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Oleanane-*type* Saponins from *Glochidion hirsutum* and Their Cytotoxic Activities

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Abstract

Five new oleanane-*type* saponins, hirsutosides A-E, were isolated from the leaves of *Glochidion hirsutum* (Roxb.) Voigt. Their structures were elucidated as 21*β*-benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-*O*-*β*-D-glucopyranoside (**1**), 21*β*-benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-

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glucopyranoside (**2**), 21β-benzoyloxy-3β,16β,23,28-tetrahydroxyolean-12-ene 3-*O*-6-acetyl-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (**3**), 21β-benzoyloxy-3β,16β,23,28-tetrahydroxyolean-12-ene 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside (**4**), and 21β-benzoyloxy-3β,16β,23-trihydroxyolean-12-ene-28-al 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside (**5**). All isolated compounds were evaluated for cytotoxic activities on four human cancer cell lines, HepG-2, A-549, MCF-7, and SW-626 using the SRB assay. Compounds **1**, **2**, **4**, and **5** showed significant cytotoxic activities against all human cancer cell lines with IC_{50} values ranging from 3.4 to 10.2 μM. Compound **3** containing acetyl group at glc C(6″) exhibited weak cytotoxic activity with IC_{50} values ranging from 47.0 to 54.4 μM.

Keywords: *Glochidion hirsutum,* Euphorbiaceae, hirsutosides A-E, oleanane-*type* saponin, cytotoxic activity.

Introduction

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. Accordingly, the development of new and efficient anticancer drugs has been interested from the scientists around the world. Natural products are potential sources of novel drugs with a broad range of biological and pharmacological activities, including anticancer activities.[1] There are more than 60% of currently anticancer drugs from natural sources.[2] Moreover, many oleanane triterpene-*type* saponins from various plants such as *Bolbostemma paniculatum*,[3] *Platycodon grandiflorum,*[4] *Glochidion eriocarpum*[5] exhibited cytotoxic activities.

Glochidion is a large genus of the Euphorbiaceae family, comprising more than 250 species in the world. *Glochidion hirsutum* (Roxb.) Voigt is a shrub or small tree distributed throughout Southeast Asia. The leaves of *G. hirsutum* have been used in folk medicine to treat toothaches; the roots are used as medicine for rheumatism and pneumonia.[6]

Phytochemical studies of *G. hirsutum* have shown the presence of flavonols.[7, 8] Previous our investigation program on cytotoxic constituents of *Glochidion* genus identified cytotoxic oleanane saponins from *G. eriocarpum*[5] and *G. glomerulatum.*[9, 10] Herein, we reported the isolation, structural elucidation of oleanane-*type* saponins from the leaves of *G. hirsutum*, and their cytotoxic activity against four human cancer cell lines, HepG-2, A-549, MCF-7, and SW-626.

Results and Discussion

Structure Elucidation

The methanol extract of the *G. hirsutum* leaves was suspended in water and then partitioned with CH_2Cl_2 and EtOAc to obtain three layers. The EtOAc layer was separated using a combination of silica gel and RP-18 column chromatographic steps to afford five new oleanane-*type* saponins (*Fig. 1*). Their structures were elucidated by extensive spectroscopic methods including 1D and 2D NMR experiments, as well as by HR ESI MS analysis.

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined as $C_{43}H_{64}O_{11}$ by HR ESI MS ion at *m/z* 779.4370 $[M + Na]^+$ (calcd for $C_{43}H_{64}O_{11}Na$, 779.4346). The ^1H NMR spectrum of **1** showed the signals of six methyl groups at δ(H) 0.75, 0.96, 1.04, 1.06, 1.17, and 1.34 (each, 3H, s) and one olefinic proton at δ(H) 5.37 (1H, t, J = 3.0 Hz), which indicated an oleanane aglycone. In addition to these, protons of a benzoyloxy were observed at δ(H) 7.51 (2H, t, J = 8.0 Hz), 7.62 (1H, t, J = 8.0 Hz), and 8.04 (2H, d, J = 8.0 Hz). One anomeric proton at δ(H) 4.43 (1H, d, J = 8.0 Hz) showed the presence of a sugar moiety. The ^{13}C NMR and DEPT spectra of **1** showed the presence of 43 carbons, including 1 carbonyl, 8 quaternary carbons, 17 methines,11 methylenes, and 6 methyl carbons (Table 1). The analysis of ^1H and ^{13}C NMR spectroscopic data indicated that the aglycone of **1** was similar to those of 21β-(benzoyloxy)olean-12-ene-3β,16β,23,28-tetraol, an oleanane-*type* triterpene isolated from *Glochidion assamicum.*[11]

