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Congmujingnosides B-G, triterpene saponins from the stem of *Aralia chinensis* and their protective effects against H_2O_2-induced myocardial cell injury

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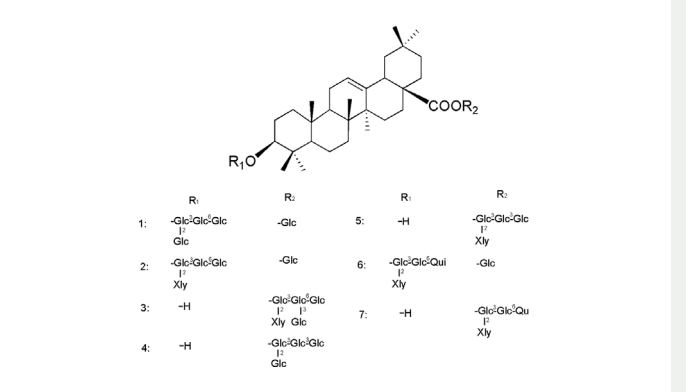
ABSTRACT

Phytochemical investigation of the stem of *Aralia chinensis* yielded six new oleanane-type triterpene saponins named as congmujingnosides B-G (**1**–**6**). The new ones were elucidated on the basis of the chemical and spectroscopic analysis. Protective effects of compounds **1**–**6** were tested against H_2O_2-induced H9c2 cardiomyocyte injury, and the data showed that compounds **1** and **5** had significant cell-protective effects. No significant DPPH radical scavenging activity was observed for compounds **1**–**6**.



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1. Introduction

The genus *Aralia Linn* belongs to the *Araliaceae* family is mainly distributed in Asia and less so in the North America. In China, there are approximately 30 species in this genus (He and Zeng 1978). *Aralia chinensis* as one species of this genus, its buds are the major source of wild vegetables, the roots and barks are found as folk medicine for rheumathism, lumbago, hepatitis, bruise nephritis and oedema (Sakai et al. 1994). Previous phytochemical investigations indicated that *Aralia chinensis* species contain a rich source of oleanane-type triterpene saponins (Saito et al. 1990; Yoshikawa et al. 1994; Sakai et al. 1994; Zou et al. 2001). Among these triterpene saponins, some displayed significant biological activities such as cytotoxic (Tomatsu et al. 2003), anti-inflammatory (Suh et al. 2007), live-protecting (Du and Chi 2005) and antioxidative (Zhang et al. 2006). However, the chemistry of the stems of *A. chinensis* Linn. has been little researched. As a result, the phytochemical investigation of the stems of *A. chinensis* Linn. was performed, and obtained six new triterpene saponins (Figure 1). In this paper, we report the isolation and structural elucidation of the new compounds, as well as the protective effects of the isolates against cardiomyocyte injury induced by H_2O_2. In addition, their radical scavenging activity was evaluated by DPPH assay.

2. Results and discussion

2.1. New compound structure elucidation

Compound **1** was obtained in the form of a white amorphous powder with $[\alpha]_D^{20}$ −20.6 (*c* 0.1, MeOH), and the Molish and Liebermann–Burchard reactions were positive. The HRESIMS spectrum showed a quasi-molecular ion at *m/z* 1259.6067 (calcd for $C_{59}H_{96}O_{27}Na$ $[M + Na]^+$, 1259.6037), from which in conjunction with NMR data, the molecular formula was established as $C_{59}H_{96}O_{27}$. The NMR spectra (Table S1) of compound **1** showed the characteristic signals of a triterpenoid saponin. Seven signals for tertiary methyl groups at δ_H 0.84, 1.08, 1.07, 0.86, 1.24, 1.27 and 0.87 which agreed with the information obtained from the ^{13}C NMR spectrum (seven sp^3 carbons at δ_C: 16.1, 16.9, 17.9, 24.1, 26.6, 28.3 and 33.6) as determined by an HSQC technique. The 1D NMR data also revealed one trisubstituted olefinic proton at δ_H 5.39 (1H, br s, H-12) with two typical olefinic carbon signals at δ_C 122.3 and 144.6. The downfield chemical shift of C-3 (δ_C 89.9) and the upfield chemical shift of C-28 (δ_C 177.0) showed that **1** was a bisdesmosidic glycoside, and the chemical shift of C-3 (δ_C 89.9) indicated an *α*-orientation for H-3 (Liang et al. 2011). The ^1H and ^{13}C NMR spectra of **1** showed five anomeric signals at δ_H 5.47 (1H, d, *J* = 7.8 Hz), 4.80 (1H, d, *J* = 7.8 Hz,), 5.36 (1H, d, *J* = 7.8 Hz), 5.02 (1H, d, *J* = 7.8 Hz), and 6.25 (1H, d, *J* = 7.8 Hz) and carbon signals at δ_C 105.3, 105.9, 105.7, 105.7 and 96.1, which further revealed that compound **1** was a oleanane-type glycoside with five sugars attached to the C-3 and C-28 positions. Through detailed analysis of the 2D NMR spectra (HSQC and ^1H–^1H COSY), the spin systems for the sugars were assigned. In contrast, the HMBC spectrum (Figure S1) of **1** showed a proton signal at δ_H 5.47 (glc-H-1′) that correlated with the carbon signal at δ_C 89.9 (C-3) of the aglycone moiety, and the anomeric proton signals at δ_H 5.02 (xyl-H-1′′′′), 4.80 (glc-H-1′′) and 5.36 (glc-H-1′′′) showed correlations with the carbon signals at δ_C 79.5 (glc-C-2′), 85.3 (glc-C-3′) and 69.8 (glc-C-6′′), respectively, of the inner sugars, which suggests glycosylation at C-3 of aglycone with a [glc (1 → 3)-glc (1 → 6)]-[xyl (1 → 2)]-glc moiety. In addition an anomeric proton signal at δ_H 6.25 (glc-H-1′′′′) showed correlation with the carbon signal at δ_C 177.0 (C-28) indicating the presence of a glucose

