Natural Product Sciences
21(3) : 196-204 (2015)

Chemical Constituents from Leaves of *Acanthopanax henryi* (II)

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Abstract – Nineteen compounds, including one organic acid (**1**), one anthraquinone (**2**), one amide (**3**), and sixteen triterpenoid saponins (**4 - 19**) were isolated from the leaves of *Acanthopanax henryi* (Oliv.) Harms (Araliaceae). Their structures were determined on the basis of physicochemical properties and spectral analyses (HR-MS and NMR). Among them, compounds **2**, **3**, **7**, **12** and **19** were new within Araliaceae. Compounds **4**, **5**, **9 - 11**, **13**, **14**, **16** and **18** were reported for the first time from the *Acanthopanax* genus. Except for compounds **4** and **9**, other compounds were isolated from *A. henryi* (Oliv.) Harms for the first time. The rare anthraquinone, compound **2**, significantly decreased the production of NO and the levels of other inflammatory factors, such as TNF-α and IL-6, in lipopolysaccharide (LPS)-stimulated macrophages in a dose-dependent manner. This is the first time to report anti-inflammatory effect of this compound.
Keywords – Araliaceae, *Acanthopanax henryi*, Triterpenoid saponins, Anthraquinone, Nitric oxide, Anti-inflammatory

Introduction

Acanthopanax henryi (Oliv.) Harms (AH) belongs to the family of Araliaceae, which is a deciduous scrubs or tree that grows in forest margins or shrubbery up to an altitude of 1000 - 3200 m. As a Chinese endemic plant, it is distributed mainly in provinces including Hunan, Sichuan, Anhui, and Zhejiang.[1] *Acanthopanax spp*. plays an important role in traditional medicine in countries like China, Korea and Japan. Its dried roots and stem barks are famous traditional medicine, used for the treatment of rheumatism, arthritis, paralysis, sinew and bone pain. Leaves of *Acanthopanax spp*. are generally consumed as tea or a versatile vegetable soup material in folk medicine.[2] In Hunan Province, the root bark of AH as "Wu jia pi" was included at Hunan local medicinal materials standard, and has been used as a traditional oriental medicine for treatment of paralysis, arthritis, rheumatism, lameness, edema, injure from fall, hernia, abdominal pain, and so on.[3] In the previous study, lignans, diterpenes, flavonoids, caffeoyl quinic acid derivates and other compounds were isolated from the bark or leaves of AH. The cytotoxicity, acetyl cholinesterase inhibitory and antioxidant effects of their extracts from the root barks of AH were reported.[4]

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In the present study, we report isolation and identification of nineteen compounds (**1 - 19**) from the leaves of AH including one organic acid (**1**), one anthraquinone (**2**), one amide (**3**), and sixteen triterpenoid saponins (**4 - 19**). Their structures were determined by physicochemical properties and spectroscopic methods. Moreover, this paper first focused on anti-inflammatory activity on LPS-induced production of NO and the inhibition effect of production of TNF-α and IL-6 in RAW 264.7 macrophages and cytotoxic activity of the rare anthraquinone (**2**).

Experimental

General experimental procedures – Melting points (uncorrected) were measured using a Boetius micromelting point apparatus. ^{1}H-NMR (600 MHz), ^{13}C-NMR (150 MHz) and 2D-NMR were recorded at room temperature in CD_3OD or pyridine-d_5 using Bruker ACF-500 NMR spectrometer and chemical shifts were given in δ (ppm) value relative to TMS as the internal standard. Mass spectra were obtained on MS Agilent 1200 Series LC/MSD Trap Mass spectrometer (ESI-MS). Column chromatography was carried out on silica gel (200 - 300 mesh and 100 - 200 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (Merck) and D101 macroporous resin (Tianjin Guangfu Chemical Co., Ltd., Tianjin, China). RP-TLC was performed on a precoated

RP-18F_{254s} (Merck) plates. TLC was conducted on self-made silica gel G (Qingdao Marine Chemical Industry, Qingdao, China) plates and spots were visualized by spraying with 10% H_2SO_4 in ethanol (v/v) followed by heating at 105 °C. The murine macrophage cell line RAW 264.7 was obtained from American Type Culture Collection (ATCC, USA). Lipopolysaccharide (LPS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and Griess reagent, were purchased from Sigma-Aldrich (USA). Dulbecco's Modified Eagle Medium (DMEM) was purchased from Gibco (USA).

Plant materials – The leaves of AH were collected in Xinhua, Hunan Province of China, in October 2012 and were botanically identified by one of the coauthors (X.Q.L.). The voucher specimen (NO.20121125) has been deposited in school of pharmacy, Hunan University of Chinese Medicine.

