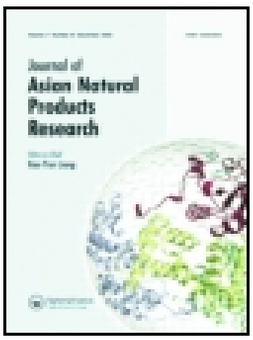


Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: http://www.tandfonline.com/loi/ganp20

# Platycosides P and Q, two new triterpene saponins from *Platycodon grandiflorum*

Lu Qiu, Yu Xiao, Yao-Qi Liu, Lian-xin Peng, Wan Liao & Qiang Fu

**To cite this article:** Lu Qiu, Yu Xiao, Yao-Qi Liu, Lian-xin Peng, Wan Liao & Qiang Fu (2018): Platycosides P and Q, two new triterpene saponins from *Platycodon grandiflorum*, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2018.1488835

**To link to this article:** https://doi.org/10.1080/10286020.2018.1488835

Published online: 02 Jul 2018.

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH, 2018
https://doi.org/10.1080/10286020.2018.1488835



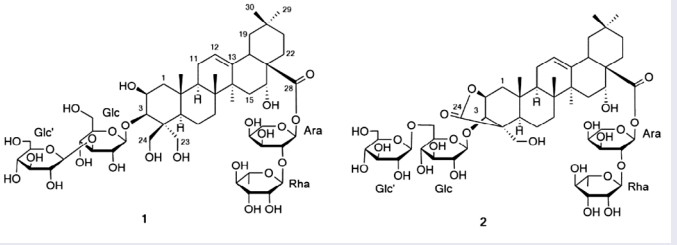
# Platycosides P and Q, two new triterpene saponins from *Platycodon grandiflorum*

Lu Qiu[a], Yu Xiao[a], Yao-Qi Liu[a], Lian-xin Peng[a,b], Wan Liao[c] and Qiang Fu[a,b]

[a]College of Pharmacy and Bioengineering, Chengdu University, Chengdu 610106, China; [b]Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture, Chengdu 610106, China; [c]State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources, College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

**ABSTRACT**

The EtOH extract of the roots of *Platycodon grandiflorum* afforded two new triterpene saponins platycoside P (**1**) and platycoside Q (**2**). Their structures were elucidated based on spectroscopic means and hydrolysis products. These compounds were evaluated for inhibitory activity against LPS-induced TNF-α production in RAW 246.7 macrophages. Compounds **1** and **2** showed inhibitory activity with the inhibition ratios (%) of 38.6 and 44.1 at 50 μM, respectively.




**ARTICLE HISTORY**
Received 5 April 2018
Accepted 10 June 2018

**KEYWORDS**
Campanulaceae; *Platycodon grandiflorum*; triterpene saponins; TNF-α production inhibitory activity

## 1. Introduction

*Platycodon grandiflorum* A. DC. (Campanulaceae) is distributed widely in northeast Asia. Its dried roots are a source of traditional Chinese medicine "Jiegeng," with claims being made concerning its anti-tumor, hepatoprotective, immunoregulatory, and anti-oxidant properties [1,2]. Chemical investigations on this plant afforded triterpene saponins, flavonoids, phenolic acids, polyacetylene, and sterols [2–5]. Several biological effects, such as anti-inflammation, anti-viral, and anti-tumor, may be attributed to the characteristic markers of triterpene saponins based upon platycodigenin [5–7].

In our previous work, a systematic investigation of bioactive components for the treatment of inflammation, particularly on the triterpene saponins from traditional Chinese

**CONTACT** Qiang Fu peter028@126.com

medicine were implemented [8–10]. As part of our systematic investigation, a research on the roots and rhizomes of *P. grandiflorum* led to the isolation of two new triterpene saponins (Figure 1). Although *P. grandiflorus* aqueous extracts showed a clear inhibitory effect on LPS-induced inflammation and a decrease in the expression of the inflammatory genes TNF-α, iNOS, and COX-2, no investigation on anti-inflammation bioactivity of triterpene saponin from *P. grandiflorus* was reported. Herein, we report the isolation, structure elucidation, and anti-inflammation bioactivity of two new triterpene saponins.