The HMBC correlations between H-C(18) (δ(H) 2.51) and C(13) (δ(C) 143.0)/C(16) (δ(C) 67.9)/C(17) (δ(C) 44.7)/C(28) (δ(C) 66.6) as well as the COSY correlations between H-C(15) (δ(H) 1.40-1.46 and 1.80-1.84) and H-C(16) (δ(H) 4.36) confirmed the positions of two hydroxyl groups at C(16) and C(28) (*Fig. 2*). The β configuration (*equatorial* orientation) of the hydroxyl group at C(16) was confirmed by NOESY correlations between H-C(16) (δ(H) 4.36) and H-C(27) (δ(H) 1.34)/Hα- C(19) (δ(H) 2.10) as well as by the coupling constant of H-C(15) and H-C(16), $J_{eq\text{-}ax}$ = 5.0 Hz and $J_{ax\text{-}ax}$ = 12.0 Hz. The location of a oxygenated group at C(21) was assigned based on the HMBC correlations between H-C(29) (δ(H) 0.96)/H-C(30) (δ(H) 1.17) and C(19) (δ(C) 48.0)/C(20) (δ(C) 36.6)/C(21) (δ(C) 78.2). Furthermore, the esterification location of benzoic acid at C-21 was confirmed by HMBC correlation between H-C(21) (δ(H) 5.16) and Bz C(7′) (δ(C) 167.9). The configuration of the benzoyloxy group was determined as β by the NOESY observations between H-C(21) (δ(H) 5.16) and Hα-C(19) (δ(H) 2.08-2.12)/H-C(29) (δ(H) 0.96). The HMBC correlations from H-C(3) (δ(H) 3.67) to C(4) (δ(C) 43.9)/C(5) (δ(C) 48.1)/C(23) (δ(C) 64.8)/C(24) (δ(C) 13.4), from H-C(23) (δ(H) 3.31 and 3.67)/H-C(24) (δ(H) 0.75) to C(3) (δ(C) 83.3)/C(4) (δ(C) 43.9)/C(5) (δ(C) 48.1) suggested the location of the oxygenated and hydroxyl groups at C(3) and C(23), respectively. The α-orientations of H-C(3) and the hydroxylmethylene group at C(4) were determined by the NOESY observation of H-C(3) (δ(H) 3.67) and H-C(5) (δ(H) 1.63-1.69)/H-C(23) (δ(H) 3.31 and 3.67) and of H-C(24) (δ(H) 0.75) and H-C(25) (δ(H) 1.04). Acid hydrolysis of **1** afforded D-glucose as sugar component (identified as TMS derivatives by GC). Also, the HMBC correlation from glc H-C(1″) (δ(H) 4.43) to C(3) (δ(C) 83.3) confirmed the location of glucopyranosyl moiety at C(3). Consequently, the structure of **1** was elucidated to be 21β-benzoyloxy-3β,16β,23,28-tetrahydroxyolean-12-ene 3-*O*-β-D-glucopyranoside and named hirsutoside A.

The molecular formula of **2** was determined as $C_{49}H_{74}O_{16}$ by the HR ESI MS ion at *m/z* 941.4896 $[M + Na]^+$ (calcd for $C_{49}H_{74}O_{16}Na$, 941.4875). The ^1H and ^{13}C NMR spectra exhibited the presence of one oleanane aglycone, one benzoyloxy, and two sugar moieties (Table 1). The NMR data of **2** were similar to those of hirsutoside A (**1**), except for the addition of a sugar moiety at glc C(3″). The aglycone was recognized to be 21*β*-(benzoyloxy) olean-12-ene-3*β*,16*β*,23,28-tetraol.[11] The configurations of functional groups for aglycone **2** were similar to those of **1**, confirmed by NOESY experiments. Acid hydrolysis of **2** gave D-glucose (identified as TMS derivatives by GC). The HMBC correlations between glc H-C(1‴) (*δ*(H) 4.57) and glc C(3″) (*δ*(C) 88.3); glc H-C(3″) (*δ*(H) 3.55) and glc C(1‴) (*δ*(C) 105.3) confirmed the sequence of sugar linkages as 3-*O*-*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranoside. The sequence of sugar linkages at C(3) of aglycone was proved by HMBC correlations between glc H-C(1″) (*δ*(H) 4.49) and C(3) (*δ*(C) 83.4). Consequently, the structure of **2** was determined as 21*β*-benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside and named hirsutoside B.

The molecular formula of **3** was deduced as $C_{51}H_{76}O_{17}$ by the HR ESI MS ion at *m/z* 983.4965 $[M + Na]^+$ (calcd for $C_{51}H_{76}O_{17}Na$, 983.4980). Analysis of the NMR data of **3** indicated that the structure of aglycone was similar to those of **1** and **2**. The HMBC correlations between glc H-C(1‴) (*δ*(H) 4.53) and glc C(3″) (*δ*(C) 83.4); between glc H-C(6″) (*δ*(H) 4.18 and 4.36) and acetyl group (*δ*(C) 172.8) confirmed the sugar linkages to be 3-*O*-6-acetyl-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside. Moreover, the sequence of sugar linkages was located at C(3) of aglycone by HMBC correlation between glc H-C(1″) (*δ*(H) 4.68) and C(3) (*δ*(C) 83.3). Based on the above evidence, compound **3** was defined as 21*β*-benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-*O*-6-acetyl-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside and named hirsutoside C.