Extraction and isolation – The air-dried leaves of AH (10 kg) were cut into small pieces and extracted with MeOH for three times (3 × 100 L, 7 days each time) under soak at room temperature, and concentrated to give a dark-green residue (0.8 kg) under reduced vacc., which was suspended in H_2O and partitioned with petroleum ether. The water portion was fractionated by column chromatography (CC) on macroporous resin (D101) with a gradient EtOH/H_2O (0, 30%, 50%, 75%, and 95%) into five fractions (1 - 5). Fraction 1 (100 mg) was subjected to Sephadex LH-20 CC eluted with H_2O to give **1** (30.0 mg). Fraction 2 (14.0 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (25:1:0/1:1:0.2) to give fifteen subfractions (A-O). The subfraction B (260.0 mg) yielded small crystals, which were collected by removal of the supernatant liquid and recrystallized from $CHCl_3$ yielding **2** (7.0 mg). Subfraction C (119.0 mg) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (15:1:0/6:1:0.1) and purified on reversed phase gel (MeOH/H_2O, 10:90/100:0) to give **4** (12.0 mg). Subfraction D (0.8 g) was re-fractionated on Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to yield **5** (45.0 mg). Subfraction E (113.8 mg) was further purified by silica gel CC using $CHCl_3$/MeOH/H_2O (10:1:0/5:1:0.1) to give **6** (20.0 mg), **7** (12.0 mg) and **3** (8.0 mg). Subfraction F (115.0 mg) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (10:1:0.1/5:1:0.1) to yield **8** (20.0 mg). Subfraction G (1.2 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (10:1:0.1/6:1:0.1) and recrystallizations with MeOH to give **9** (600.0 mg) and **10** (200.0 mg). Subfraction H (613.0 mg) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (7:1:0.1/4:1:0.1) and followed by Sephadex LH-20 (MeOH) to give **11** (35.0 mg). Subfraction I (410.0 mg) yielded needle crystals, which were collected by recrystallized from MeOH yielding **12** (30.0 mg). Subfraction J (0.58 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (7:1:0.1/3:1:0.1) and purified by Pre-HPLC (Acetonitrile/H_2O, 6:4) yield **13** (22.0 mg). Subfraction K (0.95 g) was refractionated on silica gel CC eluted with $CHCl_3$/MeOH/H_2O (6:1:0.1/2:1:0.1) to produce six sub-fractions (K1-K6). K2 and K3 (106.0 mg) were subjected to silica gel CC and then purified by Sephadex LH-20 (MeOH) to yield **14** (35.0 mg), **15** (35.0 mg) and **16** (30.0 mg). Subfraction L (1.1 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (4:1:0.1/1:1:0.2) to give four sub-fractions (L1-L4), and L2-L4 were further separated by Sephadex LH-20 (MeOH) to afford **17** (15.0 mg), **18** (51.0 mg) and **19** (60.0 mg).

MTT assay for cell viability – RAW 264.7 cells were cultured in 96-well plates for 4 h, followed by treatment with compound **2** at different concentrations (2.5 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and 40 μg/mL). After 24 h incubation, MTT was added to the medium for 4 h. Finally, the supernatant was removed and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm.

Nitrite assay – The RAW 264.7 cells were dispensed to a 96 well plate and cultured for 4 h. Then compound **2** at different concentrations (2.5 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and 40 μg/mL) were adhered and incubated for 1 h. After pre-incubation of RAW 264.7 cells (5×10^4 cells/mL) with LPS (0.5 μg/mL) for 24 h, the quantity of nitrite in the culture medium was measured as an indicator of NO production. Amounts of nitrite, a stable metabolite of NO, were measured using Griess reagent. Briefly, 100 μL of cell culture medium were mixed with 100 μL of Griess reagent. Subsequently, the mixture was incubated at room temperature for 10 min and the absorbance at 540 nm was measured in a microplate reader. The quantity of nitrite was determined from a sodium nitrite standard curve.

TNF-α and IL-6 assay – RAW 264.7 cells were pretreated with sample (2.5 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and 40 μg/mL) for 30 min, and then were incubated with or without LPS (0.5 μg/mL) for 24 h. The production of TNF-α and IL-6 was measured using ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Results from three independent experiments were used for statistical analyses.

Statistical analysis – All values are expressed as the mean ± S.D. Differences between mean values of normally distributed data were assessed with one-way ANOVA

(Newman Keuls t-test). Statistical significance was accepted at $P < 0.05$.

Fumaric acid (1) – Colorless needles; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 13.11 (1H, brs, -COOH), 6.50 (1H, s, H-2); ^{13}C-NMR (150 MHz, pyridine-d_5): δ 165.93 (C-1), 133.95 (C-2).

