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New Triterpenoids and Other Constituents from the Fruits of *Benincasa hispida* (Thunb.) Cogn.

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Supporting Information

ABSTRACT: *Benincasa hispida* (Thunb.) Cogn. fruits are widely consumed in China and tropical countries. This study identifies three new triterpenoids, 3α,29-*O*-di-*trans*-cinnamoyl-D:C-friedooleana-7,9(11)-diene (**1**), oleanolic acid 28-*O*-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**2**), and oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**), together with 12 known compounds, multiflorenol (**4**), isomultiflorenyl acetate (**5**), stigmasterol (**6**), stigmasterol 3-*O*-β-D-glucopyranoside (**7**), α-spinasterol (**8**), α-spinasterol 3-*O*-β-D-glucopyranoside (**9**), β-sitosterol (**10**), daucosterol (**11**), arbutin (**12**), nicotinic acid (**13**), (+)-pinonesinol (**14**), and ethyl β-D-glucopyranoside (**15**). The structures of compounds **1**−**15** were determined by spectroscopic and chemical methods. All the compounds with the exception of **4**, **5**, and **9**−**11** were isolated from *B. hispida* for the first time. The anticomplement activities of compounds **1**−**15** were assessed by Mayer's modified method. Compounds **1**−**15** showed no significant cytotoxic activity against HeLa human cervical, HL-60 human hepatoma, and SMMC-7721 human hepatoma cell lines.

KEYWORDS: *Benincasa hispida (Thunb.) Cogn., triterpenoids, anticomplement activity, cytotoxic activity*

INTRODUCTION

Benicasa hispida (Thunb.) Cogn., which belongs to the Cucurbitaceae family, is a widely used vegetable in China and tropical countries.[1] It is commonly known as ash gourd, ash pumpkin, white gourd, white pumpkin, gourd melon, winter melon, gourd tallow, wax gourd, Chinese watermelon, or Chinese preserving melon.[2] Around the world, this plant is known as Kondol (Philippines), Kundur (Malay), Bhuru Kolu or Safed Kolu (Gujarati), Petha (Hindi), Calabaza china or Calabaza blanca (Spanish), Kushmanda (Sanskrit), dōngguā (Chinese), Fak kio (Thailand), and Bleego (Indonesian). The mature fruits of *B. hispida* are sources of dietary fiber (27.5% of the fruit dry weight),[3] amino acids, proteins,[4] enzymes,[5−7] vitamins (B_1 and C), sterols, flavonoid C-glycoside, terpenes,[8,9] phenolic acids, sugars (e.g., glucose, rhamnopyranose, and mannitol), and trace minerals.[10]

The complement system is a key component of the innate immune system. When excessively or abnormally activated, the complement system contributes to ischemia, asthma, autoimmune disorders, neurodegenerative diseases, and certain types of cancer.[11] Anticomplement agents derived from natural products represent safe therapeutic approaches against inflammatory and allergic disorders.[12−14] In this study, we isolated and identified triterpenoids and other compounds from *B. hispida* mature fruits. The anticomplement and cytotoxic activities of these compounds were assessed.

MATERIALS AND METHODS

General. In this study, melting points were determined using the XT5 micro-melting-point system (Beijing Optical Instrument Factory, Beijing, China). Specific rotation values were determined using a model 241 polarimeter (PerkinElmer Inc., Waltham, MA). Infrared (IR) spectra were recorded with a 983 G spectrometer (PerkinElmer Inc.). ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and two-dimensional NMR spectra were recorded with an Inova 500 spectrometer (Varian Inc., Palo Alto, CA) in C_5D_5N using tetramethylsilane (TMS) as the internal standard. HR-ESI-MS spectra were recorded with a Q-TOF2 spectrometer (Micromass Corp., London, U.K.).

Chromatography. High-performance liquid chromatography (HPLC) analyses and purification were performed on a Shimadzu HPLC system equipped with an LC-20AT pump and an SPD-20A detector (Shimadzu Corp., Kyoto, Japan) at 203 nm. Medium-pressure liquid chromatography (MPLC) purification was performed using a flash chromatography system equipped with a C-650 pump and a flash column [230 mm × 26 mm (inside diameter); Büchi Corp., Flawil, Switzerland)]. The column used for semipreparative HPLC consisted of a Zorbax SB-C18 column [250 mm × 9.4 mm (inside diameter), 5 μm] attached to a guard column of the same material [4 mm × 4 mm (inside diameter); Agilent Corp., Palo Alto, CA]. Both the silica gel (column chromatography) and precoated silica gel [thin layer chromatography (TLC) plates] were obtained from Qingdao Marine Chemical Factory (Qingdao, China). Compounds for TLC were treated with 10% sulfuric acid in alcohol. Octadecylsilane (ODS) for MPLC was purchased from Merck KGaA (Darmstadt, Germany). Sephadex LH-20 for column chromatography was purchased from GE Corp. (Piscataway, NJ). D101 macroporous resin for column chromatography was purchased from Xi'an Sunresin New Materials Co., Ltd. (Xi'an, China). Gas chromatography (GC) was performed in a GC-14C system (Shimadzu Corp.) coupled to a flame ionization detector (FID). The GC system consisted of a DB-5 column [30 m × 0.25 μm (inside diameter); Suzhou Huitong Chromatography Technology Co., Ltd., Suzhou, China] coupled to a FID detector. The column temperature was 210 °C, the injection temperature 270

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Table 1. NMR Spectroscopic Data of 1 (in $CDCl_3$)[a]