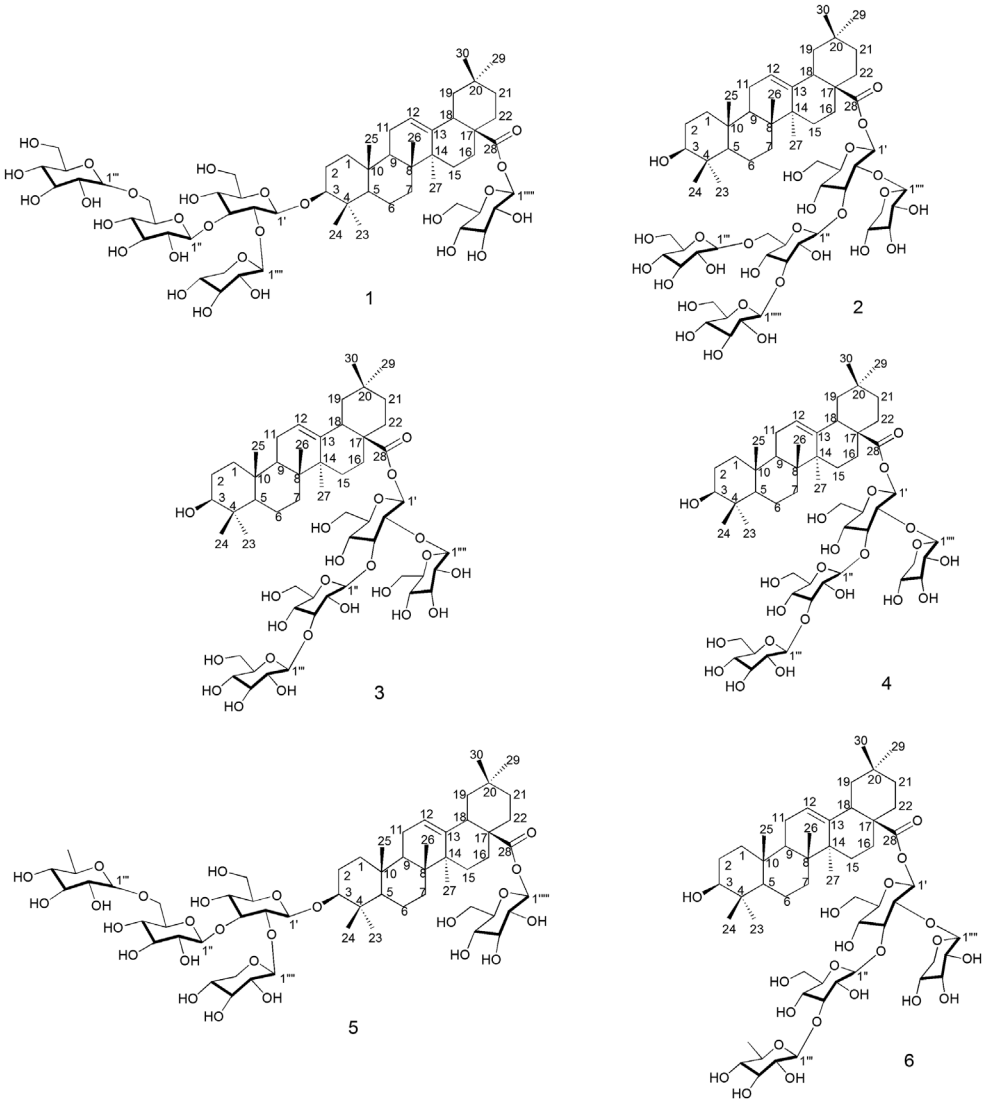

Figure 1. Chemical structure of *Congmujingnosides* B-G.

