Three new triterpenoid saponins from *Aralia echinocaulis*

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**A B S T R A C T**

**Objective:** To study the active ingredients in the root bark of *Aralia echinocaulis*.

**Methods:** Three triterpenoid saponins were separated from the 70% ethanol extracts and purified by column chromatography and their structures were determined by spectroscopic analysis. Compound 1 and 3 were evaluated for antioxidant activity by the in vitro DPPH free radical scavenging ability and the protective effect of OH⁻ induced DNA oxidative damage.

**Results:** Compound 1 is a new type of triterpenoid saponin, named as echinocaulisaglycone 3-O-β-D-glucopyranoside (echinocaulisaponin A), and it had good antioxidant activity. Compound 2 was similar to compound 1, named as 1-hydroxyl-echinocaulisaglycone 3-O-β-D-glucopyranoside (echinocaulisaponin B). Compound 3 was also a new type of triterpenoid saponin, named as echinocaulisaglycone II 3-O-α-L-arabinopyranosyl-(1°→4°)-β-D-glucopyranosiduronic acid (echinocaulisaponin C), and its antioxidant activity was weaker than compound 1.

**Conclusion:** In this study, three new compounds were discovered and two of them were carried out in vitro anti-oxidation studies, laying the foundation for further research on the treatment of related diseases (cardiovascular disease, arthritis, age-related macular degeneration, etc.) through anti-oxidation or quenching free radical function.

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**1. Introduction**

*Aralia echinocaulis* Hand.-Mazz., is a small tree widely distributed in central and south of China and has been used as a traditional Chinese medicine to treat fractures, rheumatism, arthritis, and so on (Fang et al., 2007; He and Zeng, 1978). In recent decades, the constituents in the rhizones of *A. echinocaulis* had been investigated, including saponins, flavonoids, essential oils, and trace elements (Chen et al., 2013; Jia et al., 1990; Pei et al., 2009; Yan, 2018; Zheng and Pei, 2012). *A. echinocaulis* could enhance cell viability, increase superoxide dismutase (SOD) activity and cell membrane fluidity, reduce reactive free oxygen (ROS) and lipid peroxide (LPO) content, thereby improving the oxidative damage of H₂O₂ to MC3T3-E1 osteoblasts (Pei et al., 2010; Wang et al., 2016). In order to further explore the active substances in the plant, we reported here the isolation and identification of three new triterpenoid saponins (Fig. 1) and their antioxidant activities in vitro. Compound 1 is a new type of triterpenoid saponin, named as echinocaulisaglycone 3-O-β-D-glucopyranoside (echinocaulisaponin A). Compound 2 is similar to compound 1, named as 1-hydroxyl-echinocaulisaglycone 3-O-β-D-glucopyranoside (echinocaulisaponin B). Compound 3 is also a new type of triterpenoid saponin, named as echinocaulisaglycone II 3-O-α-L-arabinopyranosyl-(1°→4°)-β-D-glucopyranosiduronic acid (echinocaulisaponin C).

**2. Materials and methods**

2.1. General experimental procedures

NMR spectra were obtained with a Varian VNMRS 600 Spectrometer (Utah, USA) operating at 600 MHz for 1H NMR and 150 MHz for 13C NMR, respectively. Chemical shifts were reported in parts per million on the 0 scale with TMS as internal standard. IR spectra were recorded on Thermo Scientific Nicolet iS5 FTIR Spectrometer with ATR detection (Massachusetts, USA). Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Massachusetts, USA). HR-ESI-MS spectra were measured on Shimadzu LCMS-IT-TOF Mass Spectrometer (Tokyo, Japan). Semi-preparative HPLC was carried out with Shimadzu LC-20AT Liquid Chromatograph with SPD-M20A and ELSD-LT II detector (60 °C, gas flow: 1.6 L/min) and an X Aqua HPLC column (5 μm, 4.6 mm x 250 mm, Huapu Xinchuang Technology Co., Ltd., Zhejiang, China). Macroporous resin MCI-GEL (Mitsubishi Chemical, Tokyo,
Japan), silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), and ODS silica gel (120 Å, 50 μm, YMC, Tokyo, Japan) were used for column chromatography. HPLC grade acetonitrile, formic acid, and methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Deionized water was purified by Milli-Q system (Bedford, MA). Other reagents for purification, such as methanol, chloroform, dichloromethane, acetonitrile, and n-butanol, were of analytical grade bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Plant materials