	δ_C	δ_H		δ_C	δ_H		δ_C	δ_H
1	30.5	1.76 m, 1.48 m	17	31.6		3′	144.4	7.62 d (16.0)
2	23.2	1.99 m, 1.84 m	18	44.6	1.74 m	4′	134.5	
3	78.5	4.82 t (4.5)	19	28.3	1.70 m, 1.50 m	5′	128.1	7.48 m
4	37.0		20	31.6		6′	128.8	7.34 m
5	43.1	1.64 m	21	30.0	1.56 m, 1.43 m	7′	130.1	7.34 m
6	23.8	2.20 m, 2.09 m	22	34.2	1.78 m, 0.94 m	8′	128.8	7.34 m
7	118.2	5.52 br s	23	27.4	1.04 s	9′	128.1	7.48 m
8	142.0		24	22.1	0.90 s	29-*O*-Cin-1″	167.3	
9	144.5		25	20.5	0.97 s	2″	118.4	6.45 d (16.0)
10	36.2		26	21.9	0.95 s	3″	144.6	7.68 d (16.0)
11	114.0	5.28 br s	27	19.6	0.93 s	4″	134.5	
12	39.4	2.12 m, 1.77 m	28	31.1	1.12 s	5″	128.0	7.46 m
13	37.4		29	72.4	4.04 br s	6″	128.8	7.32 m
14	40.1		30	30.4	1.07 s	7″	130.2	7.32 m
15	27.4	1.75 m, 1.40 m	3-*O*-Cin-1′	166.7		8″	128.8	7.32 m
16	36.7	1.77 m, 1.50 m	2′	118.8	6.43 d (16.0)	9″	128.0	7.46 m

[a]Data in parentheses are *J* values (in hertz). Cin refers to the cinnamoyl group.

°C, the detection temperature 300 °C, and the carrier gas (N_2) at 2.0 kPa.

Animals. Healthy Hartley guinea pigs (n = 5; 300−350 g), which were obtained from the Experimental Animal Center of Soochow University, were housed in plastic cages with *ad libitum* access to food and water. Plastic cages were kept at 22 ± 2 °C, and the animals were exposed to 12 h light−dark cycles. Guinea pig serum (10.0 mL) was collected according to the Local Guidelines for the Care and Use of Laboratory Animals. The study was approved by the Ethics Committee of the Experimental Animal Centre of Soochow University (86-2-1).

Materials. Fresh mature *B. hispida* fruits were collected in Jinghong City (Yunnan Province, China) in September 2009 and authenticated by C.-y. Liu (Soochow University). In this study, 10.2 kg fruits were cut into flakelets, spread on bamboo mats, and sun-dried. A voucher specimen (09-09-25-01) was deposited in the herbarium of the College of Pharmaceutical Science at Soochow University.

Extraction and Isolation. Sun-dried fruits (2.0 kg) was extracted three times in 70% EtOH (50 L). The solvent was removed under reduced pressure. The resulting residue (250 g) was subsequently dissolved in distilled water and fractionated in petroleum ether, $CHCl_3$, or *n*-BuOH. The *n*-BuOH fraction (86.5 g) was dissolved in distilled water, passed through the D101 macroporous resin column, and eluted with an EtOH gradient (0, 30, 60, and 90%; 4.0 L each). The 60% EtOH eluate (16.2 g) was subjected to the MPLC/ODS column and eluted with a MeOH/H_2O mixture (40:60, 60:40, 80:20, and 90:10; 1000 mL each) at a rate of 20 mL/min; five fractions were obtained. Compounds **2** (8 mg; t_R = 15.27 min) and **3** (21 mg; t_R = 12.25 min) were obtained from fraction 1 (97 mg) subjected to semipreparative RP-HPLC and eluted with a MeOH/H_2O mixture (30:70) at a rate of 2 mL/min. Fraction 2 (345 mg) was subjected to Sephadex LH-20 gel column chromatography [100 cm × 3 cm (inside diameter)] and eluted with pure MeOH, resulting in compounds **12** (52 mg), **13** (26 mg), and **14** (15 mg). Compounds **9** (8 mg; t_R = 16.74 min), **7** (6 mg; t_R = 18.61 min), and **11** (2.75 g; t_R = 22.70 min) were obtained from fraction 3 (4.1 g) following semipreparative RP-HPLC purification with a MeOH/H_2O mixture (80:20) as the eluant. Fraction 4 (1.6 g), which was subjected to semipreparative RP-HPLC and eluted with a MeOH/H_2O mixture (90:10), yielded compounds **8** (15 mg; t_R = 18.42 min), **10** (726 mg; t_R = 21.76 min), **6** (23 mg; t_R = 24.04 min), and **4** (16 mg; t_R = 25.62 min). Compounds **1** (17 mg; t_R = 28.16 min) and **5** (28 mg; t_R = 30.43 min) were obtained from fraction 5 (102. mg) following semipreparative RP-HPLC purification with a MeOH/H_2O mixture (90:10) as the eluant.

3α,29-O-Di-trans-cinnamoyl-D:C-friedooleana-7,9(11)-diene (1). White needles: $[\alpha]^{25}_D$ −31.7 (c = 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 215 (4.06), 243 (4.32), 326 nm (4.14); IR (KBr) ν_{max} 3015, 2963, 2923, 1705, 1702, 1633, 1587, 1585, 1514, 817 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectroscopic data in Table 1; HR-ESI-MS (positive ion mode) m/z 723.4370 ([M + H]$^+$, calcd for $C_{48}H_{60}O_4Na$, 723.4389).