unit at C-28. The anomeric configurations of the sugar moieties were determined to be *β* because of the J_{H-H} values (7.8 Hz). The absolute configuration of the monosaccharides was determined to be D through GC analysis of the chiral derivatives of the monosaccharides in the hydrolysate. Finally, the structure of **1** was established as 3-O-{[β-D-xylopyranosyl-(1 → 2)]-[β-D-glucopyranosyl- (1 → 6)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl} oleanolic acid 28-O-β-D-glucopyranosyl ester and named congmujingnoside B (Figure 1).

Compound **2** was obtained as a white amorphous powder, and its elemental formula of $C_{59}H_{96}O_{27}$ Na was evident on the basis of positive HRESIMS $[M + Na]^+$ ion peak at *m/z* 1259.6034 (calcd. for 1259.6037), which was the same as that of **1**. The acid hydrolysis of **2** liberated D-glucose and D–xylose. The ^1H and ^{13}C NMR spectra of **2** were similar to those of

1, except for the signals due to the upfield chemical shifts of C-3 (δ_C 79.1), which suggests C-3 without sugar unit. And the sugar linkages were determined from the HMBC spectrum. The long-range couplings observed between the proton signal at δ_H 6.24 (glc-H-1′) and the carbon signal at δ_C 177.0 (C-28) of the aglycone moiety, and the anomeric proton signals at δ_H 5.02 (xyl-H-1′′′′), 4.81 (glc-H-1′′), 5.58 (glc-H-1′′′′), and 5.36 (glc-H-1′′′) showed correlations with the carbon signals at δ_C 79.8 (glc-C-2′), 90.1 (glc-C-3′), 89.3 (glc-C-3′′), and 69.8 (glc-C-6′′) of the inner sugar, respectively, confirm the glycosylation at C-28 of the aglycone with a {[glc-(1 → 6)-glc-(1 → 3)]-glc-(1 → 3)}-[xyl-(1 → 2)]-glc moiety. Therefore, the structure of **2** was established as [*β*-D-xylopyranosyl-(1 → 2)]-{[*β*-D-glucopyranosyl- (1 → 3)]-[*β*-D-glucopyranosyl-(1 → 6)]-*β*-D-glucopyranosyl-(1 → 3)}28-*O*-*β*-D-glucopyranosyl oleanolic acid ester and named congmujingnoside C. (Figure 1)

Compound **3,** which was isolated as a white acicular crystal. In the positive-ion HRFABMS analysis revealed the molecular formula of **3** to be $C_{54}H_{88}O_{23}$. By the acid hydrolysis of **3**, D-glucose were identified by TLC analysis. The ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of **2**, apart from the lack of signals of a β-D-xylopyranosyl. In contrast, long-range couplings were observed from a proton signal at δ_H 6.32 (glc-H-1′) to the carbon signal at δ_C 176.9 (C-28), δ_H 5.33 (glc-H-1′′′′) to δ_C 80.1 (glc-C-2′), δ_H 4.83 (glc-H-1′′) to δ_C 89.8 (glc-C-3′), and δ_H 5.57 (glc-H-1′′′) to δ_C 89.1 (glc-C-3′′) in the HMBC spectrum of **3**, which suggests glycosylation at C-28 with a{ [glc (1 → 3)]-glc-(1 → 3)]}-[glc(1 → 2)]-glc moiety. This result agrees with the above observations and suggests that compound **3** is [*β*-D-glucopyranosyl-(1 → 2)]-{[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl-(1 → 3)}28-*O*-*β*-D-glucopyranosyl oleanolic acid ester and named congmujingnoside D. (Figure 1).

Compound **4** was obtained as an amorphous powder. The HRESIMS showed a deprotonated molecular ion peak at *m/z* 1097.5500 $[M + Na]^+$, indicating the molecular formula to be $C_{53}H_{86}O_{22}$. The ^{1}H and ^{13}C NMR spectroscopic data (Table S2) were closely related to those of **3** except for one D-glucose in **3** was replaced by D-xylose in **4**. The sugar moieties and linkages were the same as **3** on the basis of the similar ^{13}C NMR and HMBC correlations, which were further confirmed by HSQC and ^{1}H–^{1}H COSY experiments. All of the correlations showed that compound **4** was an oleanolic acid glycoside at C-28 with a {[glc (1 → 3)]-glc-(1 → 3)]}-[xyl-(1 → 2)]-glc moiety. Therefore, the structure of **4** was established as [*β*-D-xylopyranosyl-(1 → 2)]-{[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl-(1 → 3)}28-*O*-*β*-D-glucopyranosyl oleanolic acid ester and named congmujingnoside E. (Figure 1).

