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Triterpenoid Saponins from *Stauntonia chinensis*

Yu Chen, Fang Yang,† Sha Wang,† De-bin Wang,† Jing Xu,† and Guang-zhong Yang†,*

College of Chemistry and Material Sciences, South Central University for Nationalities, Wuhan 430074, P.R. China
*†Laboratory for Natural Product Chemistry, College of Pharmacy, South Central University for Nationalities, Wuhan 430074, P.R. China. *E-mail: yanggz888@126.com*
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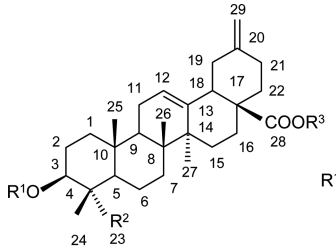
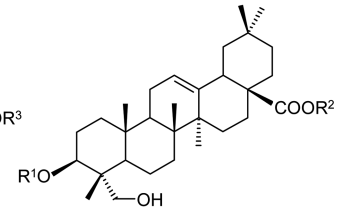
Stauntonia chinensis DC. (Lardizabalaceae), an evergreen herb growing in southern China, commonly known as "Ye Mu Gua", has been used as a traditional Chinese medicine, especially for its anti-inflammatory and analgesic effects.[1] The chemical constituents of *S. chinensis* were previously reported to contain nortriterpenoid saponins,[2] neolignan glycosides,[3] flavonoids.[4] To find the biologically active compounds, a detailed phytochemical investigation was carried out on the stem of *S. chinensis*, which resulted in isolation of two new triterpenoid saponins, named as yemuoside $YM_{36\text{-}37}$. Their structues were established on the basis of 2D-NMR experiments and mass spectrometry.

In addition, eight known triterpene glycoside 3-*O*-α-L-arabinopyranosyl-30-norhederagenin (**3**),[5] sinofoside A (**4**),[5] yemuoside YM11 (**5**),[2g] 3-*O*-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-akebonic acid (**6**),[2d] 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin (**7**),[6] 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin (**8**),[7] hederasaponin D (**9**)[8] and 28-*O*-β-D-glucopyranosyl-(16)-β-D-glucopyranosyl-hederagenin (**10**)[9] were isolated from this plant. Compounds **3-4** and **7-10** were isolated from thisplant for the first time.



1-6



7-10

1 R^1 = H; R^2 = CH_2OH; R^3 = glc(1→6)glc
2 R^1 = xyl(1→3)-rha-(1→2)-ara; R^2 = CH_2OH; R^3 = H
3 R^1 = ara; R^2 = CH_2OH; R^3 = H
4 R^1 = ara; R^2 = CH_2OH; R^3 = rha(1→4)-glc-(1→6)-glc
5 R^1 = ara; R^2 = CH_3; R^3 = glc(1→6)-glc
6 R^1 = ara(1→3)-rha-(1→2)-ara; R^2 = CH_3; R^3 = H
7 R^1 = rha(1→2)-ara; R^2 = H
8 R^1 = xyl(1→3)-rha-(1→2)-ara; R^2 = H
9 R^1 = ara; R^2 = rha(1→4)-glc-(1→6)-glc
10 R^1 = H; R^2 = glc(1→6)-glc
glc: β-D-glucopyranosyl xyl: β-D-xylopyranosyl
rha: α-L-rhamnopyranosyl ara: α-L-arabinopyranosyl

Figure 1. Their structures of compounds **1-10**.

Compound **1** was obtained as white amorphous powder. The molecular formula was determined as $C_{41}H_{64}O_{14}$ from the $[M+Na]^+$ signal at *m/z* 803.4186 by HR-ESI-MS. After acid hydrolysis of **1**, the gas chromatography analysis of acetylated aldononitriles revealed the presence of D-glucose. The ^{1}H-NMR spectrum of **1** showed signals for four tertiary methyl groups at δ_H 1.02, 1.07, 1.16 and 1.17 (each 3H, s), an exomethylene group at δ_H 4.66 (1H, s) and 4.71-4.72 (1H, m), an olefinic proton at δ_H 5.46 (br s). The ^{13}C-NMR data of the aglycone spectra of **1** revealed the presenceof five sp^3 quaternary carbons, four methyl groups, four methine groups (including one oxygenated methine at δ_C 73.8), 11 methylene groups (including one oxygenated methylene at δ_C 68.3), a COOH function at δ_C 176.2, and two C=C moiety at δ_C 123.4 (d), 143.9 (s), 107.4 (t) and 148.8 (s), which were characteristic of 30-nor-olean-12-en-28-oic acid type triterpenoid. The detailed NMR data analysis and comparison with reference data indicated that the aglycon should be 30-norhederagenin.[2e] The ^{1}H- and ^{13}CNMR data exhibited singals for two anomeric protons at δ_H 6.24 (1H, d, J = 8.5 Hz) and 5.03 (1H, d, J = 7.5 Hz) and the related anomeric carbon singals at δ_C 95.8 and 107.4. The β configuration of two glucoses were determined from the coupling constants of the anomeric protons. In comparison of ^{13}C-NMR data of **1** with those of 30-norhederagenin, the chemical shift of C-28 showed upshift from 179.4 to 176.2 ppm. It suggested that compound **1** was a diglycoside with an ester linkage to C-28 of the aglycone which was furtherconfirmed by the HMBC correlations of H-1" at δ 5.03 of the terminal β-D-glucose to C-6' at δ 69.9; and H-1' at δ 6.24 of the inner β-D-glucose to C-28 at δ 176.2. Thus, the structure of **1** was identified as 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-30-norhederagenin, named as yemuoside YM_{36}.

