	38.3
23	29.4	71.4	24.9
24	22.6	17.8	180.7
25	17.2	17.5	15.5
26	17.8	17.7	17.6
27	24.9	24.8	24.6
28	178.7	178.7	178.6
29	24.7	27.5	27.2
30	16.7	16.6	16.7
1’	95.9	95.8	95.8
2’	73.6	73.8	73.8
3’	78.3	78.2	78.2
4’	71.1	71.0	71.0
5’	77.9	77.9	77.7
6’	69.6	69.4	69.7
1”	104.8	104.7	104.7
2”	75.2	75.2	75.2
3”	78.3	78.1	78.2
4”	71.6	71.6	71.5
5”	78.1	78.0	78.0
6”	62.8	62.8	62.8

Acid hydrolysis of **1**-**3** and sugar determination

Compound **1** (2 mg) was shaken with 1 mL of 1 mol L^{-1} HCl for 1 h at 90 °C. After cooling, the hydrolyzate was extracted with $CHCl_3$ and the extract was evaporated *in vacuo* to yield 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (**1a**), which was identified by comparing its ^{1}H NMR data with those reported in literature. The sugar in water layer appeared to be glucose by co-TLC comparison ($CHCl_3$:MeOH:H_2O = 2:1:0.2, R_f value: 0.2) with a glucose standard (Aldrich), which was confirmed by gas chromatography-mass spectrometry (GC-MS) as follows. The sugars obtained from the hydrolysis of compounds **1**-**3** were dissolved in anhydrous pyridine (0.1 mL) and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between hexane and H_2O (0.3 mL each). The H_2O layer was neutralized by passage through an Amberlite IRA-67 column (Rohm and Haas) and was repeatedly evaporated to give D-glucose, identified by co-injection of the hydrolyzate with standard silylated samples, giving a GC-MS single peak at 9.712 min. Compounds **2** (2 mg) and **3** (5 mg) were treated using the same method to give 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**2a**) and 2α,3β,19α-trihydroxy-urs-12-ene-24,28-dioic acid (**3a**).

2α,3α,19α-Trihydroxyurs-12-en-28-oic acid (**1a**)

Colorless gum; ^{1}H NMR (700 MHz, pyridine-d_5) δ 5.55 (brs, 1H, CH), 4.27 (dt, 1H, *J* 10.0, 3.5 Hz, CHOH), 3.72 (d, 1H, *J* 2.5 Hz, CHOH), 3.11 (ddd, 1H, *J* 13.5, 13.0, 4.5 Hz, CH_2), 3.01 (s, 1H, CH), 2.29 (ddd, 1H, *J* 13.5, 13.0, 4.0 Hz, CH_2), 1.59 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.07 (d, 3H, *J* 6.0 Hz, CH_3), 1.05 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.85 (s, 3H, CH_3).

2α,3α,19α,23-Tetrahydroxyurs-12-en-28-oic acid (**2a**)

Colorless gum; ^{1}H NMR (700 MHz, CD_3OD) δ 5.31 (brs, 1H, CH), 3.89 (ddd, 1H, *J* 12.0, 5.0, 3.0 Hz, CHOH), 3.62 (d, 1H, *J* 3.0 Hz, CHOH), 3.55 (d, 1H, *J* 11.0 Hz, CHOH), 3.41 (d, 1H, *J* 11.0 Hz, CHOH), 2.58 (td, 1H, *J* 13.0, 4.0 Hz, CH_2), 2.53 (s, 1H, CH), 1.37 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.94 (d, 3H, *J* 7.0 Hz, CH_3), 0.88 (s, 3H, CH_3).