Oleanolic Acid 28-O-β-D-Xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (2). White amorphous powder: $[\alpha]^{25}_D$ −32.2 (c = 0.1, MeOH); IR (KBr) ν_{max} 3408, 2953, 1742, 1645, 1460, 1055, 887 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data in Tables 2 and 3; HR-ESI-MS (positive ion mode) m/z 1021.5306 ([M + Na]$^+$, calcd for $C_{51}H_{82}O_{19}$, 1021.5348).

Oleanolic Acid 28-O-β-D-Glucopyranosyl-(1→3)-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (3). White amorphous powder: $[\alpha]^{25}_D$ −36.5 (c = 0.1, MeOH); IR (KBr) ν_{max} 3386, 2945, 1742, 1648, 1455, 1052, 886 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectroscopic data in Tables 2 and 3; HR-ESI-MS (positive ion mode) m/z 1183.5865 ([M + Na]$^+$, calcd for $C_{57}H_{92}O_{24}$, 1183.5876).

Acid Hydrolysis of Compounds 2 and 3. In this experiment, 4 mg of each compound was dissolved in a 1 N HCl (dioxane/H_2O, 1:1, 2.0 mL) solution and heated at 90 °C for 2 h. After the mixture had cooled, silver carbonate (10 mg) was added, and the solvent was evaporated to dryness under N_2. The mixture was extracted with $CHCl_3$ and H_2O; the aqueous layer was concentrated to dryness. The residue was dissolved in 0.2 mL of dry pyridine. L-Cysteine methyl ester hydrochloride in dry pyridine (0.06 M, 0.2 mL) was added, and the mixture was heated at 60 °C for 2 h. In this experiment, 0.2 mL of (trimethylsilyl)imidazole dissolved in dry pyridine was added and evaporated to dryness at 60 °C for 2 h. The residue was partitioned with *n*-hexane and H_2O (0.1 mL each). The *n*-hexane fraction was subjected to GC. Sugar units were identified by comparing their retention times (t_R) with those of known standards: 10.49 min for D-glucose, 8.46 min for L-xylose, 11.10 min for L-glucose, 9.16 min for D-rhamnose, 8.52 min for D-arabinose, 8.33 min for L-arabinose, and 8.89 min for L-rhamnose.

Anticomplementary Activity Assay. The anticomplementary assay was performed following the method reported by Mayer.[12] Briefly, sensitized erythrocytes were prepared by incubating sheep erythrocytes (4.0 × 10^8 cells/mL; Shanghai Kang-Run Biotech Co., Ltd., Shanghai, China) in the presence of rabbit anti-sheep erythrocyte antibody (EA), donated by D.-f. Chen (College of Pharmacy, Fudan University, Shanghai, China) in veronal buffer [VBS^{2+}, i.e., barbitone sodium hydrochloric acid buffer solution (pH 7.4) with 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} (Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China)]. Stock solutions of isolated compounds (samples) were prepared in dimethyl sulfoxide (DMSO) and diluted at different

Table 2. NMR Spectroscopic Data for Aglycone Moieties of 2 and 3 (in C_5D_5N)[a]

	2		3	
	δ_C	δ_H	δ_C	δ_H
1	39.2	1.01 m, 1.58 m	38.2	0.88 m, 1.48 m
2	28.4	1.89 m, 2.17 m	27.0	1.76 m, 2.02 m
3	79.1	3.46 d (11.0, 4.5)	76.8	3.02 dd (11.0, 4.5)
4	39.4		38.4	
5	56.0	0.89 d (12.0)	54.8	0.67 d (12.0)
6	19.0	1.39 m, 1.52 m	18.0	1.28 m, 1.46 m
7	33.5	1.34 m, 1.54 m	32.4	1.24 m, 1.38 m
8	40.0		39.0	
9	47.4	1.74 m	47.1	1.47 m
10	37.5		36.6	
11	23.3	1.99 m, 2.06 m	22.2	1.57 m, 1.94 m
12	122.9	5.51 br s	121.9	5.20 br s
13	144.3		143.4	
14	42.3		41.3	
15	28.8	1.28 m, 2.12 m	27.2	1.03 m, 1.78 m
16	23.9	1.98 m, 2.20 m	22.9	1.53 m, 161 m
17	46. 5		46.2	
18	41.8	3.38 d (4.5)	40.7	2.79 d (3.5)
19	48.3	1.39 m, 1.89 m	45.4	1.04 m, 1.63 m
20	30.9		30.4	
21	34.3	1.31 m, 1.58 m	33.2	1.14 m, 1.32 m
22	33.2	1.92 m, 2.22 m	31.7	1.45 m, 1.62 m
23	28.2	1.24 s	28.2	0.89 s
24	16.5	1.17 s	16.0	0.88 s
25	15.7	0.96 s	15.2	0.67 s
26	17.6	1.04 s	16.7	0.85 s
27	26.1	1.30 s	25.5	1.05 s
28	176.2		175.1	
29	32.8	0.98 s	32.8	0.68 s
30	23.8	1.07 s	23.4	0.88 s

[a]Data in parentheses are J values (in hertz).