Compound **4** was also obtained as a white amorphous powder. The molecular formula was determined as $C_{48}H_{72}O_{15}$ by the HR ESI MS ion peak at *m/z* 911.4779 $[M + Na]^+$ (calcd for $C_{48}H_{72}O_{15}Na$, 911.4769). The ^1H, ^{13}C NMR, and DEPT spectra of compound **4** showed one olean-12-ene triterpene aglycone, one benzoyloxy, and two sugar moieties (Table 2). The aglycone of **4** was found to be similar to those of hirsutoside A (**1**). Acid hydrolysis and GC analysis of **4** confirmed the presence of D-glucose and L-arabinose. In addition, the coupling constants of ara H-C(1″) and ara H-C(2″), J = 7.5 Hz; glc H-C(1‴) and glc H-C(2‴), J = 8.0 Hz, confirmed the configurations of the *O*-glycoside bonds as *β*-D-glucopyranosyl and *α*-L-arabinopyranosyl. The sequence of sugar linkages was as 3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranoside confirming by the HMBC correlation from glc H-C(1‴) (δ(H) 4.57) to ara C(3″) (δ(C) 84.2). Furthermore, the location of sugar at C(3) of aglycone was confirmed by the HMBC correlation between ara H-C(1″) (δ(H) 4.38) and C(3) (δ(C) 83.3). Consequently, compound **4** was defined as 21*β*-benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranoside and named hirsutoside D.

The molecular formula of compound **5** was determined as $C_{48}H_{70}O_{15}$ by the HR ESI MS ion at *m/z* 909.4602 $[M + Na]^+$ (calcd for $C_{48}H_{70}O_{15}Na$, 909.4612). Analysis of the NMR data of **5** indicated that the structure of **5** was similar to those of **4** except for the presence of aldehydic group instead of hydroxylmethylene at C(17). The HMBC correlations from H-C(16) (δ(H) 4.45)/H-C(22) (δ(H) 1.45 and 2.46) to C(28) (δ(C) 207.4); from H-C(18) (δ(H) 2.92) to C(12) (δ(C) 125.2)/C(13) (δ(C) 142.1)/C(16) (δ(C) 66.1)/C(17) (δ(C) 42.7) confirmed the positions of hydroxyl and aldehyde groups at C(16) and C(17), respectively. In addition, the *β* configuration of the hydroxyl group at C(16) was confirmed by NOESY correlations between H-C(16) (δ(H) 4.45) and H-C(27) (δ(H) 1.32). The location of a benzoyloxy group at C(21) was proved by the HMBC correlations between H-C(29) (δ(H) 0.99)/H-C(30) (δ(H) 1.17) and C(19) (δ(C) 47.8)/C(20) (δ(C) 36.5)/C(21) (δ(C) 77.1);

between H-C(21) (δ(H) 5.15) and Bz C(7′) (δ(C) 167.7). The configuration of this benzoyloxy group was determined as β by the NOESY observations between H-C(21) (δ(H) 5.15) and H-C(29) (δ(H) 0.99). The orientation of remaining functional groups of aglycone were based on NOESY experiments and coupling constant analysis. The position and sequence of sugar linkages were similar to those of compound **4**. Consequently, the structure of **5** was elucidated to be 21β-benzoyloxy-3β,16β,23-trihydroxyolean-12-ene-28-al 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside, and named hirsutoside E.

Biological Studies

All compounds were evaluated against four human cancer cell lines, HepG-2 (human liver hepatocellular carcinoma), A-549 (human lung carcinoma), MCF-7 (human breast carcinoma) and SW-626 (human ovarian carcinoma) using the SRB assay. Ellipticine, an anticancer agent, was used as a positive control with IC_{50} values ranging from 1.4 to 2.1 μM for all the human cancer cell lines (Table 3).

Comparing to ellipticine, compounds **1**, **2**, **4**, and **5** showed significant cytotoxic activities against all human cancer cells with IC_{50} values ranging from 3.4 to 10.2 μM. Compound **3** containing acetyl group at glc C(6″) exhibited weak cytotoxic activity with IC_{50} values ranging from 47.0 to 54.4 μM. In the structure-activity relationship of isolated compounds **1**-**3**, when additional sugar moiety at glc C(3″) (**2**), the cytotoxic activity exhibited stronger, however, when acetyl group at glc C(6″) (**3**) the cytotoxic activity decreased. The current study demonstrated that the cytotoxic activity of **2** on all tested human cancer cell lines comparable to those of ellipticine. This work has thus provided a further example of the importance of oleanane-*type* saponins contain a benzoyloxy group at C(21) as potential anticancer agents.