2α,3β,19α-Trihydroxy-urs-12-ene-24,28-dioic acid (**3a**)

Colorless gum; ^{1}H NMR (700 MHz, CD_3OD) δ 5.51 (brs, 1H, CH), 4.69 (m, 1H, CHOH), 3.34 (d, 1H, *J* 9.0 Hz, CHOH), 3.02 (m, 1H, CH_2), 2.33 (dd, 1H, *J* 13.0, 4.0 Hz, CH_2), 1.68 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.06 (d, 3H, *J* 6.0 Hz, CH_3), 1.05 (s, 3H, CH_3).

Cytotoxicity assays

A sulforhodamine (SRB) bioassay was used to determine compound cytotoxicity against cultured human

tumor cell lines A549, SK-OV-3, SK-MEL-2, and HCT-15.[5] The assays were performed at the Korea Research Institute of Chemical Technology. Doxorubicin was used as a positive control. The IC_{50} values of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines were 0.029, 0.036, 0.001, and 2.041 μM, respectively.

Results and Discussion

The stems of *F. simplex* were extracted with 80% aqueous MeOH. Chemical investigation of the extract using successive column chromatography over silica gel and Sephadex LH-20, and preparative HPLC resulted in the isolation and identification of three new ursane triterpene saponins (**1**-**3**), together with twelve known ursane triterpenes (**4**-**15**). Their structures were elucidated as follows.

Compound **1** was obtained as a colorless gum, and its molecular formula $C_{42}H_{67}O_{15}$ was inferred from the negative HR-FABMS ion at *m/z* 811.4474 $[M–H]^-$. The IR absorption bands at 3385 and 1732 cm^{-1} implied the presence of hydroxyl and carboxylic functionalities. The ^1H NMR spectrum (Table 1) of **1** showed the signals for an olefinic proton at δ_H 5.33 (t, 1H, *J* 3.5 Hz, H-12), two oxygenated methine protons at δ_H 3.95 (m, 1H, H-2) and 3.38 (m, 1H, H-3), one methine proton at δ_H 2.54 (brs, 1H, H-18), six tertiary methyl protons at δ_H 1.36 (s, 3H, H-27), 1.22 (s, 3H, H-29), 1.02 (s, 3H, H-25), 1.01 (s, 3H, H-23), 0.89 (s, 3H, H-24) and 0.79 (s, 3H, H-26), one secondary methyl proton at δ_H 0.95 (d, 1H, *J* 7.0 Hz, H-30), and two anomeric protons at δ_H 5.31 (d, 1H, *J* 8.0 Hz, H-1') and 4.37 (d, 1H, *J* 8.0 Hz, H-1''). The ^{13}C NMR (Table 2), DEPT, and HMQC spectral data revealed forty-two signals, which included seven methyl carbon signals at δ_C 29.4 (C-23), 24.9 (C-27), 24.7 (C-29), 22.6 (C-24), 17.8 (C-26), 17.2 (C-25), and 16.7 (C-30), two olefinic carbon signals at δ_C 139.8 (C-13) and 129.7 (C-12), two oxygenated methine carbon signals at δ_C 80.2 (C-3) and 67.3 (C-2), eight methylene carbon signals at δ_C 42.6 (C-1), 38.5 (C-22), 34.1 (C-7), 29.8 (C-15), 27.3 (C-21), 26.3 (C-16), 24.9 (C-11), and 19.4 (C-6), four methine carbon signals at δ_C 55.0 (C-18), 49.7(C-5), 48.3 (C-9), and 43.0 (C-20), one carbonyl carbon at δ 178.7 (C-28), six quaternary carbon signals at δ_C 73.8 (C-19), 49.3 (C-17), 42.9 (C-14), 41.5 (C-8), and 39.5 (C-4), and two anomeric carbons at δ_C 104.8 (C-1'') and 95.9 (C-1').

The NMR data of **1** were very similar to those of the ursane **4**,[6] with the exception of an additional sugar moiety in **1**. The linkage of the disaccharide moiety to the pentacyclic scaffold, and the attachment position between the two sugar units were assigned from the following HMBC correlations: δ_H 4.37 (d, *J* 8.0 Hz, H-1'') to δ_C 69.6 (C-6'), and δ_H 5.31 (d, *J* 8.0 Hz, H-1') to δ_C 178.7 (C-28) (Figure 2).