Table 3. NMR Spectroscopic Data for Sugar Moieties of 2 and 3 (in C_5D_5N)[a]

	2		3	
	δ_C	δ_H	δ_C	δ_H
Ara-1	93.5	6.51 d (7.0)	91.6	5.57 d (7.0)
2	75.7	4.63 m	73.8	3.62 m
3	69.6	4.59 m	67.8	3.78 m
4	66.0	4.45 m	64.4	3.65 m
5	62.9	4.56 m, 3.95 m	61.2	3.37 m, 3.71 m
Rha-1	100.8	5.75 br s	99.2	4.79 br s
2	71.6	4.85 m	70.2	4.25 m
3	82.4	4.65 m	79.5	3.69 m
4	78.3	4.66 m	76.3	3.59 m
5	68.8	4.52 m	67.1	3.51 m
6	18.6	1.82 d (6.0)	17.7	1.12 d (6.2)
Xyl-1	105.9	5.15 d (7.6)	104.2	4.46 d (7.0)
2	75.4	3.93 m	73.7	3.06 m
3	78.5	4.59 m	87.7	3.27 m
4	71.0	4.11 m	68.6	3.40 m
5	67.2	4.19 m, 3.43 m	65.7	3.70 m, 3.06 m
Xyl-1′	105.3	5.41 d (7.2)	102.4	4.71 d (7.5)
2′	75.1	3.93 m	72.6	3.11 m
3′	78.5	4.13 m	76.4	3.17 m
4′	71.3	4.19 m	69.6	3.27 m
5′	67.3	4.10 m, 3.42 m	65.0	3.77 m, 3.09 m
Glc-1			103.7	4.30 d (8.1)
2			73.7	3.06 m
3			76.1	3.09 m
4			69.8	3.77 m
5			76.8	3.18 m
6			61.2	3.71 m, 3.68 m

[a]Data in parentheses are J values (in hertz). Ara refers to arabinose, Rha to rhamnose, Xyl to xylose, and Glc to glucose.

concentrations (10, 20, 50, 100, 200, 500, and 1000 μM) in VBS^{2+}. The final concentration of DMSO was <1%. The diluted serum of guinea pig (1:80) was selected for submaximal lysis in the absence of anticomplement agents. Different sample concentrations (200 μL) were preincubated with 200 μL of diluted serum of guinea pig at 37 °C for 10 min and mixed with 200 μL of sensitized erythrocytes. The resultant mixture was incubated at 37 °C for 30 min. The controls were incubated under the same conditions: (1) vehicle control, 200 μL of VBS^{2+}; (2) 100% lysis control, 200 μL of EA (erythrocyte antibody) in 400 μL of water; and (3) sample control, 200 μL of a diluted sample in 400 μL of VBS^{2+}. Following the 30 min incubation, the mixture was centrifuged and the optical density (OD) of the resulting supernatant was measured at 405 nm. Hemolysis inhibition rates (I%) were calculated with the following equation

$$I\% = [1 - (A_{\text{sample test}} - A_{\text{sample control}})/A_{\text{vehicle control}}] \times 100\%$$

The 50% inhibitory concentration (CP_{50}) was calculated using the straight line fitting method.[12] Heparin sodium salt (>98% pure; Sigma-Aldrich, St. Louis, MO) was used as a positive control. Anticomplementary assays were repeated three times. The anticomplementary activity of the samples was expressed as CP_{50} relative to the complement-dependent hemolysis of the control.

Cytotoxic Activity Assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was performed[15] to assess the cytotoxic activities of the isolated compounds against HeLa human cervical, HL-60 human hepatoma, and SMMC-7721 human hepatoma cell lines (Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences). Briefly, 2×10^5 cells per well were seeded onto 96-well culture plates in 100 μL volumes. Different sample concentrations (2.5, 5, 10, 20, and 50 μM) were added to the wells. Each concentration represented a treatment group. No sample was added to the negative control group. The positive control group contained norcantharidin (2.5 μg/mL, >99.0% pure as determined by HPLC;[16] Nanjing Zelang Medical Technology Co. Ltd., Nanjing, China). Six parallel wells were used for each treatment or control group. Following a 24 h incubation, culture wells were treated with tetrazolium reagent. Formazan was determined by photometry at 570 nm. Results are expressed as the absorbance of control cells relative to that of the sample-treated cells. The percentage of cell proliferation was calculated from the ratio of the sample OD to the control OD. Experiments were repeated three times.

RESULTS AND DISCUSSION

The 70% EtOH extract of dried fruits of *B. hispida* was separated by being successively partitioned with petroleum ether, $CHCl_3$, and *n*-BuOH and by repeated D101 resin and ODS column chromatography to afford three new triterpenoids, **1**–**3** (Figure 1), as well as 12 other known compounds, which were identified as multiflorenol (**4**),[17] isomultiflorenyl acetate (**5**),[18] stigmasterol (**6**),[19] stigmasterol 3-*O*-*β*-D-glucopyranoside (**7**),[20] *α*-spinasterol (**8**),[21] *α*-spinasterol 3-*O*-*β*-D-glucopyranoside (**9**),[20] *β*-sitosterol (**10**),[17] daucosterol (**11**),[20] arbutin (**12**),[22] nicotinic acid (**13**),[23] (+)-pinonesinol (**14**),[24] and ethyl *β*-D-glucopyranoside (**15**),[25] based on their spectroscopic data and comparisons with reference values and authentic samples.