## 2. Results and discussion

The EtOH extract of roots of *P. grandiflorum* was suspended in water, and then extracted by *n*-butanol. The *n*-butanol extract was separated by repeated column chromatography using silica gel, Sephadex LH-20, and HPLC to give two new triterpene saponins **1** and **2** (Figure 1).

Compound **1** was obtained as white amorphous powder. The molecular formula of **1** was found to be $C_{53}H_{86}O_{25}$ by the negative HR-ESIMS ion at *m/z* 1121.5384 $[M-H]^-$. The

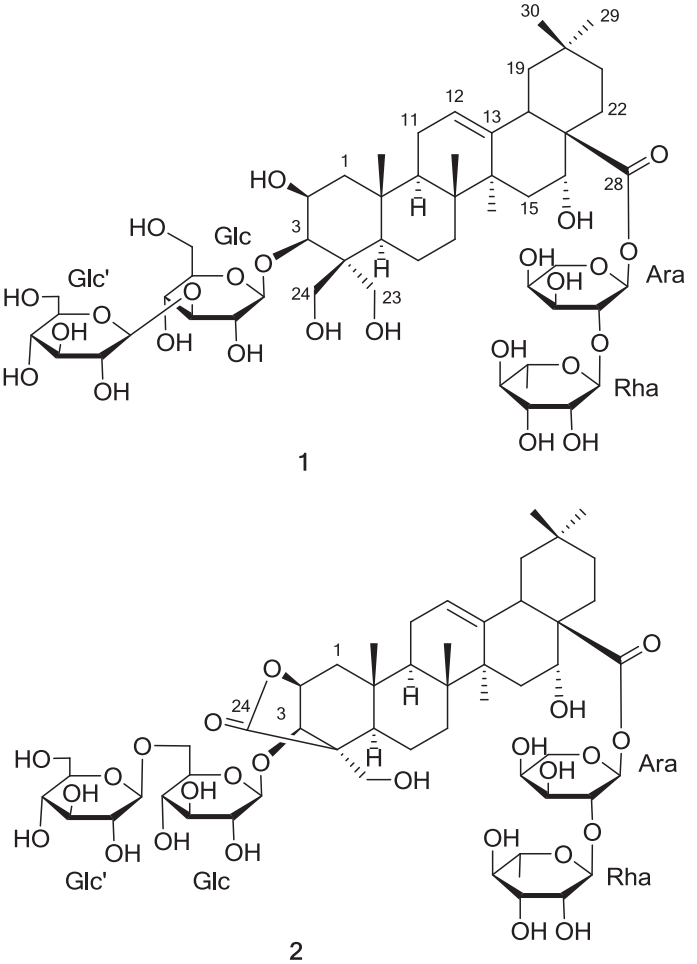



**Figure 1.** The structures of compounds **1** and **2**.

**Table 1.** $^{1}H$ and $^{13}C$ NMR spectral data for aglycone moieties of compounds **1** and **2** (500 MHz, in $C_5D_5N$).