Compound **2** was obtained as white amorphous powder. Its molecular formula was determined as $C_{45}H_{70}O_{16}$ according to the positive HRESIMS (*m/z* 889.4548$[M+Na]^+$, calcd 889.4562). After acid hydrolysis and derivatization of **2** by the same method as **1**, the GC analysis revealed the presence of L-rhamnose, L-arabinose and D-xylose. In comparison of the ^{13}C-NMR data of **2** with those of **1**, a majority of the aglycone were the same except for difference at C-3 and C-

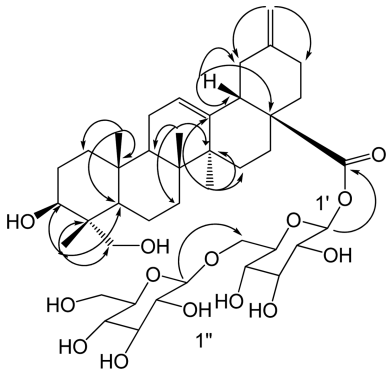


Figure 2. Significant HMBC correlations of compound **1**.

28 (δ_C 73.8 (C-3) and 176.2 (C-28) in **1**; δ_C 81.5 (C-3) and 180.0 (C-28) in **2**). Thus, the glycoside linkage was suggested to be at C-3 of the aglycone. All the above analyses revealed that the compounds **1** and **2** shared the same aglycone. By comparison of the ^{13}C NMR data of **2** with those of the moiety of the ether-linkage sugar chain of Yemuoside YM_{22} suggested that **2** possessed the same ether-linkage sugar chain as Yemuoside YM_{22}.[2g] This deduction was confirmed by HMBC correlations between H-1' at δ_H 5.09 and C-3 at δ_C 81.5; H-1" at δ_H 6.35 and C-2' at δ_C 75.5; H-1'" at δ_H 5.35 and C-3" at δ_C 83.4. According to anomeric proton signals at δ_H 5.09 (1H, d, J = 6.0 Hz), 6.35 (1H, br s) and 5.35 (1H, d, J = 7.0 Hz), the relative configurations of the sugar units were determined as α-arabinose, α-rhamnose and β-xylose. Therefore, the structure of **2** was identified as 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-30-norhederagenin, named as yemuoside YM_{37}.

Triterpenoid saponins **2**, **4**, **6-8** and **10** were evaluated for their cytotoxicities against the cancer cell lines HepG2 human hepatocellular liver carcinoma using the MTT assay method. MTT assays were used to estimate IC_{50} values, that is, the drug concentration that causes 50% of cell growth inhibition after 24 h of continuous exposure to the test molecule. Compound **6-8** exhibited moderate cytotoxic activity with an IC_{50} values of 14.97, 24.46 and 21.32 μM respectively, using cisplatin as a positive control (IC_{50} 31.60 μM). Compound **2**, **4** and **10** were inactive against tested cell lines (IC_{50} > 40 μM).

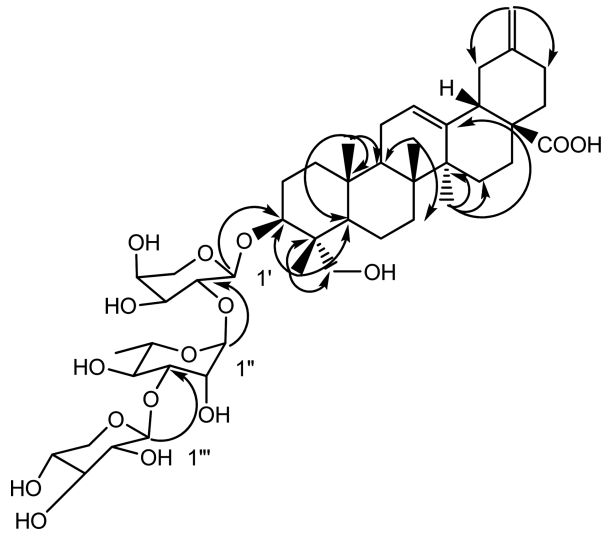


Figure 3. Significant HMBC correlations of compound **2**.

Experimental

Plant Materials. The stems of *Stauntonia chinensis* were collected from NanNing, Guangxi Zhuang Autonomous Region, P. R. China and identified by associate chief pharmacist Jin-Wei Huang at Guangxi Institute of Minority Medicine. The voucher specimen (20090801) was deposited with the Herbarium of College of Pharmacy, South Central University for Nationalities.