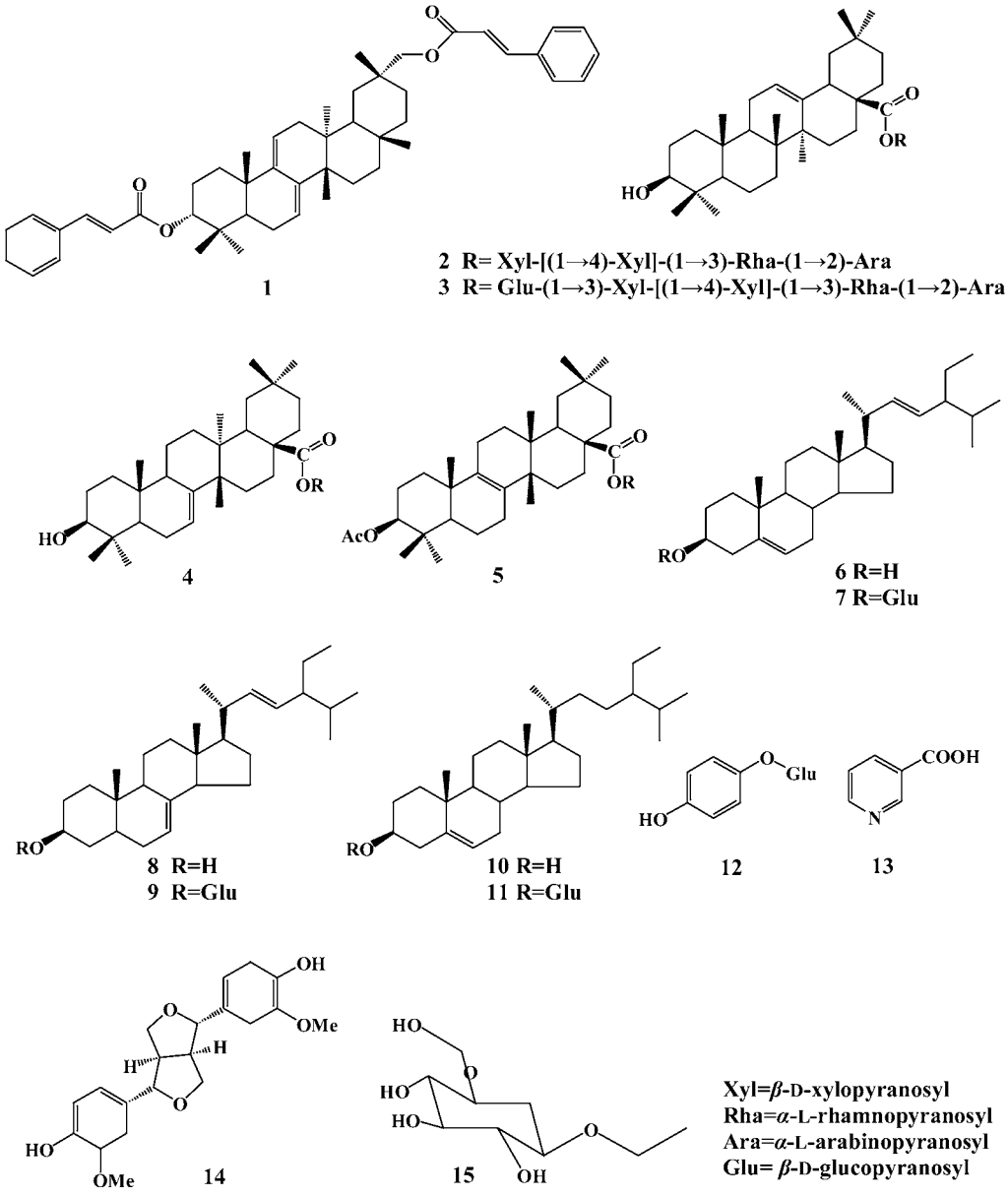

Figure 1. Structures of compounds **1**−**15**.

Compound **1** was isolated as a white powder. Its molecular formula is $C_{48}H_{60}O_4$ based on the positive HR-ESI-MS. The IR spectrum results revealed the presence of conjugated ester groups (1705 and 1702 cm^{-1}), a conjugated double bond (1633, 1585, and 817 cm^{-1}), and phenyl group(s) (1587 and 1514 cm^{-1}). The ^1H NMR spectrum (Table 1) presents seven signals at δ 1.12 (3H, s, Me-28), 1.07 (3H, s, Me-30), 1.04 (3H, s, Me-23), 0.97 (3H, s, Me-25), 0.95 (3H, s, Me-26), 0.93 (3H, s, Me-27), and 0.90 (3H, s, Me-24), which were assigned to seven tertiary methyl groups. Furthermore, two olefinic proton signals at δ 5.52 (1H, br s, H-7) and 5.28 (1H, t, J = 4.0 Hz, H-11), a methane signal at δ 4.82 (1H, t, J = 4.5 Hz, H-3), and the signal of an oxymethylene at δ 4.04 (2H, br s, H-29) suggested that compound **1** belonged to the group of D:C-friedooleanane-type triterpenes.[26,27] Its ^1H NMR spectrum also exhibited the signals of two *trans*-cinnamoyl groups (Cin) at δ [6.43 (1H, d, J = 16.0 Hz, 3-*O*-Cin-2′), 7.62 (1H, d, J = 16.0 Hz, 3-*O*-Cin-3′), 7.48 (2H, m, 3-*O*-Cin-5′,9′), 7.34 (3H, m, 3-*O*-Cin-6′,7′,8′)], and [6.45 (1H, d, J = 16.0 Hz, 29-*O*-Cin-2′), 7.68 (1H, d, J = 16.0 Hz, 29-*O*-Cin-3′), 7.46 (2H, m, 29-*O*-Cin-5′,9′), 7.32 (3H, m, 29-*O*-Cin-6′,7′,8′)].[28] In addition, the triplet peak for H-3, as well as the ^3J value of 4.5 Hz between H-3 and H-2 suggested that the *trans*-cinnamoyl group at C-3 was located at the axial position; i.e., its configuration was α. The ^{13}C NMR data (Table 1) show the resonances for 48 carbon atoms, whose substitution patterns were revealed from the distortionless enhancement by polarization transfer (DEPT) and heteronuclear single-quantum coherence (HSQC) experiments as 7 methyls, 10 methylenes, 19 methines, and 12 quaternary carbons. According to the ^{13}C NMR spectrum, there were the typical signals of four olefinic carbons at δ 118.2, 142.0, 144.5, and 114.0 corresponding to C-7, C-8, C-9, and C-11, respectively, an oxymethine carbon at δ 78.5 corresponding to C-3, and an oxymethene carbon at δ 72.4 corresponding to C-29. The two *trans*-cinnamoyl groups were located at C-3 and C-29 on the basis of the downfield shifts of H-3 at δ 4.82 (1H, t, J = 4.5 Hz) and H-29 at δ 4.04 (2H, br s), which was supported by the correlation between δ_H 4.82 (1H, t,