| | **1** | | **2** | |
|---|---|---|---|---|
| No. | $\delta_H$ | $\delta_c$ | $\delta_H$ | $\delta_c$ |
| 1 | 1.52–1.54 m | 45.1 | 1.63–1.66 m | 41.3 |
| | 1.90–1.93 m | | 2.01–2.05 m | |
| 2 | 4.03–4.06 m | 69.7 | 5.41 d (5.0) | 83.3 |
| 3 | 4.67 s | 86.6 | 4.71 s | 89.4 |
| 4 | | 48.0 | | 54.1 |
| 5 | 1.83 dd (6.5, 3.0) | 46.8 | 2.15–2.19 m | 51.7 |
| 6 | 1.52–1.55 m | 19.2 | 1.27–1.30 m | 19.4 |
| | 1.81–1.85 m | | 2.13–2.16 m | |
| 7 | 1.42–1.46 m | 33.6 | 1.41–1.45 m | 33.6 |
| | 1.62–1.64 m | | 1.73–1.75 m | |
| 8 | | 40.6 | | 40.6 |
| 9 | 1.86 t (9.0) | 47.5 | 2.02 t (9.0) | 48.2 |
| 10 | | 37.5 | | 37.7 |
| 11 | 1.94–1.97 m | 23.8 | 1.92–1.96 m | 24.5 |
| | 2.03–2.07 m | | 2.04–2.07 m | |
| 12 | 5.62 t (3.0) | 123.2 | 5.51 t (3.0) | 122.5 |
| 13 | | 144.4 | | 145.1 |
| 14 | | 42.4 | | 42.4 |
| 15 | 1.72–1.76 m | 36.1 | 1.29–1.32 m | 36.1 |
| | 2.30–2.34 m | | 2.26–2.29 m | |
| 16 | 5.24–5.27 m | 73.9 | 5.17–5.20 m | 74.0 |
| 17 | | 49.7 | | 49.6 |
| 18 | 3.54 dd (3.5, 13.5) | 41.5 | 3.50 dd (3.5, 13.5) | 41.5 |
| 19 | 1.35–1.38 m | 47.4 | 1.33–1.36 m | 47.2 |
| | 2.76–2.78 m | | 2.76–2.81 m | |
| 20 | | 31.0 | | 31.1 |
| 21 | 1.27–1.32 m | 36.1 | 1.25–1.30 m | 36.1 |
| | 2.35–2.39 m | | 2.33–2.36 m | |
| 22 | 2.14–2.17 m | 32.0 | 2.15–2.18 m | 32.1 |
| | 2.25–2.30 m | | 2.26–2.29 m | |
| 23 | 4.02–4.06 m | 63.3 | 4.33–4.36 m | 57.3 |
| | 4.48–4.52 m | | 4.74–4.78 m | |
| 24 | 4.11–4.15 m | 63.5 | | 178.4 |
| | 4.59–4.61 m | | | |
| 25 | 1.42 s | 18.4 | 1.27 s | 17.7 |
| 26 | 1.07 s | 17.5 | 1.02 s | 18.2 |
| 27 | 1.71 s | 27.2 | 1.69 s | 27.5 |
| 28 | | 175.8 | | 176.1 |
| 29 | 0.96 s | 33.1 | 0.94 s | 33.2 |
| 30 | 1.11 s | 24.6 | 1.09 s | 24.8 |

spectral features and physicochemical properties revealed **1** to be a triterpenoid saponin. The $^{1}H$ NMR spectrum displayed the signals of five tertiary methyl groups at $\delta$ 0.96, 1.07, 1.11, 1.42, and 1.71 and one olefinic proton at $\delta$ 5.62 (*t*, $J$ = 3.0 Hz). The $^{13}C$ NMR spectrum showed the presence of five $sp^3$ carbons at $\delta$ 17.5, 18.4, 24.6, 27.2, and 33.1, and two $sp^2$ olefinic carbons at $\delta$ 123.2 and 144.4, and five oxygenated methylene and methine carbons at $\delta$ 63.3, 63.5, 69.7, 73.9, and 86.6 (Table 1). Further comparison of the $^{1}H$ and $^{13}C$ NMR data of **1** with reported values revealed the aglycone of **1** to be platycodigenin (2β,3β,16α,23,24-pentahydroxyolean-12-en-28-oic acid) [5]. The downfield shift of C-3 at $\delta$ 86.6 and the upfield shift of C-28 at $\delta$ 175.8 suggested that **1** was a bisdesmosidic saponin.