Experimental Section

General

Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. The NMR spectra were recorded using a Bruker DRX 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz). The HR-ESI-MS were obtained using an Agilent 6550 iFunnel Q-TOF LC/MS system. Column chromatography was performed using silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (30 - 50 μm, Fujisilisa Chemical Ltd.), and thin layer chromatography (TLC) was performed using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ plates (0.25 mm, Merck).

Plant Material

The leaves of *Glochidion hirsutum* (Roxb.) Voigt were collected in Sondong, Bacgiang, Vietnam in December 2012 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (GH1212) was deposited at the Herbarium of the Institute of Marine Biochemistry, Hanoi, Vietnam.

Extraction and Isolation

Dried leaves of *G. hirsutum* (4.0 kg) were sonicated in MeOH (7 L × 3 times) for 15 h to yield a MeOH extract (355 g) after evaporating under reduced pressure. The MeOH extract was suspended in H_2O and successively partitioned with CH_2Cl_2 and EtOAc to obtain CH_2Cl_2 (GH1, 120.0 g), EtOAc (GH2, 50.0 g), and H_2O (GH3, 180.0 g) layers after removal of the solvents in *vacuo*. The GH2 layer was applied to a silica gel CC eluted with a gradient elution of CH_2Cl_2 – MeOH (100:1, 30:1, 10:1, 5:1, 1:1, 0:1, v/v) to give six smaller fractions, GH2A (4.0 g), GH2B (3.1 g), GH2C (3.6 g), GH2D (8.5 g), GH2E (4.3 g), and GH2F (7.0 g). The GH2C fraction was applied to a silica gel CC eluting with CH_2Cl_2 – MeOH (8:1, v/v) to give two fractions, GH2C1 and GH2C2. The GH2C1 fraction was further purified by silica gel CC eluting with EtOAc – MeOH – H_2O (12:1:0.01, v/v/v) to yield **1** (12.0 mg). The GH2D

fraction was subjected to a silica gel CC eluting with CH_2Cl_2 –MeOH – H_2O (5:1:0.1, v/v/v) to give three smaller fractions, GH2D1-GH2D3. The GH2D1 fraction was applied to a silica gel CC eluting with EtOAc – MeOH – H_2O (6:1:0.01, v/v/v) to yield compound **4** (18.0 mg). The GH2D2 fraction was subjected to an RP-18 CC eluting with MeOH – H_2O (3.5:1, v/v) to yield compound **5** (32.0 mg). The GH2D3 fraction was applied to a silica gel CC, eluted with CH_2Cl_2 – MeOH – H_2O (6:1:0.05, v/v/v) to yield compound **3** (21.0 mg). Compound **2** (9.0 mg) was obtained from GH2E fraction, using a silica gel CC eluting with CH_2Cl_2 – MeOH – H_2O (6:1:0.05, v/v/v) then an RP-18 CC eluent of MeOH – H_2O (3:1, v/v). The purity of the compounds was assessed by HPLC-DAD at 210 nm as > 95%.

21*β*-Benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-O-*β*-D-glucopyranoside (1): White amorphous powder; $[\alpha]_D^{25}$ = +30.0 (*c* 0.1, MeOH); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; $C_{43}H_{64}O_{11}$, HR ESI MS *m/z*: 779.4370 $[M + Na]^+$ (calcd for $C_{43}H_{64}O_{11}Na$, 779.4346).

21*β*-Benzoyloxy-3β,16*β*,23,28-tetrahydroxyolean-12-ene 3-O-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside (2): White amorphous powder; $[\alpha]_D^{25}$ = + 50.0 (*c* 0.1, MeOH); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; $C_{49}H_{74}O_{16}$, HR ESI MS *m/z*: 941.4896 $[M + Na]^+$ (calcd for $C_{49}H_{74}O_{16}Na$, 941.4875).

21*β*-Benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-*O*-6-acetyl-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside (3): White amorphous powder; $[\alpha]_D^{25}$ = –20.0 (*c* 0.1, MeOH); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; $C_{51}H_{76}O_{17}$, HR ESI MS *m/z*: 983.4965 $[M + Na]^+$ (calcd for $C_{51}H_{76}O_{17}Na$, 983.4980).

21*β*-Benzoyloxy-3*β*,16*β*,23,28-tetrahydroxyolean-12-ene 3-O-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranoside (4): White amorphous powder; $[\alpha]_D^{25}$ = + 41.0 (*c* 0.1, MeOH); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 2; $C_{48}H_{72}O_{15}$, HR ESI MS *m/z*: 911.4779 $[M + Na]^+$ (calcd for $C_{48}H_{72}O_{15}Na$, 911.4769).

21*β*-Benzoyloxy-3*β*,16*β*,23-trihydroxyolean-12-ene-28-al 3-O-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranoside (5): White amorphous powder; $[\alpha]_D^{25} = -9.0$ (*c* 0.1, MeOH); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 2; $C_{48}H_{70}O_{15}$, HR ESI MS *m/z*: 909.4602 $[M + Na]^+$ (calcd for $C_{48}H_{70}O_{15}Na$, 909.4612).