Glyceroyl-1,6,8-trihydroxy-3-methyl-9,10-dioxo-2-anthracene carboxylate (2) – Orange red needles; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.58 (1H, s, H-4), 7.12 (1H, d, J = 2.3 Hz, H-5), 6.58 (1H, d, J = 2.3 Hz, H-7), 4.22 (1H, dd, J = 6.2 Hz, J = 11.2 Hz, H-1'), 4.38 (1H, dd, J = 6.2 Hz, J = 11.2 Hz, H-1'), 3.76 (1H, m, H-2'), 3.66 (1H, dd, J = 5.7 Hz, J = 5.6 Hz, H-3'), 3.62 (1H, dd, J = 5.3 Hz, J = 6.0 Hz, H-3'), 12.74 (1H, s, 1-OH), 11.99 (1H, s, 6-OH), 12.45 (1H, brs, 8-OH), 5.03 (1H, brs, 2'-OH), 4.71 (1H, brs, 3'-OH), 2.39 (1H, s, 3-Me); ^{13}C-NMR (150 MHz, CD_3OD): δ 160.2 (C-1), 130.2 (C-2), 146.3 (C-3), 121.9 (C-4), 134.9 (C-4a), 110.6 (C-5), 166.8 (C-6), 109.2 (C-7), 166.8 (C-8), 110.4 (C-8a), 191.5 (C-9), 115.4 (C-9), 182.6 (C-10), 136.7 (C-10a), 67.7 (C-1'), 71.2 (C-2'), 64.2 (C-14), 20.3 (3-Me), 167.7 (2-COO). HR-ESI-MS *m/z*: 411.0783 $[M+Na]^+$.

1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,7*Z*)-2-(2'-hydrooxypalmitoylamino)-8-octadecene-1,3,4-triol (3) – White needles; ^{1}H-NMR (pyridine-d_5, 400 MHz): δ 0.87 (6H, m, J = 6.8 Hz, 2×Me), 1.26 [s, $(CH_2)_n$], 3.88 (1H, brs, H-5''), 4.02 (1H, t, J = 8.1 Hz, H-2''), 4.21 (3H, q, H-3, 4, 3''), 4.30 (1H, H-4''), 4.37 (1H, m, H-6''), 4.49 (1H, m, H-6''), 4.53 (1H, q, H-1), 4.59 (1H, q, H-2'), 4.72 (1H, q, H-1), 4.97 (1H, d, J = 7.7 Hz, H-1''), 5.31(1H, m, H-2), 5.47 (1H, dt, J = 5.7, 14.6 Hz, H-8), 5.52 (1H, dt, J = 5.6, 14.6 Hz, H-9), 8.58 (1H, d, J = 9.2 Hz, N-H); ^{13}C-NMR (pyridine-d_5, 100 MHz): δ 14.8 (Me), 23.4, 26.3, 27.1, 30.0, 30.1, 30.1, 30.3, 30.4, 32.6 (C-7), 33.5 (C-10), 33.8, 34.4, 36.1 (all CH_2), 52.2 (C-2), 63.1 (glc-C-6''), 71.0 (C-1), 71.9 (glc-C-4''), 72.9 (C-4, 2'), 75.3 (glc-C-2''), 76.4 (C-3), 78.9 (glc-C-3''), 79.0 (glc-C-5''), 106.1 (glc-C-1''), 131.2 (C-9), 131.3 (C-8), 176.1 (C-1'). HR-ESI-MS *m/z*: 755.0427 $[M+Na]^+$.

Ursolic acid 3-*O*-α-L-arabinopyranoside (4) – White needles; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.87, 0.99, 0.97, 1.06, 1.27, 1.31, 1.32 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.39 (1H, dd, J = 4.32, J = 11.64, H-3), 5.50 (1H, brs, H-12), 4.81 (1H, d, J = 6.96, ara-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 611.3911 $[M+Na]^+$.

Eechinocystic acid 3-*O*-α-L-arabinopyranoside (5) – White needles; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.79, 0.84, 0.87, 0.96, 0.97, 1.04, 1.37 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.13 (1H, dd, J = 2.92, J = 7.80, H-3), 5.29 (1H, brs, H-12), 4.27 (1H, d, J = 6.7 Hz, ara-H-1), 4.47 (1H, brs, H-16); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 627.3846 $[M+Na]^+$.

Eleutheroside K (6) – White amorphous powder; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.88, 0.98, 1.01, 1.04, 1.08, 1.28, 1.32 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.30 (1H, dd, J = 4.2, J = 11.6, H-3), 1.66 (3H, d, J = 6.2, rha-H-6), 5.49 (1H, brs, H-12), 4.94 (1H, d, J = 5.28, ara-H-1), 6.14 (1H, brs, rha-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 757.4474 $[M+Na]^+$.

Prosapogenin CP_{2b} (7) – White amorphous powder; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.87, 1.02, 1.03, 1.05, 1.09, 1.18, 1.27 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.26 (1H, dd, J = 4.2, J = 11.6, H-3), 5.49 (1H, brs, H-12), 4.96 (1H, d, J = 5.96, ara-H-1), 5.04 (1H, d, J = 6.68, xyl-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5) : see Table 1. HR-ESI-MS *m/z*: 743.4303 $[M+Na]^+$.