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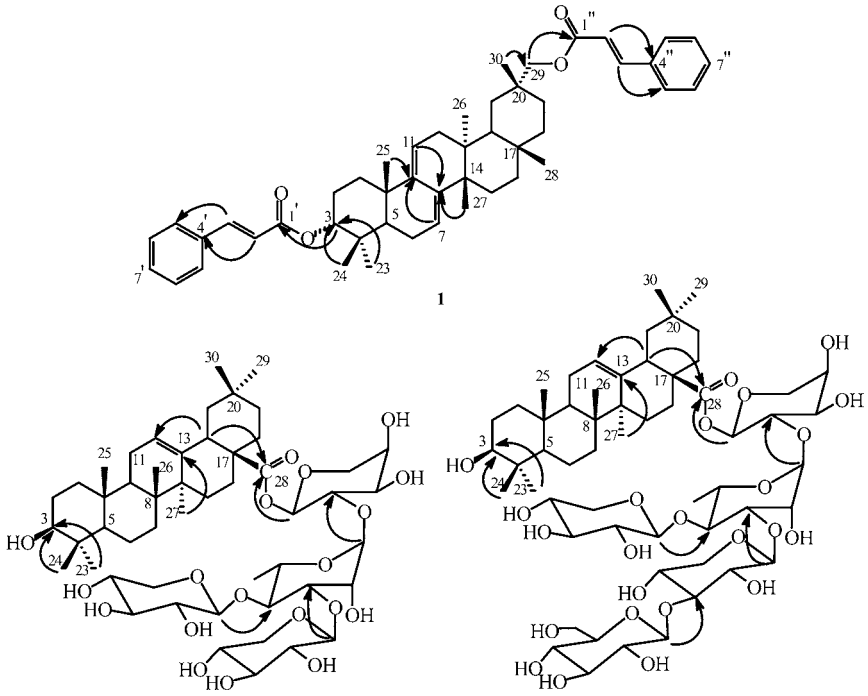

Figure 2. Key HMBC correlations for compounds **1**−**3**.

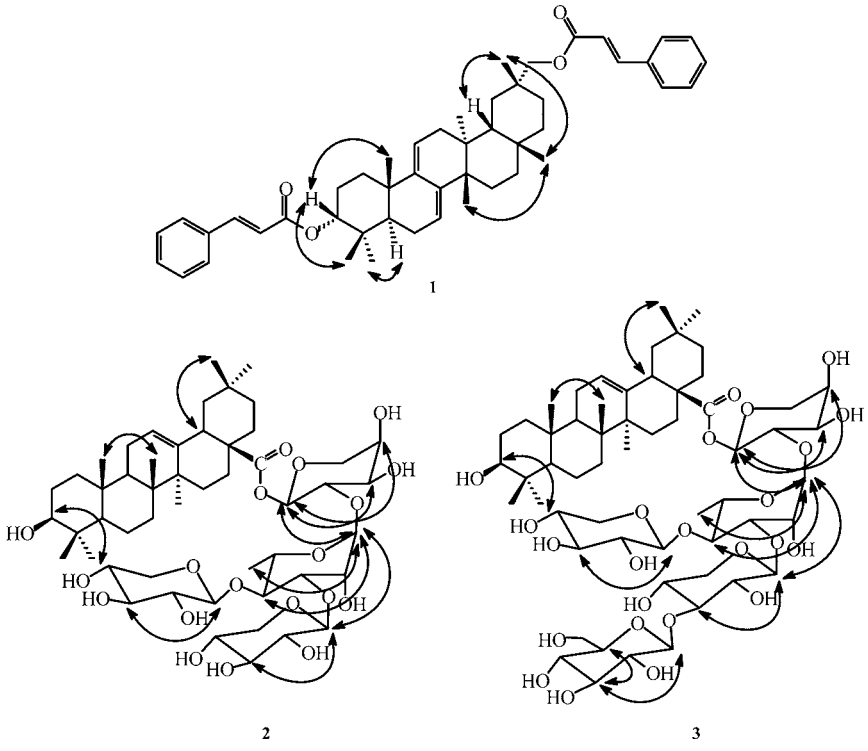

Figure 3. Key NOESY correlations for compounds **1**−**3**.