Acid Hydrolysis

Each compound (**1**-**5**, 2.0 mg) was separately dissolved in 1.0 N HCl (dioxane - H_2O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. The solvent in acidic solution was removed under an N_2 stream. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2. The residue was dissolved in dry pyridine (0.1 mL), followed by addition of L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL). The reaction mixture was heated at 6 °C for 2 h. Trimethylsilylimidazole solution (0.1 mL) was then added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL each), and the organic layer was analyzed by gas chromatography (GC): column DB-5 (0.32 mm ID × 30 m length), detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (2 mL/min). Under these conditions, the standard sugars gave peaks at t_R (min) 14.11 and 14.26 for D- and L-glucose, 9.82 and 15.24 for D- and L-arabinose, respectively. Peaks at t_R (min) 14.11 of D-glucose for **1** – **3**; 14.11 and 15.24 of D-glucose and L-arabinose for **4** and **5**, were observed.

Cytotoxic Assay

Tumour cells were cultivated in a humidified atmosphere of 5% CO_2 at 37 °C for 48 h. Cell viability was examined by SRB method for the determination of cell density, based on the measurement of cellular protein content. Viable cells were seeded in the growth medium (180 µL) into 96-well microwell plates (4×10^4 cells per well) and allowed to attach overnight. Test samples were added carefully into wells of 96-well plates and the cultivation was continued under the same conditions for another 48 h. Thereafter, the medium was removed

and the remaining cell monolayers are fixed with the cold 20% (w/v) trichloroacetic acid for 1 h at 4 °C and stained by 1X SRB staining solution at room temperature for 30 min, after which the unbound dye was removed by washing repeatedly with 1% (v/v) acetic acid. The proteinbound dye is dissolved in 10 mM Tris base solution for OD determination at 515 nm on an ELISA Plate Reader (Bio-Rad). DMSO 10% was used as blank sample while ellipticine was used as positive control. The cytotoxicity was measured at doses of 100.0, 20.0, 4.0, and 0.8 μM and estimated as a half maximal inhibitory concentration (IC_{50}), which was calculated by the program TableCurve Version 4.0. All experiments were prepared in triplicates. The inhibition rate (IR) of cells was calculated by the following formula IR% = $[100 – (absorbance_t – absorbance_0)/(absorbance_c – absorbance_0)] \times 100\%$, where IR indicates inhibition rate of cell growth, absorbancet indicates average optical density value at day 2; $absorbance_0$ indicates average optical density value at time-zero and absorbancec indicates average optical density value of the blank DMSO control sample.

Supplementary Material

1D and 2D NMR, and HR ESI MS are included for all compounds. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org//10.1002/cbdv.xxx

Notes

The authors declare no competing financial interest.