Tauroside D (8) – White amorphous powder; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.91, 0.99, 1.06, 1.09, 1.21, 1.32, 1.88 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 1.65 (3H, d, J = 6.2, rha-H-6), 3.29 (1H, dd, J = 4.16, J = 11.7, H-3), 5.28 (1H, brs, H-16α), 5.66 (1H, brs, H-12), 4.94 (1H, d, J = 5.32, ara-H-1), 6.14 (1H, brs, rha-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 773.4401 $[M+Na]^+$.

Guaianin N (9) – White amorphous powder; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.85, 0.98, 0.99, 1.01, 1.03, 1.33, 1.34 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.39 (1H, m, H-3), 5.49 (1H, brs, H-12), 4.78 (1H, d, J = 7.4, ara-H-1), 5.43 (1H, d, J = 7.8, glu-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 773.4428 $[M+Na]^+$.

Matesaponin J_2 (10) – White amorphous powder; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.85, 0.98, 0.99, 1.01, 1.04, 1.32, 1.33 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.36 (1H, m, H-3), 5.49 (1H, brs, H-12), 4.78 (1H, d, J = 7.5, ara-H-1), 5.43 (1H, d, J = 7.8, glu-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 773.4428 $[M+Na]^+$.

Echinocystic acid 3-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-arabinopyranoside (11) – White amorphous powder; ^{1}H-NMR (600 MHz, pyridine-d_5): δ 0.91, 1.00, 1.06, 1.09, 1.21, 1.32, 1.88 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.41 (1H, dd, J = 3.8, J = 7.5, H-3), 5.67 (1H, brs, H-12), 5.28 (1H, brs, H-16α), 4.76 (1H, d, J = 7.3, ara-H-1), 5.40 (1H, d, J = 7.8, glu-H-1); ^{13}C-NMR (150 MHz, pyridine-d_5): see Table 1. HR-ESI-MS *m/z*: 789.4369 $[M+Na]^+$.

Table 1. ^{13}C-NMR data (150 MHz, pyridine-d_5) of compounds **4 - 19**

aglycone	4	5	6	7	8	9	10	11
1	39.4	39.9	39.3	39.4	39.4	39.3	39.4	39.4
2	27.2	27.2	27.0	27.0	27.0	27.2	27.2	27.2
3	89.2	90.3	89.3	89.2	89.3	89.2	89.2	89.2
4	40.0	40.3	40.0	40.0	40.0	40.1	40.2	40.1
5	56.4	57.3	56.4	56.4	56.5	56.4	56.4	56.5
6	18.9	19.5	19.0	19.0	19.1	19.0	19.0	19.0
7	34.0	34.4	33.7	33.7	34.0	33.3	34.7	34.0
8	40.4	40.8	40.2	40.2	40.4	40.2	40.5	40.2
9	48.4	48.3	48.5	48.5	47.7	48.5	48.5	47.7
10	37.4	38.0	37.5	37.5	37.6	37.5	37.5	37.6
11	24.1	24.6	24.2	24.2	24.3	24.3	24.1	24.3
12	126.1	123.6	122.9	123.0	122.9	123.0	126.2	122.9
13	139.1	145.2	145.4	145.4	145.6	145.2	139.7	145.6
14	43.0	42.8	42.7	42.6	42.6	42.7	43.0	42.6
15	29.2	36.7	28.8	28.8	36.6	28.4	29.2	36.6
16	25.4	75.4	24.3	24.2	75.2	24.2	24.2	75.3
17	48.5	48.3	47.2	47.2	49.4	47.2	47.2	49.4
18	54.0	42.2	42.5	42.5	41.9	42.5	42.5	41.9
19	40.0	47.9	47.0	47.0	47.8	47.0	40.3	47.8
20	39.8	31.6	31.5	31.4	31.5	31.5	40.0	31.5
21	31.6	32.9	34.8	34.7	33.4	34.7	31.5	36.7
22	37.9	36.4	33.6	33.6	36.7	34.0	38.0	33.3
23	28.7	28.7	28.6	28.6	28.5	28.6	28.8	28.6
24	16.1	17.1	17.5	17.0	17.5	17.5	17.5	17.5
25	17.4	16.2	16.0	16.0	16.1	16.0	16.1	16.1
26	17.9	17.9	17.9	17.5	17.9	17.9	17.9	18.0
27	24.4	27.4	26.7	26.6	27.7	26.7	24.4	27.7
28	180.3	181.3	180.2	180.4	180.5	180.4	180.7	180.5
29	18.0	33.6	33.8	33.7	33.8	33.8	18.0	33.8
30	21.9	25.1	24.3	24.3	25.2	24.3	21.9	25.2
	C-3-ara	**C-3-ara**	**C-3-ara**	**C-3-ara**	**C-3-ara**	**C-3-ara**	**C-3-ara**	**C-3-ara**
1	108.0	107.3	105.3	105.8	105.3	107.9	107.9	107.9
2	73.4	73.0	76.4	81.2	76.4	72.1	72.1	72.4
3	75.1	74.5	74.3	74.3	74.3	86.6	86.6	84.6
4	70.0	69.7	69.1	69.1	69.2	69.8	69.8	69.8
5	67.2	66.5	65.2	65.2	65.2	67.5	67.5	67.5
			rha(1 → 2)ara	**xyl(1 → 2)ara**	**rha(1 → 2)ara**	**glc(1 → 3)ara**	**glc(1 → 3)ara**	**glc(1 → 3)ara**
1			102.2	106.7	102.4	106.9	106.9	106.9
2			73.1	74.6	73.1	76.2	76.2	76.2
3			72.9	74.9	72.9	78.9	78.9	78.9
4			74.6	69.7	74.6	72.1	72.1	72.1
5			70.4	67.5	70.4	79.2	79.2	79.1
6			19.1		19.1	63.2	63.2	63.2