The $^{1}H$ NMR spectrum of **1** exhibited four anomeric proton resonances at $\delta$ 4.94 (*d*, $J$ = 8.0 Hz), 5.17 (*d*, $J$ = 8.0 Hz), 6.49 (brs), and 5.80 (brs). Acid hydrolysis of **1** yielded glucose, arabinose, and rhamnose, which were detected by TLC comparison with authentic

**Table 2.** $^{1}H$ and $^{13}C$ NMR spectral data for sugar moieties of compounds **1** and **2** (500 MHz, in $C_5D_5N$).

| | | **1** | | **2** | |
|---|---|---|---|---|---|
| No. | | $\delta_H$ | $\delta_c$ | $\delta_H$ | $\delta_c$ |
| Glc | 1 | 4.94 d (8.0) | 105.5 | 5.21 d (8.0) | 105.1 |
| | 2 | 4.04–4.09 m | 74.1 | 3.93–3.96 m | 74.7 |
| | 3 | 4.06–4.10 m | 88.2 | 4.11–4.14 m | 78.2 |
| | 4 | 4.01–4.03 m | 69.8 | 4.09–4.13 m | 71.2 |
| | 5 | 3.88–3.90 m | 78.1 | 3.97–3.99 m | 77.1 |
| | 6 | 4.22 dd (12.0, 6.0) | 62.3 | 4.24 dd (12.0, 6.0) | 70.0 |
| | | 4.46 dd (12.0, 2.0) | | 4.75 dd (12.0, 2.0) | |
| Glc′ | 1 | 5.17 d (8.0) | 105.4 | 5.01 d (8.0) | 105.5 |
| | 2 | 4.03–4.05 m | 75.5 | 4.01–4.03 m | 75.3 |
| | 3 | 4.24–4.27 m | 78.2 | 4.13–4.15 m | 78.3 |
| | 4 | 4.11–4.14 m | 71.7 | 4.14–4.17 m | 71.6 |
| | 5 | 4.02–4.06 m | 78.6 | 3.86–3.89 m | 78.4 |
| | 6 | 4.20 dd (12.0, 6.0) | 62.5 | 4.27 dd (12.0, 6.0) | 62.5 |
| | | 4.60 dd (12.0, 2.0) | | 4.48 dd (12.0, 2.0) | |
| Ara | 1 | 6.49 brs | 93.6 | 6.48 brs | 93.5 |
| | 2 | 4.48–4.52 m | 75.4 | 4.48–4.51 m | 75.5 |
| | 3 | 4.49–4.51 m | 70.3 | 4.49–4.51 m | 70.1 |
| | 4 | 4.37–4.42 m | 66.2 | 4.36–4.40 m | 66.0 |
| | 5 | 3.91–3.95 m | 63.0 | 3.89–3.93 m | 63.1 |
| | | 4.52–4.57 m | | 4.52–4.56 m | |
| Rha | 1 | 5.80 brs | 101.5 | 5.81 brs | 101.4 |
| | 2 | 4.47–4.49 m | 72.3 | 4.47–4.51 m | 72.4 |
| | 3 | 4.48–4.51 m | 72.6 | 4.48–4.52 m | 72.7 |
| | 4 | 4.24–4.28 m | 73.8 | 4.26–4.29 m | 73.7 |
| | 5 | 4.54–4.56 m | 70.4 | 4.54–4.57 m | 70.5 |
| | 6 | 1.67 d (6.0) | 18.5 | 1.66 d (5.5) | 18.5 |

samples. The absolute configuration of the monosaccharides was determined to be D for glucose, and L for arabinose and rhamnose by GC analysis of chiral derivatives of the monosaccharides in the hydrolysate of each compound (see Section 3). The relatively large coupling constants (8.0 Hz) for the anomeric protons in the $^{1}H$ NMR spectrum of **1** suggested that the glucopyranosyl moiety has a β-configuration. The α-configurations of the rhamnopyranosyl and arabinopyranosyl moieties were determined from the broad singlets observed for the anomeric protons. The large $^{1}J_{H\text{-}1,C\text{-}1}$ value of the rhamnopyranosyl moiety (172 Hz) confirmed that the anomeric proton was equatorial (α-pyranoid anomeric form).