The relative stereochemistry of the aglycone was assigned from the NOESY cross-peaks of H-2/H-25, H-3/H-24, H-5/H-9, H-9/H-27, H-24/H-25, and H-25/H-26 (Figure 3).[6]

The coupling constant (*J* 8.0 Hz) of the anomeric protons of H-1' and H-1'' suggested a β-orientation.[7] Acid hydrolysis of **1** gave 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (**1a**), identified by comparison of its ^1H NMR spectrum data with of previously reported values,[8] and D-glucose, which was identified by GC-MS.[9] Thus, compound **1** was determined to be 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3α,19α-trihydroxy-12-en-28-ursolic acid.

Compound **2** was obtained as a colorless gum, and its molecular formula $C_{42}H_{67}O_{16}$ was inferred from the negative

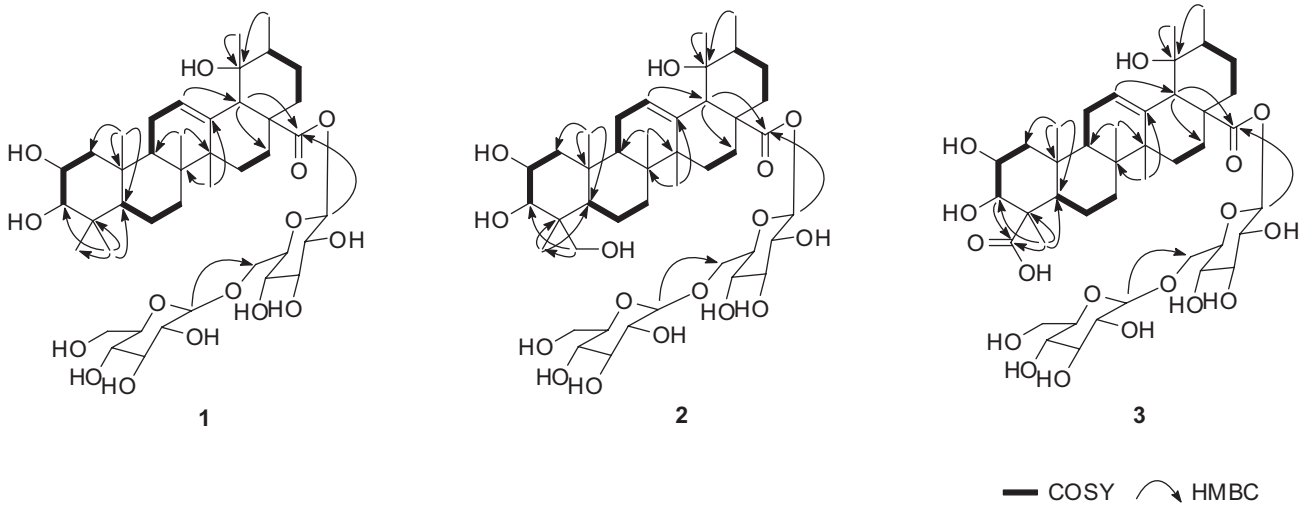
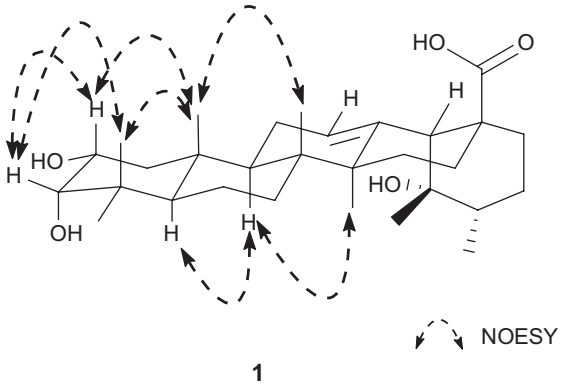


Figure 2. Key ^1H-^1H COSY and HMBC (H → C) correlations of compounds **1**-**3**.