Table 1. ^{13}C-NMR data (150 MHz, pyridine-d_5) of compounds **4 - 19** (continued)

aglycone	12	13	14	15	16	17	18	19
1	39.5	38.5	39.3	39.5	39.3	39.1	39.3	39.3
2	28.6	27.6	27.1	27.2	27.2	26.9	27.2	27.2
3	79.3	78.6	89.4	89.2	89.2	89.5	89.2	89.2
4	39.9	39.6	40.2	40.4	40.0	40.0	40.1	40.1
5	56.3	55.4	56.4	56.4	56.4	56.3	56.4	56.4
6	19.3	18.1	19.0	19.0	19.1	19.0	19.1	18.2
7	33.0	33.0	33.7	33.0	34.5	33.7	33.0	33.0
8	40.4	41.5	40.4	40.6	40.6	40.2	40.4	40.4
9	48.7	47.3	48.4	48.6	48.6	48.5	48.6	48.6
10	37.9	36.7	37.5	37.5	37.5	37.5	37.5	37.5
11	23.9	23.2	24.2	24.3	24.3	24.2	23.9	23.8
12	123.0	122.5	123.1	123.4	126.6	123.1	123.4	123.4
13	144.9	143.5	145.3	144.6	138.9	145.3	144.6	144.6
14	42.6	41.9	42.6	42.6	43.0	42.8	42.6	42.6
15	28.8	29.2	28.8	29.2	29.2	28.8	28.8	28.8
16	24.3	22.6	24.3	25.1	25.1	24.3	24.3	24.3
17	47.6	48.2	47.2	48.9	48.9	47.2	47.5	47.5
18	42.2	41.2	42.5	42.2	42.2	42.5	42.2	42.2
19	46.8	45.8	47.0	46.7	39.8	47.0	46.7	46.7
20	31.2	31.6	31.4	31.2	39.6	30.5	31.2	31.2
21	34.1	33.5	34.7	34.0	31.3	34.7	34.5	34.5
22	33.7	36.2	34.0	37.4	37.3	33.7	33.0	33.0
23	29.3	27.5	28.6	28.8	28.7	28.7	28.6	28.6
24	17.0	16.5	17.3	17.4	17.4	17.5	18.0	18.0
25	16.2	15.1	16.0	16.1	16.3	16.0	16.1	16.1
26	18.1	20.2	17.3	17.9	18.0	17.9	17.5	17.5
27	26.6	27.4	26.6	26.5	24.2	26.7	26.6	26.6
28	177.0	177.2	180.6	177.0	176.7	180.7	177.0	177.0
29	33.6	32.1	33.8	33.6	18.2	33.8	33.6	33.6
30	24.6	24.9	24.3	24.2	21.8	24.3	24.2	24.2
	C-28-glc	**C-28-glc**	**C-3-ara**	**C-3-ara**	**C-3-ara**	**C-3-gluA**	**C-3-ara**	**C-3-ara**
1	96.2	94.3	105.8	107.9	108.0	107.4	107.9	107.9
2	74.4	75.4	78.2	73.4	73.4	75.9	72.4	72.4
3	79.0	78.5	83.5	75.1	75.1	78.7	86.6	84.6
4	71.4	69.5	69.0	70.0	70.0	74.1	69.8	69.8
5	78.5	76.7	66.0	67.2	67.2	77.6	67.5	67.5
6	69.9	68.0				172.9		
	glc(1 → 6)glc	**glc(1 → 6)glc**	**gla(1 → 2)ara**	**C-28-glc**	**C-28-glc**		**glc(1 → 3)ara**	**glc(1 → 3)ara**
1	105.8	102.8	105.7	96.1	96.1		106.9	106.9
2	75.7	73.9	74.0	74.4	74.4		76.2	76.2
3	78.9	75.3	75.8	79.2	79.2		78.9	78.9
4	72.0	78.1	70.2	71.4	71.4		72.1	72.1
5	78.6	76.6	76.8	78.5	78.5		79.2	79.2
6	63.1	60.5	61.9	69.7	69.9		63.2	63.2