Compound **5** was obtained as an amorphous white powder with $[\alpha]_D^{20}$ −26.3 (*c* 0.1, MeOH). It was determined to have the molecular formula of $C_{59}H_{96}O_{26}$ on the basis of the ^{13}C NMR data and HRESIMS spectrum at *m/z* 1243.6085 $[M + Na]^+$ (Calcd for $C_{59}H_{96}O_{23}$ Na, 1243.6088). An examination of the ^{1}H and ^{13}C NMR data (Table S3) showed the structure of **5** to be similar to that of **1** except for one D-glucose in **1** was replaced by D-quinovose in **5**. Through detailed analysis of the 2D NMR spectra, the spin systems for the sugars were assigned. In the HMBC spectrum, proton signal at δ_H 5.47 (glc-H-1′) had correlation with the carbon signal at δ_C 89.2 (C-3) of the aglycone moiety, and the anomeric proton signals at δ_H 5.02 (xyl-H-1′′′′), 4.81 (glc-H-1′′), and 6.20 (qui-H-1′′′) showed correlations with the carbon signals at δ_C 79.2 (glc-C-2′), 83.7 (glc-C-3′) and 69.9 (glc-C-6′′), respectively, of the inner sugar. In addition an anomeric proton signal at δ_C 6.25 (glc-H-1′′′′) showed correlation with the carbon signal at δ_C 177.0 (C-28), which suggests glycosylation at C-3 of aglycone with a [glc (1 → 3)-qui (1 → 6)]-[xyl (1 → 2)]-glc moiety glycosylation and at C-28 with a glucose. Therefore, taking together with the NOE spectrum, the structure of **5** was

defined as 3-O-{[β-D-xylopyranosyl-(1 → 2)]-[β-D-quinovopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl} oleanolic acid 28-O-β-D-glucopyranosyl ester and named congmujingnoside F.

Compound **6** exhibited an $[M + Na]^+$ ion peak at *m/z* 1081.5567 using HRESIMS, corresponding to the molecular formula $C_{53}H_{86}O_{21}$. The ^1H and ^{13}C NMR signals (Table S3) were closely related to those of **4** except for the sugar unit. The monosaccharides obtained after aqueous acidic hydrolysis of compound **6** were identified as glucose, xylose and quinovose by TLC comparison with authentic samples, which displayed one D-glucose in **4** was replaced by D-quinovose in **6**. The absolute configuration of the monosaccharides was determined to be D by GC analysis of the chiral derivatives of the monosaccharides in the hydrolysate. The similar NOEs of **6** and **4** indicated that their absolute configurations were identical. Therefore, the structure of compound **6** was [*β*-D-xylopyranosyl-(1 → 2)]-{[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl-(1 → 3)}28-*O*-*β*-D-glucopyranosyl oleanolic acid ester and named as congmujingnoside G.

In this study, the protective effects of compounds **1**–**6** against H_2O_2-induced myocardial cell injury were tested. As shown in Table S4, compounds **1** and **5** (15–200 μM) significantly increased the viability of H9c2 induced by H_2O_2 in a dose-dependent manner, ranging from the concentrations at 15–200 μM. Compound **5** was shown to be the most efficient among the isolates. It increased the cell viability at a low concentration of 30 μM and was more efficient than vitamin E, which was used as the positive control. Compounds **1** showed moderate protective effects. They significantly improved cell viability at a concentration of 200 μM. No significant DPPH radical scavenging activity was observed for compounds **1**–**6**.

3. Conclusion

In summary, Phytochemical investigation of the stem of *Aralia chinensis* yielded six new oleanane-type triterpene saponins named as congmujingnosides B-G (**1**–**6**). Protective effects of compounds **1**–**6** were tested against H_2O_2-induced H9c2 cardiomyocyte injury, and the data showed that compounds **1** and **5** had significant cell-protective effects. No significant DPPH radical scavenging activity was observed for compounds **1**–**6**.

Supplementary material

Supplementary material relating to this article is available online, alongside Experimental, Tables S1–S5 and Figures S1–S37.

Disclosure statement

No potential conflict of interest was reported by the authors.

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