J = 4.5 Hz, H-3) and δ_C 166.7 (3-*O*-Cin-1′), as well as the correlation between δ_H 4.04 (2H, br s, H-29) and δ_C 167.3 (29-*O*-Cin-1″) in the heteronuclear multiple-bond connectivity (HMBC) experiment (Figure 2).

The relative stereochemistry of compound **1** was assessed by its nuclear Overhauser effect spectroscopy (NOESY) spectrum (Figure 3). Interactions between H-3 (δ 4.82) and Me-24 (δ 0.90) revealed that they were on the same side of the molecule, while the nuclear Overhauser effect (NOE) between Me-30 (δ 1.07) and H-18 (δ 1.74) suggested that Me-30 and H-18 were on the same side. Therefore, compound **1** was identified as 3*α*,29-*O*-di-*trans*-cinnamoyl-D:C-friedooleana-7,9 (11)-diene.

Compound **2** was isolated as a white amorphous powder. Its molecular formula is $C_{51}H_{82}O_{19}$ based on the positive HR-ESI-MS. The ^{1}H NMR spectrum had seven signals at δ 1.30 (3H, s, Me-27), 1.24 (3H, s, Me-23), 1.17 (3H, s, Me-24), 1.07 (3H, s, Me-30), 1.04 (3H, s, Me-26), 0.98 (3H, s, Me-29), and 0.96 (3H, s, Me-25), which were assigned to seven tertiary methyl

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groups; one olefinic proton at δ_H 5.51 (1H, br s) was located at C-12 and a methine proton at δ 3.46 (1H, dd, J = 11.0, 4.5 Hz, H-3). In addition, the ^{13}C NMR spectrum of compound **2** revealed one oxymethylene at δ_C 79.1 (C-3) and one carboxylic carbon at δ_C 176.2 (C-28) (Table 2). The ^{1}H NMR and ^{13}C NMR spectra indicate that compound **2** was an oleananic acid derivative.[29] The ^{1}H NMR spectroscopic data also revealed the presence of four sugar residues, with four anomeric proton signals at δ_H 6.51 (1H, d, J = 7.0 Hz, H-1 of arabinopyranose), 5.75 (1H, br s, H-1 of rhamnopyranose), 5.15 (1H, d, J = 7.5 Hz, H-1 of xylopyranose 1), and 5.41 (1H, d, J = 7.0 Hz, H-1′ of xylopyranose 1′), corresponding to four anomeric carbon signals at δ_C 93.5 (C-1 of arabinopyranose), 100.8 (C-1 of rhamnopyranose), 105.9 (C-1 of xylopyranose 1), and 105.3 (C-1′ of xylopyranose 1′), respectively, in the ^{13}C NMR spectrum (Table 3), and the upfield shift (−4.2 ppm) at δ_C 176.2 (C-28) indicated that **2** was deduced to be a monodesmosidic saponin.[25] L-Arabinopyranose, D-xylopyranose, and L-rhamnopyranose (molar ratio of 1:2:1) were the sugar residues obtained from the acid hydrolysis of compound **2**. The sequencing and points of attachment in the glycosidic chains were determined by HMBC correlations. The carbohydrate located at C-28 of the aglycone was identified as β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl, by the HMBC correlations between δ_H 6.51 (H-1 of arabinopyranose) and δ_C 176.2 (C-28), between δ_H 5.75 (H-1 of rhamnopyranose) and δ_C 75.7 (C-2 of arabinopyranose), between δ_H 4.63 (H-2 of arabinopyranose) and δ_C 100.8 (C-1 of rhamnopyranose), between δ_H 5.15 (H-1 of xylopyranose 1) and δ_C 82.4 (C-3 of rhamnopyranose), between δ_H 4.65 (H-3 of rhamnopyranose) and δ_C 105.9 (C-1 of xylopyranose 1), between δ_H 5.41 (H-1′ of xylopyranose 1″) and δ_C 78.3 (C-4 of rhamnopyranose), and between δ_H 4.66 (H-4 of rhamnopyranose) and δ_C 105.3 (C-1′ of xylopyranose 1′) (Figure 2). The two xylopyranosyl moieties were identified as having a β configuration on the basis of the large $^3J_{H\text{-}1,H\text{-}2}$ coupling constants; the rhamnopyranosyl unit was determined to have an α configuration by the NOE correlations between δ 5.75 (H-1 of rhamnopyranosyl) and δ 4.66 (H-4 of rhamnopyranosyl) and δ 1.82 (H-6 of rhamnopyranosyl), and the arabinose moiety was inferred as having an α configuration upon the observation of the NOE correlation between δ 6.51 (H-1 of arabinopyranose) and δ 4.45 (H-4 of arabinopyranose) and δ 4.56 and 3.95 (H-5 of arabinopyranose) (Figure 3).[30] On the basis of the analyses described above, **2** was elucidated as oleanolic acid 28-*O*-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Compound **3** was isolated as a white powder. Its molecular formula is $C_{57}H_{92}O_{24}$ based on the positive HR-ESI-MS. The ^{1}H NMR spectrum revealed the presence of five sugar residues, with five anomeric proton signals at δ_H 5.57 (1H, d, J = 7.0 Hz, H-1 of arabinopyranose), 4.79 (1H, br s, H-1 of rhamnopyranose), 4.46 (1H, d, J = 7.0 Hz, H-1 of xylopyranose 1), 4.71 (1H, d, J = 7.5 Hz, H-1′ of xylopyranose 1′), and 4.30 (1H, d, J = 7.5 Hz, H-1 of glucopyranose), corresponding to five anomeric carbon signals at δ_C 91.6 (C-1 arabinopyranose), 99.2 (C-1 of rhamnopyranose), 104.2 (C-1 of xylopyranose 1), 102.4 (C-1′ of xylopyranose 1′), and 103.7 (C-1 of glucopyranose), respectively, as determined by the HSQC correlations (Table 3). L-Arabinopyranose, D-xylopyranose, L-rhamnopyranose, and D-glucose (molar ratio of 1:2:1:1) were the sugar residues obtained from the acid hydrolysis of compound **3**. The ^{1}H NMR and ^{13}C NMR data of compound **3** were very similar to those of compound **2**, with the exception of additional signals attributed to one glucopyranose residue. This glucopyranose residue was determined to be located at C-3 of the xylopyranose 1 moiety, inferred by the downfield shift of C-3 at δ 87.7 (9.2 ppm) of xylopyranose 1 and the HMBC correlation between δ_H 4.30 (H-1 of glucopyranose) and δ_C 87.7 (C-3 of xylopyranose 1). Therefore, the carbohydrate present at C-28 of the aglycone was β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl. The glucopyranosyl and two xylopyranosyl moieties were identified as having a β configuration based on the large $^3J_{H\text{-}1,H\text{-}2}$ coupling constant; the rhamnopyranosyl unit had an α configuration based on NOE correlations between δ 4.79 (H-1 of rhamnopyranosyl) and δ 3.59 (H-4 of rhamnopyranosyl) and δ 1.12 (H-6 of rhamnopyranosyl), and the arabinose moiety was inferred as having an α configuration based on the observation of the NOE correlation between δ 5.57 (H-1 of arabinopyranose) and δ 3.65 (H-4 of arabinopyranose), 3.37, and 3.71 (H-5 of arabinopyranose) (Figure 3).[30] Therefore, compound **3** was elucidated as oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-[β-D-xylopyranosyl-(1→4)]-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