Extraction and Isolation. The stems of *Stauntonia chinensis* (16 kg) were extracted with 60% EtOH three times and then successively partitioned with EtOAc and *n*-BuOH. The extract of *n*-BuOH (300 g) was chromatographed on silica gel with $CHCl_3$-MeOH (100:0, 98:2, 95:5, 9:1, 8:2, 7:3, 1:1, 3:7, 0:100 v/v) to give 15 fractions (fr.1-fr.15). Fr. 10 (9.6 g) was subjected to CC on silica gel with $CHCl_3$-MeOH (1:0, 99:1, 95:5, 9:1, 0:1) and further purified by octadecylsilane CC with H_2O/MeOH (95:5→0:1) to obtain **3** (10.3 mg). Fr. 12 (30 g) was subjected to CC on silica gel with EtOAc-EtOH (1:0, 98:2, 95:5, 9:1, 8:2, 7:3, 0:1) to afford compounds **2** (13.0 mg), **6** (8.1 mg), **7** (15 mg), **8** (15.0 mg). Fr. 13 (45 g) was subjected to CC on silica gel with EtOAc-EtOH (1:0, 98:2, 95:5, 9:1, 8:2, 7:3, 0:1) to give five fractions (Frs. 13.1-13.5). Fr. 13.2 (3.6 g) was successively separated by CC (ODS; H_2O/MeOH; (95:5→0:1)) to give compound **1** (29 mg). Fr. 13.2.3 (314.8 mg) was successively separated by CC (ODS; H_2O/MeOH; (9:1→0:1)) and further purified by semi-prep. HPLC (CH_3CN/H_2O 36:74, 2.5 mL/min) to afford compound **5** (9.6 mg; t_R 15.7 min) and **10** (18 mg; t_R 24.4 min). Fr. 13.5 (14.1 g) was subjected to CC on silica gel with $CHCl_3$-MeOH (99:1, 98:2, 95:5, 9:1, 8:2, 7:3, 6:4, 0:1) and further purified by semi-prep. HPLC (CH_3CN/H_2O 30:70, 2.5 mL/min) to afford compound **4** (14.3 mg; t_R 11.8 min) and **9** (14.0 mg; t_R 19.2 min).

Yemuoside YM_{36} (1): white amorphous powder; $[\alpha]_D$ = +42.5 (c 0.48, MeOH); ^1H NMR and ^{13}C NMR (in C_5D_5N) spectroscopic data, see supporting information; HRESIMS *m/z* 803.4186 $[M+Na]^+$ (calcd. for $C_{41}H_{64}O_{14}Na$, 803.4194).

Yemuoside YM_{37} (2): white amorphous powder; $[\alpha]_D$ = +40.8 (c 0.50, MeOH); ^1H NMR and ^{13}C NMR (in C_5D_5N) spectroscopic data, see supporting information; HRESIMS *m/z* 889.4548 $[M+Na]^+$ (calcd. for $C_{45}H_{70}O_{16}Na$, 889.4562).

Acidic Hydrolysis. Compounds **1-2** (3 mg) were respectively added to trifluoro-acetic acid (TFA, 4 N) solution (6 mL), then heated for 3 h under 90 ºC. After cooled to room temperature, the hydrolytical solution was extracted with chloroform (3 × 6.0 mL). The water layer was concentrated to 0.5 mL. The completely concentrated water layer and reference substances of β-D-glucose, α-L-arabinose and β-D-xylose and α-L-rhamnose were derived by reacting with 1.5 mg hydroxylamine hydrochloride and 0.5 mL pyridine for 1 hourat 90 ºC. After cooling, 0.9 mL Ac_2O was added and the

mixture was heated at 90 ºC for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS. The samples (10 μL) were injected into a HP-1 30 m × 0.2 mm chromatographic column (Agilent, Santa Clara, CA, USA). The t_R values of β-D-glucose, β-D-xylose, α-L-arabinose and and α-L-rhamnose derivatives were 11.04, 9.08, 8.98 and 8.86 min, respectively.

Cytotoxic Activity. Cell viability was measured using the MTT assay. The HepG2 cells were cultured in DMEM medium containing heat inactivated 10% (v/v) fetal bovine serum (FBS) and 100 U/mL penicillin, 100 μg/mL streptomycin. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C and pass-aged every 3 days by trypsinization (0.25%). For experiments, HepG2 cells were in-cubated in complete medium with 10% fetal bovine serum in 96-well plates (1000 cells/well). Cells were treated with or without different concentrations of saponins (dissolved in DMSO) when the cells reached 70-80% confluence. Test compounds and blank group had four repetitions. After 24 h, the medium was removed and the serum free DMEM containing 10% MTT (5 mg/mL) were added to each wells. 4 hours later, the culture medium containing MTT was removed and 100 μL DMSO was add to each well to dissolve the formazan and placed on a shaker for 10s. The absorbance values was measured at a wavelength of 492 nm using a microplate reader, and the absorbance values were expressed as a percentage of un-treated control cells (control = 100%).

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Supporting Information. NMR spectral data of compounds **1** and **2** are available as Supporting Information.

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