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **1 – 3**

C	1		2		3	
	δ(C)	δ(H) (mult., *J*, in Hz)	δ(C)	δ(H) (mult., *J*, in Hz)	δ(C)	δ(H) (mult., *J*, in Hz)
Aglycone						
1	39.6	0.98 – 1.02 (m) 1.64 – 1.68 (m)	39.6	1.01 – 1.05 (m) 1.64 – 1.70 (m)	39.7	0.99 – 1.03 (m) 1.64 – 1.68(m)
2	26.3	1.70 – 1.76(m) 1.95 – 2.01 (m)	26.3	1.75 – 1.79 (m) 1.95 – 2.01 (m)	26.4	1.74 – 1.80 (m) 1.97 – 2.03 (m)
3	83.3	3.67 (dd, 3.5, 13.0)	83.4	3.68 (m)	83.3	3.65 (dd, 4.5, 12.0)
4	43.9	-	43.9	-	44.1	-
5	48.1	1.63 – 1.69 (m)	48.2	1.64 – 1.70 (m)	47.8	1.63 – 1.70 (m)
6	18.8	1.42 – 1.46 (m) 1.55 – 1.59 (m)	18.9	1.45 – 1.49 (m) 1.55 – 1.59(m)	18.9	1.42 – 1.46 (m) 1.52 – 1.56 (m)
7	33.3	1.33 – 1.39 (m) 1.72 – 1.76 (m)	33.3	1.35 – 1.41 (m) 1.73 – 1.79 (m)	33.3	1.35 – 1.41 (m) 1.72 – 1.79 (m)
8	41.1	-	41.1	-	41.1	-
9	48.1	1.25 – 1.30 (m)	48.5	1.25 – 1.30 (m)	48.2	1.25 – 1.30 (m)
10	37.5	-	37.5	-	37.5	-
11	24.7	1.94 – 2.00 (m)	24.7	1.94 – 2.00 (m)	24.7	1.94 – 2.00 (m)
12	124.9	5.37 (t, 3.0)	124.9	5.37 (br s)	125.0	5.37 (t, 3.0)
13	143.0	-	143.0	-	143.0	-
14	44.6	-	44.6	-	44.6	-
15	36.5	1.40 – 1.46 (m) 1.80 – 1.84 (m)	36.5	1.40 – 1.46 (m) 1.81 – 1.85 (m)	36.5	1.40 – 1.46 (m) 1.80 – 1.84 (m)
16	67.9	4.36 (dd, 5.0, 12.0)	67.9	4.36 (dd, 5.0, 12.0)	67.9	4.36 (dd, 5.0, 12.0)
17	44.7	-	44.8	-	44.8	-
18	43.6	2.51 (dd, 4.5, 14.0)	43.6	2.52 (dd, 4.5, 13.5)	43.7	2.52 (dd, 4.5, 13.0)
19	48.0	1.30 – 1.36 (m) 2.08 – 2.12 (m)	48.0	1.30 – 1.35 (m) 2.00 – 2.06 (m)	48.0	1.30 – 1.35 (m) 2.00 – 2.06 (m)
20	36.6	-	36.6	-	36.6	-
21	78.2	5.16 (dd, 5.0, 12.0)	78.2	5.16 (dd, 4.5, 12.0)	78.2	5.16 (dd, 5.0, 12.5)
22	30.2	1.73 (dd, 12.0, 13.5) 2.39 (dd, 5.0, 13.5)	30.2	1.73 (dd, 12.0, 13.5) 2.38 (dd, 4.5, 13.5)	30.2	1.73 (dd, 12.5, 13.5) 2.38 (dd, 4.5, 13.5)
23	64.8	3.31 (d, 13.0) 3.67 (d, 13.0)	65.0	3.32 (d, 13.0) 3.68 (d, 13.0)	64.3	3.23 (d, 11.0) 3.83 (d, 11.0)
24	13.4	0.75 (s)	13.4	0.75 (s)	13.5	0.71 (s)
25	16.6	1.04 (s)	16.6	1.05 (s)	16.6	1.04 (s)
26	17.5	1.06 (s)	17.5	1.07 (s)	17.5	1.06 (s)
27	27.4	1.34 (s)	27.4	1.32 (s)	27.4	1.32 (s)
28	66.6	3.42 (d, 11.0) 3.73 (d, 11.0)	66.6	3.42 (d, 10.5) 3.73 (d, 10.5)	66.6	3.41 (d, 11.0) 3.72 (d, 11.0)
29	29.4	0.96 (s)	29.4	0.96 (s)	29.4	0.96 (s)
30	18.9	1.17 (s)	18.9	1.17 (s)	18.9	1.17 (s)
21-*O*-Bz						
1′	134.2	-	134.2	-	134.2	-
2′, 6′	130.4	8.04 (d, 8.0)	130.4	8.05 (d, 8.0)	130.4	8.05 (d, 8.0)
3′, 5′	129.6	7.51 (t, 8.0)	129.6	7.51 (t, 8.0)	129.6	7.51 (t, 8.0)
4′	131.9	7.62 (t, 8.0)	131.9	7.63 (t, 8.0)	131.9	7.63 (t, 8.0)
7′	167.9	-	167.8	-	167.8	-
CH_3CO					172.8	-
CH_3CO					20.9	2.08 (s)
3-*O*-Glc						
1″	105.7	4.43 (d, 8.0)	105.3	4.49 (d, 8.0)	105.3	4.68 (d, 7.5)

2″	75.6	3.20 (t, 8.0)	74.9	3.38 – 3.42 (m)	76.2	3.26 – 3.30 (m)
3″	77.7	3.34 – 3.38 (m)	88.3	3.53 – 3.57 (m)	83.4	3.43 – 3.49 (m)
4″	71.6	3.29 – 3.33 (m)	70.0	3.40 – 3.46 (m)	71.3	3.30 – 3.36 (m)
5″	78.3	3.28 – 3.32 (m)	77.4	3.30 – 3.34 (m)	77.6	3.26 – 3.32 (m)
6″	62.7	3.69 (dd, 4.5, 12.0) 3.86 (dd, 2.0, 12.0)	62.4	3.71 (dd, 6.0, 11.5) 3.90 (dd, 2.0, 11.5)	64.9	4.18 (dd, 5.0, 12.0) 4.36 (dd, 2.0, 12.0)
3″-*O*-Glc						
1‴			105.3	4.57 (d, 8.0)	104.4	4.53 (d, 8.0)
2‴			75.5	3.29 – 3.33(m)	75.4	3.42 – 3.48 (m)
3‴			77.8	3.38 – 3.42 (m)	77.8	3.37 – 3.41(m)
4‴			71.6	3.37 – 3.36(m)	71.3	3.31 – 3.35(m)
5‴			78.2	3.32 – 3.38(m)	78.6	3.56 – 3.60(m)
6‴			62.4	3.64 (dd, 6.0, 11.5) 3.88 (dd, 2.0, 11.5)	62.7	3.67 (dd, 5.0, 12.0) 3.86 (dd, 2.0, 12.0)

Assignments were done by HSQC, HMBC, COSY, and NOESY experiments. Ara, arabinopyranosyl; Bz, benzoyl; Glc, glucopyranosyl.