An excessive or abnormal activation of the complement system contributes to a variety of inflammatory and anaphylactic disorders.[31] In this study, the anticomplement activities of compounds **1**−**15** were assessed. The results (Table 4) were expressed as CP_{50} (minimal concentration to produce a hemolysis ratio of 50%). Triterpenoids and steroids from herbs have immunoregulatory properties.[32] Even though the anticomplement activities of compounds **1**−**15** were lower than those of HN saponin F[33] and clionasterol,[34] our results revealed that triterpenoids and steroids isolated from *B. hispida* fruits possess anticomplement activities.

Table 4. Anticomplementary Activities of Compounds 1−15

	CP_{50} (μM)[a]
1	>300
2	182.6 ± 17.2
3	157.1 ± 14.8
4	234.5 ± 21.3
5	271.6 ± 25.6
6	115.6 ± 10.4
7	189.2 ± 18.0
8	121.3 ± 11.8
9	197.8 ± 18.4
10	118.0 ± 12.5
11	175.3 ± 16.9
12	247.6 ± 23.5
13	>300
14	282.4 ± 27.1
15	>300
heparin sodium salt[b]	32.3 ± 2.5

[a]Data were obtained from three independent experiments. Data are expressed as means ± the standard deviation. [b]Positive control.

Furthermore, the cytotoxic activities of compounds **1**−**15** were assessed by the MTT assay. The maximal inhibitory rates of the compounds are listed in Table 5. The inhibitory rates of compounds **1**−**15** were <35% against the three tumor cell lines. These results reveal that at 2.5−50 μM, compounds **1**−**15** did

Table 5. Maximal Inhibitory Rates of Proliferation of Tumor Cells by 1−15 *in Vitro*

compd[a]	HeLa	7721	HL-60
1	11.18 ± 2.14	5.16 ± 1.02	3.14 ± 3.07
2	31.71 ± 2.35	20.75 ± 3.89	28.17 ± 2.45
3	27.48 ± 3.61	15.90 ± 4.76	12.19 ± 3.10
4	22.26 ± 4.26	11.47 ± 2.15	16.13 ± 2.66
5	21.02 ± 3.06	10.52 ± 1.07	14.15 ± 3.12
6	24.47 ± 1.87	15.12 ± 2.65	10.56 ± 2.15
7	33.74 ± 2.20	8.96 ± 1.78	7.98 ± 3.16
8	24.19 ± 1.93	24.52 ± 3.56	7.14 ± 2.04
9	32.16 ± 2.35	12.98 ± 2.31	11.96 ± 2.82
10	4.12 ± 2.63	2.01 ± 4.05	3.15 ± 3.05
11	21.45 ± 4.58	18.14 ± 2.76	21.05 ± 3.43
12	23.13 ± 3.76	12.17 ± 2.45	8.18 ± 2.79
13	15.81 ± 4.61	8.19 ± 2.36	47.18 ± 3.07
14	34.14 ± 2.51	20.12 ± 3.43	17.12 ± 3.25
15	32.16 ± 2.37	7.15 ± 2.16	15.12 ± 3.11
cantharidin	91.2 ± 4.57	93.42 ± 3.17	92.78 ± 2.35

[a]For **1−15**, the test concentration range was from 2.5 to 50 μM; for cantharidin, the test concentration was 5.0 μM.

not have significant cytotoxic activity against HeLa human cervical, HL-60 human hepatoma, and SMMC-7721 human hepatoma cell lines.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HR-ESI-MS and NMR spectra of compounds **1−3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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