The root bark of A. echinocaulis was collected from Hubei Shi Entang Pharmaceutical Co., Ltd. (Enshi Chinese Medicinal Materials Co., Ltd. subpackage), OP 091101, identified as A. echinocaulis by Professor Yaojun Yang, Department of Pharmacy of Beijing University of Traditional Chinese Medicine.

2.3. Extraction and isolation

Ground root bark of A. echinocaulis (2 kg) were refluxed with 70% EtOH (21 L) for three times and each time for 2 h. The extracts were filtered and then concentrated under reduced pressure to yield a crude extract of ethanol (2.5 L), which was isolated on MCI-GEL macroporous resins to give nine fractions (Frs. A–I, water, 80%, 85%, 90%, 95% EtOH). Other reagents for purification, such as methanol, chloroform, dichloromethane, acetonitrile, and formic acid, and methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Deionized water was purified by Milli-Q system (Bedford, MA). Other reagents for purification, such as methanol, chloroform, dichloromethane, acetonitrile, and n-butanol, were of analytical grade bought from Sinopharm Chemical Reagent Co., Ltd. subpackage), OP 091101, identified as A. echinocaulis by Professor Yaojun Yang, Department of Pharmacy of Beijing University of Traditional Chinese Medicine.

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3. Results

Compound 1 was obtained as a white amorphous powder, and gave positive Molish reaction and Liebermann–Burchard reaction. Its formula C_{36}H_{58}O_{10} was deduced from HR-ESI-MS for the [M + COOH]^+ ion peak at m/z 650.4030. The ^1H NMR spectrum of compound 1 (Table 1) showed two alkene proton signal at δ_1 4.78 (1H, o) and 4.88 (1H, o), which prompted that there was a terminal double bond. One glycoside proton signal at δ_1 4.97 (1H, d, J = 7.7 Hz, H–1), which suggested that the structure contained D-glucopyranosyl compared the NMR data with the literature (Fang et al., 1992). Combining the coupling constant of the glycoside proton, the configuration of glucose was determined β-D. Six methyl signals at δ_1 0.93 (3H, s), 1.13 (3H, s), 1.40 (3H, s), 1.67 (3H, s), 2.01 (3H, s), and 1.25 (3H, s) (Tang et al., 1997), and several methylene and methyline signals. Its ^13C NMR spectrum showed two olefinic carbon (δ 157.18 and 106.06), four carbon–oxygen groups (δ 89.52, 67.64, 70.75 and 98.75), and six methyl groups (δ 31.46, 17.04, 17.62, 18.07, 17.68 and 22.41). In the HMBC spectrum (Fig. 2), long-range correlation between the following carbons and protons was observed: H–3 (δ 4.97, s) with C–3 (δ 89.52), C–4 (δ 40.29), and C–24 (δ 157.18); H–25 (δ 0.93, s) with C–5 (δ 61.37) and C–10 (δ 38.99); H–26 (δ 1.13, s) with C–7 (δ 54.43) and C–8 (δ 42.71); H–27 (δ 1.67) with C–13 (δ 40.96) and C–14 (δ 42.81). The glycosyl group located at C–3 was confirmed by HMBC correlations of Glc H–1 (δ 4.97, d) with C–3 (δ 89.52). The H–30 (δ 1.25, s) had long-range correlation with C–20 (δ 157.18), which were terminal double bond signals. The H–21 (δ 4.84) had long-range correlation with C–29 (δ 106.06), so the carbon–21 signal was determined. The H–21 (δ 4.84) had long-range correlation with C–17 (δ 54.43) and C–28 (δ 98.13), so the signals of carbon-17, 21, 22 and 28 were determined combined with ^1H–^1H COSY spectrum, and they formed a ring structure. The aglycon of compound 1 was a new aglycon, named as echinocaulisaglycone. Therefore, compound 1 was characterized as echinocaulisaglycone 3-O-β-D-glucopyranoside (echinocaulisapponin A, Fig. 1).