The spin–spin coupling system of individual monosaccharide units was identified by analysis of 1D TOCSY and 2D NMR spectra. $^{1}H$ NMR spectral data of individual monosaccharide units were obtained by selective irradiation of the anomeric protons or methyl groups of rhamnopyranosyl units in a series of 1D TOCSY experiments. Analysis of the $^{1}H$-$^{1}H$ COSY spectrum resulted in sequential assignment of all proton resonances of the four monosaccharide units, including the identification of most of their multiple splitting patterns and coupling constants, as shown in Table 2. In the HSQC experiment, proton resonances were correlated with those of the corresponding carbons, and associated anomeric protons were correlated with their respective carbon atoms from HSQC-TOCSY data, leading to unambiguous assignments of the carbons in each monosaccharide unit (Table 2). Considering the known effects of *O*-glycosylation, **1** contains two D-glucopyranosyl units (Glc), one L- arabinopyranosyl unit (Ara), and one L- rhamnopyranosyl unit (Rha). In the HMBC spectrum, the anomeric proton signals at $\delta$ 4.94 (Glc-H-1), 5.17 (Glc′-H-1), 6.49 (Ara-H-1), and 5.80 (Rha-H-1) showed cross-peaks with the carbon signals at $\delta$ 86.6

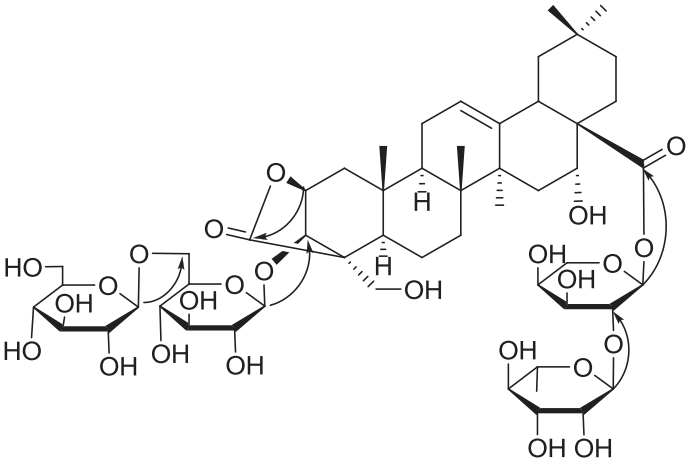



**Figure 2.** Selected HMBC correlations of compound **2.**

(Aglycone-C-3), 88.2 (Glc-C-3), 175.8 (Aglycone-C-28), and 75.4 (Ara-C-2), respectively. These signals provide evidence to determine the linkages between the sugars, and the sugar and the aglycone. These linkages were also confirmed by NOESY correlations. From the above evidence, the structure of **1** was established as 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl platycodigenin 28 *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, and named platycoside P.

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was found to be $C_{53}H_{82}O_{25}$ by the negative HR-ESIMS ion at *m/z* 1117.5063 $[M\text{-}H]^-$. The IR spectrum exhibited absorptions at 3433 (OH) and 1740 (*γ*-lactone carbonyl). The $^1H$ and $^{13}C$ NMR spectroscopic data (Table 1) of **2** indicated that **2** has an olean-12-en skeleton. Comparison of NMR data for **2** and **1** revealed that two compounds are similar. The differences found were the signals of C-24 and C-2, which appeared at down-field *δ* 178.4 and 83.3 in **2** instead of *δ* 63.5 and 69.7 in **1**, respectively. The lactone carbon of C-24 was observed to be correlated with H-2 (*δ* 5.41) based on the HMBC spectrum (Figure 2). Further comparison NMR data for **2** with reported values revealed the aglycone of **2** to be platycogenic acid A lactone (3β,16α,23-trihydroxyolean-12-en-2,24-lacton-28-oic acid), produced via intramolecular esterification between the 24-carboxylic acid and the 2-hydroxy group of platycogenic acid A [5,11]. Signals due to sugar moieties linked to C-3 and C-28 of aglycone were almost superimposable between **1** and **2** except for the linkage site of the Glc′, which was connected to Glc-C-6 in **2** instead of to Glc-C-3 in **1** according to HMBC correlations observed between Glc′-H-1 and Glc-C-6. This was further supported by NOE correlations observed between Glc′-H-1 and Glc-H-6. Thus, the structure of **2** was characterized as 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl platycogenic acid A lactone 28 *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, and named platycoside Q.