Table 1. ^{13}C-NMR data (150 MHz, pyridine-d_5) of compounds **4 - 19** (continued)

	rha(1 → 4)glc	glc(1 → 3)ara	glc(1 → 6)glc	glc(1 → 6)glc	C-28-glc	C-28-glc
1	101.5	105.4	105.4	105.5	96.2	96.1
2	70.9	75.9	75.8	75.8	74.4	74.2
3	72.4	78.8	77.7	77.7	78.9	78.8
4	72.4	72.0	78.8	78.8	71.4	71.4
5	69.3	79.0	77.0	77.0	78.5	78.5
6	16.1	63.1	61.8	61.8	69.9	69.9
			rha(1 → 4)glc	rha(1 → 4)glc	glc'(1 → 6)glc	glc'(1 → 6)glc
1			103.2	103.2	105.8	105.8
2			73.1	73.1	75.6	75.8
3			73.3	73.3	78.9	77.7
4			74.5	74.3	72.0	79.2
5			70.8	70.8	79.2	77.0
6			19.0	19.0	63.1	61.8
						rha(1 → 4)glc'
1						103.3
2						73.1
3						73.3
4						74.5
5						70.8
6						19.0

Hemslonin A (12) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.89, 0.90, 0.95, 1.05, 1.15, 1.21, 1.24 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.46 (1H, dd, J = 4.9, J = 9.6, H-3), 5.81 (1H, brs, H-12), 5.46 (1H, brs, glu1-H-1), 6.29 (1H, d, J = 8.0, glu2-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 803.4530 $[M+Na]^+$.

Cussonoside B (13) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.78, 0.92, 0.95, 0.95, 0.98, 1.16, 1.24 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.15 (1H, J = 4.1, J = 10.9, H-3), 5.28 (1H, brs, H-12), 1.28 (3H, d, J = 6.2, rha-H-6), 4.87 (1H, brs, rha-H-1), 4.41 (1H, d, J = 7.9, glu1-H-1), 5.36 (1H, d, J = 8.2, glu2-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 803.4530 $[M+Na]^+$.

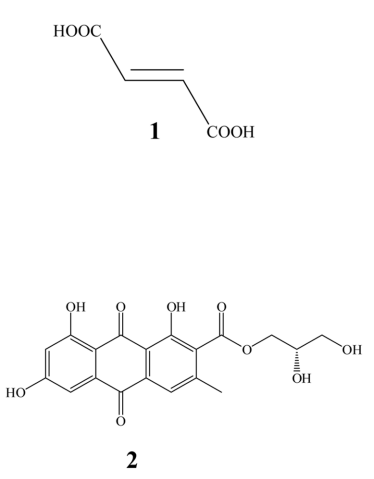
Oleanolic acid 3-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranosyl-(1→2)-*O*-α-L-arabinopyranoside (14) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.85, 0.97, 1.00, 1.03, 1.13, 1.30, 1.34 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.30 (1H, m, H-3), 5.28 (1H, brs, H-12), 4.85 (1H, brs, ara-H-1), 5.28 (1H, d, J = 7.7, glu1-H-1), 5.40 (1H, d, J = 7.8, gla-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 935.4941 $[M+Na]^+$.

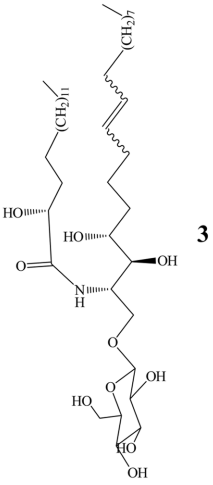
Ciwujianoside C_3 (15) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.92, 0.92, 0.96, 0.99, 1.16, 1.27, 1.30 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.37 (1H, dd, J = 4.8, J = 11.4, H-3), 5.44 (1H, brs, H-12), 1.72 (3H, d, J = 6.0, rha-H-6), 4.79 (1H, d, J = 4.5, ara-H-1), 5.88 (1H, s, rha-H-1), 4.98 (1H, d, J = 6.6, glu'-H-1), 6.26 (1H, d, J = 8.0, glu"-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 1081.5537 $[M+Na]^+$.

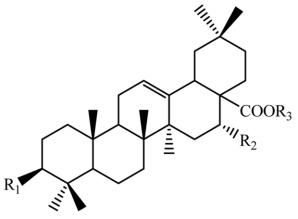
Ursolic acid 3-*O*-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1 → 4)- *O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside (16) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.91, 0.92, 0.96, 0.99, 1.12, 1.27, 1.29 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.37 (1H, m, H-3), 5.45 (1H, brs, H-12), 1.73 (3H, d, J = 6.1, rha-H-6), 4.80 (1H, s, ara-H-1), 5.88 (1H, s, rha-H-1), 4.99 (1H, d, J = 6.7, glc'-H-1), 6.28 (1H, d, J = 7.9, glc"-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 1081.5532 $[M+Na]^+$.