Compound 2 was obtained with retention time of 5.0 min longer than that of compound 1 by HPLC (25% CH_{3}CN). It also was obtained as a white amorphous powder, and gave positive Molish reaction and Liebermann–Burchard reaction. Its formula C_{36}H_{58}O_{10} was deduced from HR-ESI-MS for the [M + COOH]^+ ion peak at m/z 711.3954 (Calcd. for [M + COOH], 666.3979). The NMR data (Table 2) of compound 2 were similar to compound 1 data. There was a typical terminal double bond signal, the carbon-17, 21, 22 and 28 formed a ring structure. The glycosyl group of the compound 2 was also located at C–3, and the configuration of glucose was determined to be β-D from its ^1H and ^13C NMR data according to the literature comparison (Fang et al., 1992). The mass fraction of compound 2 was 16 more than that of compound 1, indicating that compound 2 has one more hydroxyl group. In the ^1H–^1H COSY spectrum, the proton signal at δ_1 3.12
(dd, $J = 4.7, 11.8$ Hz, H-3) had range correlation with signal at $\delta_{H}$ 1.68 and 1.97 (o, H-2), and the proton signal at $\delta_{H}$ 1.68 and 1.97 (o, H-2) had range correlation with signal at $\delta_{H}$ 4.37 (o, H-1), so carbon signal at $\delta_{C}$ 65.56 was the signal of carbon-1. Thus, the structure of compound 2 was characterized as 1-hydroxyl-echinocaulisaglycosides 3-0-$\beta$-D-glucopyranoside (echinocaulisaponin B, Fig. 1).

Compound 3 was obtained as a yellow amorphous powder, and gave positive Molish reaction and Liebermann-Burchard reaction. Its formula $C_{41}H_{64}O_{14}$ was deduced from HR-ESI-MS for the coside proton signals at 1.68 and 1.97 (o, H-2), and the proton signal at (dd, $J = 4.7, 11.6$ Hz, H-1) had range correlation with signal at $\delta_{H}$ 4.67 (o) had range correlation with signal at $\delta_{H}$ 1.71/2.61 (o, H-15), so the carbon signal at $\delta_{C}$ 69.83 was the signal of carbon-16. In the HMBC spectrum (Fig. 2), long-range correlation between the following carbons and protons was observed: $H_{2}$-23 ($\delta_{H}$ 0.93, s) with C-3 ($\delta_{C}$ 79.03), C-4 ($\delta_{C}$ 40.87), and C-24 ($\delta_{C}$ 29.27); $H_{2}$-25 ($\delta_{H}$ 0.71, s) with C-1 (0.93), C-5 ($\delta_{C}$ 79.03); C-9 ($\delta_{C}$ 85.48) was the signal of carbon-16. The arabinose group located at C-3 was confirmed by HMBC correlations of $\delta_{C}$ 69.83 and 85.48, and seven methyl groups ($\delta_{C}$ 19.00, 29.27, 17.39, 17.61, 18.00, 25.51 and 19.99). In $^{13}$C NMR spectrum, the proton signal at $\delta_{H}$ 4.67 (o) had range correlation with signal at $\delta_{H}$ 1.71/2.61 (o, H-15), so the carbon signal at $\delta_{C}$ 69.83 was the signal of carbon-16. In the HMBC spectrum (Fig. 3), long-range correlation between the following carbons and protons was observed: $H_{2}$-23 ($\delta_{H}$ 0.93, s) with C-3 ($\delta_{C}$ 79.03), C-4 ($\delta_{C}$ 40.87), and C-24 ($\delta_{C}$ 29.27); $H_{2}$-25 ($\delta_{H}$ 0.71, s) with C-1 ($\delta_{C}$ 40.18), C-5 ($\delta_{C}$ 59.17), C-9 ($\delta_{C}$ 85.48) and C-10 (38.99); $H_{2}$-26 ($\delta_{H}$ 0.90,s) with C-7 ($\delta_{C}$ 35.54), C-8 ($\delta_{C}$ 42.23), and C-9($\delta_{C}$ 50.86); $H_{2}$-27 ($\delta_{H}$ 1.51) with C-13 ($\delta_{C}$ 44.79), C-14 ($\delta_{C}$ 42.87) and C-15 ($\delta_{C}$ 36.49). The arabinose group located at C-4 was confirmed by HMBC correlations of Ara H-1 ($\delta_{H}$ 6.14, s) with C-4 ($\delta_{C}$ 77.91) and glucuronic acid group located at C-3 was confirmed by HMBC correlations of GlcA H-1 ($\delta_{H}$ 4.97, d) with C-3 ($\delta_{H}$ 89.52). The $H_{2}$-29 ($\delta_{H}$ 1.29, s) and $H_{2}$-30 ($\delta_{H}$ 0.91, s) had long-range correlation with carbon signal at $\delta_{C}$ 85.48, so the carbon signal at $\delta_{C}$ 85.48 was the signal of carbon-16. The aglycon of compound 3 was a new aglycon, which was named echinocaulisaglycone II. Therefore, the structure of compound 3 was characterized as echinocaulisaglycone II 3-0-2-L-arabinopyranosyl-(1' ->4')-2-D-glucopyranosiduronic acid (echinocaulisaponin C, Fig. 1).