Compounds **1** and **2** were evaluated for inhibitory activity against LPS-induced TNF-α production in RAW 246.7 macrophages (See Section 3). Curcumin was used as positive control ($IC_{50}$ = 7.4 ± 1.4 μM). Compounds **1** and **2** showed inhibitory activity with the inhibition ratios (%) of 38.6 and 44.1 at 50 μM, respectively.

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on a Bruker IFS-55 plus spectrometer (Bruker, Ettlingen, German). NMR spectra were recorded on an Inova 500 spectrometer (Bruker, Waltham, MA, USA). HRESI/MS measurements were performed on a Bruker-Daltonics APES-III 7.0 TESLA FTMS spectrometer (Bruker, Billerica, MA, USA). High-performance liquid chromatography (HPLC) separation was carried out on an octadecylsilanized column (YMC-pack ODS-A, 250 × 10 mm, i.d. 5 μm, YMC, Kyoto, Japan) with a photo-diode array detector (Waters, Millford, MA, USA). GC was performed with a SHIMADZU GC-14D. Column chromatography was performed with silica gel (Merck, Darmstadt, Germany) and C18 silica gel (Merck).

### 3.2. Plant material

The roots of *Platycodon grandiflorum* were collected in Aug 2016 in Taihe, Anhui Province, China. The identification of the plant was performed by the author (Qiang Fu). A voucher specimen (PG 201608) is maintained in the herbarium of College of Pharmacy and Bioengineering, Chengdu University.

### 3.3. Extraction and isolation

The dried roots of *P. grandiflorum* (5.5 kg) were extracted with 95% EtOH (40 L). After removing the solvent, the residue (849.3 g) was suspended in $H_2O$ and extracted with *n*-BuOH (233.1 g). The *n*-BuOH extract was subjected to silica gel column chromatography (CC) using eluent mixtures of $CHCl_3$/MeOH (10:1 → 1:2) to afford fractions 1–5. Fraction 3 (23.9 g) was subjected to $C_{18}$ silica gel chromatography and eluted in a gradient of MeOH–$H_2O$ (10:90 → 95:5) to afford sub-fractions 1–4 (3.9, 1.7, 12.7, and 1.8 g, respectively). Sub-fraction 4 (1.8 g) was subjected to Sephadex LH-20 column chromatography (MeOH) and prep-HPLC (ACN-$H_2O$, 15:85 → 40:60, 2.0 ml/min, UV detection at 210 nm), affording compounds **1** (33.9 mg, 24.8 min) and **2** (29.4 mg, 28.3 min).

#### 3.3.1. Platycoside P (1)

White amorphous powder; $[\alpha]_D^{20}$ − 23.3 (*c* 1.0, MeOH); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3425, 1746, and 1072; $^1H$ and $^{13}C$ NMR spectral data, see Tables 1 and 2. HR-ESIMS: *m/z* 1121.5384 $[M\text{-}H]^-$ (calcd for $C_{53}H_{85}O_{25}$,1121.5380).

#### 3.3.2. Platycoside Q (2)

White amorphous powder; $[\alpha]_D^{20}$ − 18.4 (*c* 1.0, MeOH); IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3433, 1740, and 1070; $^1H$ and $^{13}C$ NMR spectral data, see Tables 1 and 2. HR-ESIMS: *m/z* 1117.5063 $[M\text{-}H]^-$ (calcd for $C_{53}H_{81}O_{25}$, 1117.5067).