1

Figure 3. Key NOESY correlations of compound **1**.

HR-FABMS ion at *m/z* 827.4423 $[M–H]^-$. The ^1H and ^{13}C NMR spectra were close to those of **1** (Tables 1 and 2). The major differences were the disappearance of a methyl signal [δ_H 1.01 (s, 3H, H-23); δ_C 29.4] in **1**, and the presence of the oxymethylene signal [δ_H 3.56 (d, 1H, *J* 11.0 Hz, H-23a), 3.41 (m, 1H, H-23b); δ_C 71.4] in **2**. This was confirmed by the HMBC experiment showing correlations from the oxymethine proton (δ_H 3.56) to C-3, C-4, C-5, and C-24. The nature and position of the disaccharide moiety revealed to be the same as for compound **1**, as indicated by the HMBC correlations H-1''/C-6' and H-1'/C-28 (Figure 2). As for compound **1**, the relative configuration of **2** determined by the NOESY spectrum also indicated an ursane pentacyclic system. Acid hydrolysis of **2** gave 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**2a**) and D-glucose, which were identified by GC analysis and TLC comparison with authentic D-glucose.[9,10] Thus, compound **2** was determined to be 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3α,19α,23-tetrahydroxy-12-en-28-ursolic acid.

Compound **3** was obtained as a colorless gum. The molecular formula was determined to be $C_{42}H_{65}O_{17}$ from the deprotonated molecule $[M–H]^-$ at *m/z* 841.4216 in the negative-ion HR-FABMS data. The ^1H and ^{13}C NMR data of **3** were very similar to those reported for **9**,[11] except for the presence of an additional sugar unit The connectivities of the two sugar units were deduced by the HMBC cross peaks H-1''/C-6' and H-1'/C-28 (Figure 2). The relative stereochemistry of **3** was assigned by NOESY cross-peaks H-2/H-25, H-3/H-23, H-5/H-9, H-9/H-27, and H-25/H-26. Acid hydrolysis of **3** yielded 2α,3β,19α-trihydroxyurs-12-ene-24,28-dioic acid (**3a**) and D-glucose.[9,12] Thus, compound **3** was determined to be 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-2α,3β,19α-trihydroxyurs-12-ene-24,28-dioic acid.

Compounds **4**-**15** were identified by comparing their ^1H NMR, ^{13}C NMR, and MS spectra with the literature data. They were determined to be kaji-ichigoside F1 (**4**),[6] niga-ichigoside F2 (**5**),[13] euscaphicacid (**6**),[14] myrianthic acid (**7**),[15] kakisaponin A (**8**),[16] trachelosperoside A-1 (**9**),[12] pormolic acid-28-*O*-β-D-glucopyranosyl ester (**10**),[17] niga-ichigoside F1 (**11**),[13] 23-hydroxyursolic acid (**12**),[18] 2α,3α,24-trihydroxyurs-12-en-28-oic acid-28-*O*-β-D-glucopyranosyl ester (**13**),[19] arjunolic acid (**14**),[20] and 2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid (**15**).[21]

Compounds **1**-**15** were evaluated for their cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 human tumor cell lines using the SRB assay.[5] Compound **12** was cytotoxic against all the tested human cell lines with IC_{50} values of 11.96, 13.24, 14.11, and 12.27 μM, respectively, whereas the other compounds were inactive ($IC_{50} > 30$ μM).

Conclusions

This is the first study investigating the cytotoxic activities of triterpene derivatives (**1**-**15**) isolated from *Firmiana simplex*. Among them, compound **12**, which showed a significant cytotoxicity against the human tumor cell lines, could be a potentially valuable source for the development of anti-tumor agents.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

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