Compound 1 and 3 were evaluated for antioxidant activity by the in vitro DPPH free radical scavenging ability and the protective effect of OH- induced DNA oxidative damage, but compound 2 has not been tested due to the loss of the sample. The specific opera-
The scavenging method is detailed in the reference (Xue, 2020). Results showed that the scavenging rate range of DPPH were 35.62% to 92.16% (compound 1), 27.86%–85.12% (the water-soluble of compound 3), 30.18%–89.40% (the alcohol-soluble substances of compound 3), and 51.12%–96.70% (vitamin C), and IC₅₀ were 0.1985 mg/ml (compound 1), 0.3012 mg/ml (the water-soluble of compound 3), and 0.2652 mg/ml (the alcohol-soluble substances of compound 3), respectively. The protective effect of the com-

Table 2

| No. | δc  | δh(f in Hz) | No. | δc  | δh(f in Hz) |
|-----|-----|-------------|-----|-----|-------------|
| 1   | 65.56 | 4.37(o)    | 3   | Glc | 105.50 | 4.31(d, 7.8) |
| 2   | 25.51 | 1.68(o), 1.97(o) | 1'  | 74.29 | 3.19(o) |
| 3   | 89.37 | 3.12(dd, 4.7, 11.8) | 2'  | 76.85 | 3.33(o) |
| 4   | 39.40 | –          | 3'  | 70.23 | 3.29(o) |
| 5   | 60.51 | 0.89(o)    | 4'  | 76.24 | 3.24(o) |
| 6   | 67.53 | 3.94(o)    | 5'  | 61.40 | 3.64(o), 3.84(o) |
| 7   | 45.57 | 1.54(o), 1.62(o) | 6'  | 41.75 | 1.89(o) |
| 8   | 48.60 | 1.42(o)    |     | 38.39 | –         |
| 9   | 21.37 | 1.65(o)    |     | 27.41 | 1.73(o) |
| 10  | 40.42 | 1.75(o)    |     | 42.04 | –         |
| 11  | 34.02 | 1.46(o), 1.80(o) |     | 78.06 | 3.66(o) |
| 12  | 56.24 | –         |     | 40.45 | 1.89(o) |
| 13  | 31.80 | 2.07(o)    |     | 151.27 | –         |
| 14  | 87.38 | 4.47(s)   |     | 38.36 | 0.99(o), 1.68(o) |
| 15  | 29.84 | 1.35(br s) |     | 15.41 | 1.01(br s) |
| 16  | 16.85 | 0.93(br s) |     | 16.47 | 1.10(br s) |
| 17  | 16.38 | 1.27(br s) |     | 98.75 | 5.33(s) |
| 18  | 107.98 | 4.78(o), 4.86(o) |     | 20.60 | 1.14(s) |

*Note: (o) Overlapped with other signals.