### 3.4. Acid hydrolysis

Compounds **1** and **2** (5.0 mg, respectively) in 2 M of trifluoroacetic acid (1.0 ml) were heated at 110 °C for 2 h, and then dried by $N_2$ gas. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 resin (OH–form) and the resin was filtered. After removal of the solvent from the filtrate under pressure, the residue was passed through a Sep-Pak C18 cartridge with $H_2O$ and MeOH. The $H_2O$ eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (2.0 mg) in pyridine (1.0 ml) at 60 °C for 2 h. After being dried under N2 gas, the residue was treated with *N*-(trimethylsilyl) imidazol (0.2 ml) at 60 °C for 1 h. The reaction was ended by adding water (1.0 ml), and extracted with cyclohexane (1.0 ml, thrice). The cyclohexane layer was collected and concentrated to 1.0 ml for GC analysis. Separations were carried out on a HP-5 column (28 m × 0.32 mm, Agilent, Santa Clara, CA, USA). Highly pure He was employed as carrier gas (1.0 ml·min$^{-1}$ flow rate), and the FID detector was operated at 260 °C (column temp 180 °C). The retention times of the monosaccharide derivatives were as follows: L-Rha, 7.13 min; D-Glc, 12.84 min; and L-Ara, 6.31 min.

### 3.5. Assay for inhibitory activity against LPS-induced TNF-α production

Tested compounds were separately dissolved in DMSO, and diluted with phosphate buffered saline (PBS) to a final concentration of 10 mM. Such solution was then diluted to various test concentrations. RAW 264.7 macrophages were seeded in 96 well plates ($1 \times 10^5$ cells/well). Cells were co-incubated with tested samples and lipopolysaccharide (LPS, 1.0 μg/ml) for 24 h. TNF-α production levels were determined using a commercially available TNF-α ELISA kit (BioLegend, Inc., CA) according to the protocol provided by the manufacturers.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was supported by the Innovation Team Foundation of Educational Commission of Sichuan Province of China [grant number 18TD0049].

## References

[1] Editorial Committee of Chinese Pharmacopeia, *Chinese Pharmacopoeia [S]* ***1***, 277 vols. (China Medical Science Press, Beijing, 2015).
[2] L. Zhang, Y.L. Wang, D.W. Yang, C.H. Zhang, N. Zhang, M.H. Li and Y.Z. Liu, *J. Ethnopharmacol.* **164**, 147 (2015).
[3] I. Mazol, M. Glensk and W. Cisowski, *Acta Pol. Pharm.* **61**, 203 (2004).
[4] J.C. Ahn, B. Hwang, H. Tada, K. Ishimaru, K. Sasaki and K. Shimomura, *Phytochemistry*. **42**, 69 (1996).
[5] Y.H. Choi, D.S. Yoo, M.R. Cha, C.W. Choi, Y.S. Kim, S.U. Choi, K.R. Lee and S.Y. Ryu, *J. Nat. Prod*. **73**, 1863 (2010).
[6] Z.D. He, C.F. Qiao, Q.B. H, W.C Ye and H.X. Xu, *Terahedron*. **61**, 2211 (2005).
[7] K.S. Ahn, E.J. Noh, H.L. Zhoa, H. Jung, S.S. Kang and Y.S. Kim, *Life Sci*. **76**, 2315 (2005).
[8] Q. Fu, K. Zan, M.B. Zhao, S.X. Zhou, S.P. Shi, Y. Jiang and P.F. Tu, *J. Asian Nat. Prod. Res*. **15**, 610 (2013).
[9] Q. Fu, H.M. Yuan, J. Chen and J.Y. Shi, *Phytochem. Lett*. **16**, 169 (2016).
[10] Q. Fu, M. Yang, Y. Ma, J. Chen and H.M. Yuan, *Chin. J. Nat. Med*. **16**, 131 (2018).
[11] W.W. Fu, N. Shimizu, T. Takeda, D.Q. Dou and B.H. Chen, *Chem. Pharm. Bull*. **54**, 1285 (2006).