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds **4** and **5**

C	**4**		**5**	
	δ(C)	**δ(H) (mult., *J*, in Hz)**	**δ(C)**	**δ(H) (mult., *J*, in Hz)**
Aglycone				
1	39.6	0.98 – 1.02 (m) 1.64 – 1.68 (m)	39.5	0.98 – 1.02 (m) 1.64 – 1.68 (m)
2	26.3	1.70 – 1.76 (m) 1.96 – 2.00 (m)	26.3	1.73 – 2.00 (m) 1.88 – 1.92 (m)
3	83.3	3.67 (dd, 3.5, 13.0)	83.4	3.61 – 3.67(m)
4	43.9	-	43.9	-
5	48.1	1.63 – 1.66 (m)	48.1	1.62 – 1.68 (m)
6	18.8	1.44 – 1.48 (m) 1.52 – 1.56 (m)	18.8	1.42 – 1.46 (m) 1.52 – 1.56(m)
7	33.3	1.35 – 1.41 (m) 1.72 – 1.78 (m)	33.6	1.35 – 1.41 (m) 1.69 – 1.75 (m)
8	41.1	-	40.9	-
9	48.1	1.25 – 1.29 (m)	48.3	1.25 – 1.29 (m)
10	37.5	-	37.6	-
11	24.7	1.94 – 1.98 (m)	24.6	1.95 – 1.99 (m)
12	124.9	5.37 (br s)	125.2	5.42 (t, 3.0)
13	143.0	-	142.1	-
14	44.6	-	44.9	-
15	36.5	1.41 – 1.45 (m) 1.80 – 1.84 (m)	38.1	1.53 – 1.60 (m) 1.81 – 1.85 (m)
16	67.9	4.36 (dd, 5.0, 12.0)	66.1	4.45 (dd, 5.0, 12.0)
17	43.9	-	42.7	-
18	43.6	2.51 (dd, 4.5, 14.0)	43.9	2.92 (dd, 4.5, 14.0)
19	48.0	1.30 – 1.36 (m) 2.09 – 2.11 (m)	47.8	1.37 – 1.43 (m) 2.09 – 2.11 (m)
20	36.6	-	36.5	-
21	78.2	5.16 (dd, 5.0, 12.0)	77.1	5.15 (dd, 4.5, 12.0)
22	30.2	1.73 (dd, 12.0, 13.5) 2.39 (dd, 4.5, 13.5)	28.3	1.45 (dd, 12.0, 13.0) 2.46 (dd, 4.5, 13.0)
23	64.8	3.31 (d, 13.0) 3.67 (d, 13.0)	65.1	3.33 (d, 13.0) 3.67 (d, 13.0)
24	13.4	0.75 (s)	13.4	0.75 (s)

25	16.6	1.04 (s)	16.5	1.03 (s)
26	17.5	1.06 (s)	17.7	0.87 (s)
27	27.4	1.34 (s)	26.9	1.32 (s)
28	66.6	3.42 (d, 11.0)	207.4	9.78 (s)
		3.73 (d, 11.0)		
29	29.4	0.96 (s)	29.2	0.99 (s)
30	18.9	1.17 (s)	18.8	1.17 (s)
21-*O*-Bz				
1′	134.2	-	134.4	-
2′, 6′	130.4	8.04 (d, 8.0)	130.5	8.04 (d, 8.0)
3′, 5′	129.6	7.51 (t, 8.0)	129.6	7.50 (t, 8.0)
4′	131.8	7.63 (t, 8.0)	131.6	7.64 (t, 8.0)
7′	167.8	-	167.7	-
3-*O*-Ara				
1″	106.1	4.38 (d, 7.5)	106.1	4.37 (d, 7.5)
2″	72.1	3.70 – 3.74 (m)	72.1	3.69 – 3.73 (m)
3″	84.2	3.65 – 3.71 (m)	84.3	3.63 – 3.69 (m)
4″	69.5	4.06 (br s)	69.6	4.06 (br s)
5″	66.9	3.60 (br d, 12.0)	66.9	3.60 (br d, 14.0)
		3.88 (br d, 12.0)		3.88 (dd, 2.0, 14.0)
3″-*O*-Glc				
1‴	105.5	4.57 (d, 8.0)	105.5	4.56 (d, 8.0)
2‴	75.3	3.30 – 3.34 (m)	75.3	3.30 – 3.34 (m)
3‴	77.6	3.37 – 3.43 (m)	77.7	3.37 – 3.43 (m)
4‴	71.1	3.34 – 3.40 (m)	71.2	3.31 – 3.37 (m)
5‴	77.9	3.29 – 3.33 (m)	78.0	3.29 – 3.33 (m)
6‴	62.3	3.71 (dd, 5.0, 12.0)	62.4	3.71 (dd, 5.0, 12.0)
		3.87 (br d, 12.0)		3.85 (dd, 2.0, 12.0)

Assignments were done by HSQC, HMBC, COSY, and NOESY experiments. Ara, arabinopyranosyl, Bz, benzoyl, Glc, glucopyranosyl.