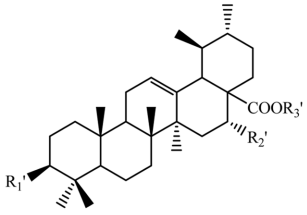
Momordin Ib (17) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5) δ 0.82, 0.99, 0.99, 1.04, 1.22, 1.32, 1.34 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.31 (1H, dd, J = 4.8, J = 13.4, H-3), 5.49 (1H, brs, H-12), 5.94 (1H, s, glcUA-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 655.8215 $[M+Na]^+$.

Araliasaponin II (18) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.90, 0.90, 0.91, 1.00,









Comp.	R_1	R_2	R_3
5	Ara-	OH	H
6	Rha-^{2}-Ara-	H	H
7	Xyl-^{2}-Ara-	H	H
8	Rha-^{2}-Ara-	OH	H
9	Glc-^{3}-Ara-	H	H
11	Glc-^{3}-Ara-	OH	H
12	OH	H	Glc-^{6}-Glc-
13	OH	H	Rha-^{4}-Glc-^{6}-Glc-
14	Glc-^{3}-Ara- |^{2} Gal	H	H
15	Ara-	H	Rha-^{4}-Glc-^{6}-Glc-
17	GluA-	H	H
18	Glc-^{3}-Ara-	H	Glc-^{6}-Glc-
19	Glc-^{3}-Ara-	H	Rha-^{4}-Glc-^{6}-Glc-

Comp.	R_1'	R_2'	R_3'
4	Ara-	H	H
10	Glc-^{3}-Ara-	H	H
16	Ara-	H	Rha-^{4}-Glc-^{6}-Glc-

Fig. 1. Chemical structures of the isolated compounds **1 - 19**.

1.12, 1.27, 1.36 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.37 (1H, dd, J = 4.2, J = 11.5, H-3), 5.42 (1H, brs, H-12), 4.77 (1H, d, J = 7.3, ara-H-1), 5.04 (1H, d, J = 7.7, glc-H-1), 5.44 (1H, d, J = 7.2, glc'-H-1), 6.27 (1H, d, J = 8.0, glc"-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 1097.5466 $[M+Na]^+$.

Begoniifolide A (19) – White amorphous powder; ^{1}H-NMR (600 MHz, pyrindine-d_5): δ 0.90, 0.91, 0.91, 1.00, 1.11, 1.27, 1.36 (each 3H, s, H-23, 24, 25, 26, 27, 29 and 30), 3.37 (1H, dd, J = 4.2, J = 11.2, H-3), 5.42 (1H, brs, H-12), 1.72 (3H, d, J = 6.0, rha-H-6), 4.77 (1H, d, J = 7.2, ara-H-1), 5.86 (1H, s, rha-H-1), 5.01 (1H, d, J = 8.1, glc1-H-1), 6.20 (1H, d, J = 7.9, glc'-H-1), 6.25 (1H, d, J = 8.0, glc"-H-1); ^{13}C-NMR (150 MHz, pyrindine-d_5): see Table 1. HR-ESI-MS *m/z*: 1097.5466 $[M+Na]^+$.

Results and Discussion

Compounds (**1 - 19**) (Fig. 1) were determined as fumaric acid (**1**), glyceroyl-1,6,8-trihydroxy-3-methyl-9,10-dioxo-2-anthracene carboxylate (**2**), 1-*O*-*β*-D-glucopyranosyl-(2*S*, 3*S*, 4*R*, 7*Z*)-2-(2'-hydrooxypalmitoylamino)-8-octadecene-1,3,4-triol (**3**), ursolic acid 3-*O*-*α*-L-arabinopyranoside (**4**), echinocystic acid 3-*O*-*α*-L-arabinopyranoside (**5**), eleutheroside K (**6**), prosapogenin CP_{2b} (**7**), tauroside D (**8**), guaianin N (**9**), matesaponin J_2 (**10**), echinocystic acid 3-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*α*-L-arabinopyranoside (**11**), hemslonin A (**12**), cussonoside B

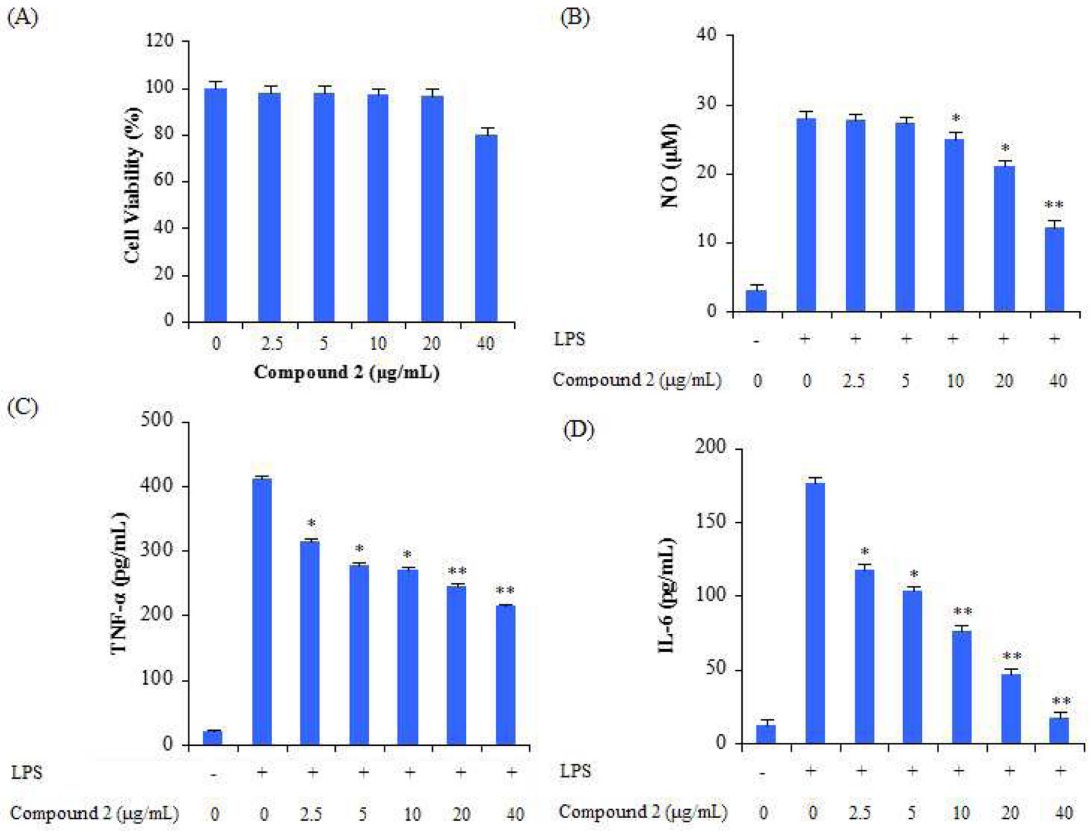


Fig. 2. Effect of compound **2** on LPS-induced NO, TNF-α and IL-6 levels in RAW 264.7 macrophages. **P* value of < 0.05, ***P* value of < 0.01. RAW 264.7 cells were treated with 40 μg/mL of compound **2** and LPS (0.5 μg/mL) for 24 h. (A) Cell viability was assessed by using MTT assay. (B) NO production was determined in the culture supernatant by using the Griess reagent. The concentrations of (C) TNF-α and (D) IL-6 were determined by using the ELISA kit. Results are expressed as the mean ± S.D. of three independent experiments. **P* value of < 0.05, ***P* value of < 0.01.

(**13**), oleanolic acid 3-*O*-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-galactopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside (**14**), ciwujianoside C_3 (**15**), ursolic acid 3-*O*-*α*-L-arabinopyranosyl-28-*O*-*α*-L-rhamnopyranosyl-(1 → 4)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside (**16**), momordin Ib (**17**), araliasaponin II (**18**), and begoniifolide A (**19**) by comparing the 1D, 2D-NMR and mass spectral data with the literature values.[7-21] To the best of our knowledge, compounds **2**, **3**, **7**, **12** and **19** were new within the family of Araliaceae. Compounds **4**, **5**, **9-11**, **13**, **14**, **16** and **18** were reported for the first time from the *Acanthopanax* genus. Except for compounds **4** and **9**, other compounds were isolated from *A. henryi* (Oliv.) Harms for the first time.

The rare anthraquinone, compound **2**, isolated from AH was tested for cytotoxicity and anti-inflammatory activity on LPS-induced production of NO and the inhibition effect of production of TNF-α and IL-6 in RAW 264.7 macrophages. As shown in Fig. 2, there was slight effect of compound **2** on cell viability at the concentration lower than 40 μg/mL, however, the production of NO and the levels of other inflammatory factors, such as TNF-α and IL-6, decreased significantly in a dose-dependent manner in lipopolysaccharide (LPS)-stimulated macrophages. The result may support that compound **2** could be considered as a potential anti-inflammatory substance for further investigation and this is the first time to report anti-inflammatory effect of this unusual compound.

Acknowledgments

The authors are thankful to Hunan provincial Natural Science Foundation (11JJ2042), Science of Chinese materia medica of Twelfth Five-Year Key Discipline Projects of Hunan province, Changsha City Science and Technology Bureau Key Projects (K1207010-21), and Science of Pharmaceutical Analysis of Twelfth Five-Year Key Discipline Projects of Hunan University of Chinese Medicine for a grant.

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Received March 25, 2015
Revised May 27, 2015
Accepted May 30, 2015