Table 3. Effects of **1**–**5** from *G. hirsutum* on the Growth of Human Cancer Cells

Compound	IC_{50} (μM)			
	HepG-2	A-549	MCF-7	SW-626
1	8.2 ± 1.3	9.3 ± 0.3	9.2 ± 0.5	8.5 ± 1.3
2	3.4 ± 0.3	4.4 ± 0.7	4.7 ± 0.6	6.6 ± 1.0
3	47.0 ± 5.6	49.3 ± 4.1	51.9 ± 3.7	54.4 ± 1.5
4	7.6 ± 0.8	8.0 ± 2.2	8.8 ± 1.3	9.1 ± 1.1
5	9.9 ± 3.1	8.6 ± 1.3	10.2 ± 2.4	10.1 ± 1.9
Ellipticine	1.4 ± 0.2	1.8 ± 0.3	2.0 ± 0.3	2.1 ± 0.3

Ellipticine was used as a positive control.

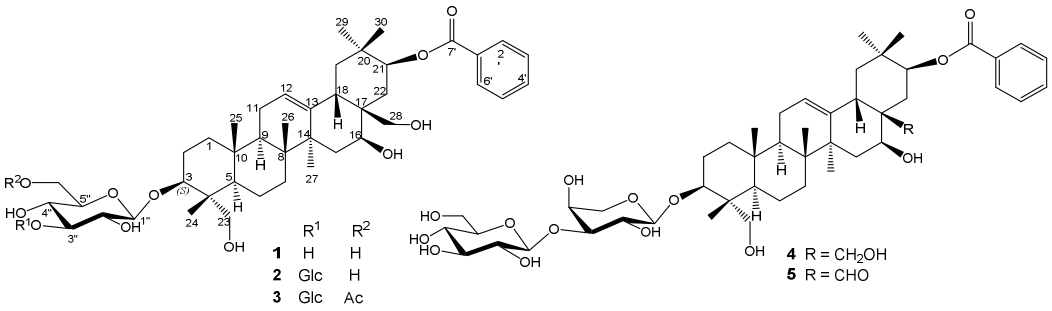

Figure 1. Chemical Structures of Compounds **1** – **5** from *G. hirsutum*

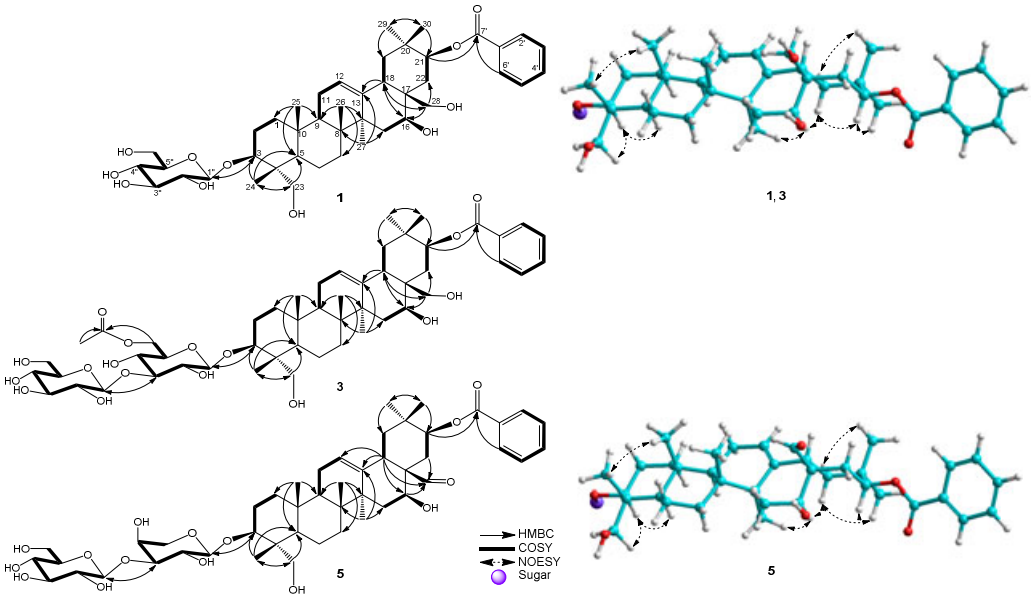

Figure 2. The Key HMBC, ^{1}H–^{1}H COSY, and NOESY Correlations of **1**, **3**, and **5**