Table 3

| No. | δc  | δh(f in Hz) | No. | δc  | δh(f in Hz) |
|-----|-----|-------------|-----|-----|-------------|
| 1   | 40.18 | 0.78(o), 1.44(o) | 3   | Glc | 108.33 | 4.98(o) |
| 2   | 28.03 | 1.85(o), 2.21(o) | 1'  | 76.63 | 4.09(o) |
| 3   | 90.37 | 3.34(dd, 4.09, 11.68) | 2'  | 77.50 | 4.30(o) |
| 4   | 40.87 | –          | 3'  | 77.91 | 4.80(o) |
| 5   | 57.11 | 0.68(o)    | 4'  | 77.50 | 4.70(o) |
| 6   | 19.62 | 1.25(o), 1.42(o) | 5'  | 173.96 | –         |
| 7   | 35.54 | 1.36(o), 1.44(o) | 6'  | 4'  | –         |
| 8   | 42.23 | –          | 5'  | 173.96 | –         |
| 9   | 50.86 | 1.39(o)    | 6'  | 173.96 | –         |
| 10  | 38.25 | –          | 7'  | 19.99 | 0.91(s) |
| 11  | 22.45 | 1.08(o), 1.44(o) |     | 42.23 | –         |
| 12  | 25.78 | 1.10(o), 1.60(o) |     | 36.49 | 2.61(o) |
| 13  | 44.79 | 1.36(o)    |     | 69.83 | 4.67(o) |
| 14  | 42.87 | –          |     | 48.22 | 1.64(o) |
| 15  | 36.49 | 1.71(o)    |     | 43.16 | 1.68(o) |
| 16  | 69.83 | 4.67(o)    |     | 45.13 | 1.64(o) |
| 17  | 48.22 | –          |     | 43.16 | 1.68(o) |
| 18  | 45.13 | –          |     | 36.49 | 2.61(o) |
| 19  | 43.16 | 1.64(o)    |     | 42.87 | –         |
| 20  | 85.48 | –          |     | 42.87 | –         |
| 21  | 28.56 | 1.87(o)    |     | 25.51 | 1.29(br s) |
| 22  | 28.87 | 1.55(o), 1.70(o) |     | 19.99 | 0.91(s) |
| 23  | 18.00 | 0.93(br s) |     | 25.51 | 1.29(br s) |
| 24  | 29.27 | 1.22(br s) |     | 19.99 | 0.91(s) |
| 25  | 17.39 | 0.71(br s) |     | 25.51 | 1.29(br s) |
| 26  | 17.61 | 0.90(br s) |     | 19.99 | 0.91(s) |
| 27  | 18.00 | 1.51(br s) |     | 19.99 | 0.91(s) |
| 28  | 177.91 | –         |     | 25.51 | 1.29(br s) |
| 29  | 19.99 | 0.91(s)    |     | 25.51 | 1.29(br s) |
| 30  | 19.99 | 0.91(s)    |     | 25.51 | 1.29(br s) |

*Note: (o) Overlapped with other signals.
compounds on $\text{O}_2^-$ induced DNA oxidative damage, results showed that the fluorescence intensity range were 0.335–0.465 (compound 1), 0.221–0.365 (the water-soluble of compound 3), 0.306–0.412 (the alcohol-soluble substances of compound 3), and 0.360–0.512 (vitamin C), respectively. Therefore, both compound 1 and compound 3 had antioxidant activity and compound 1 was stronger than compound 3.

4. Discussion

In this study, three new compounds were discovered and two of them carried out in vitro anti-oxidation studies, laying the foundation for further research on the treatment of related diseases (cardiovascular disease, arthritis, age-related macular degeneration, etc.) through anti-oxidation or quenching free radical function. Compared with the oleanolic acid saponins (Jiang et al., 1992), the carboxyl group of echinocaulisaponin A on C-28 was opened and connected with C-21 to form a new ring structure. The double bond on the nucleus was located at the end of the C-20 position instead of the C-12 and C-13 positions in the carbon rings, and multiple hydroxy groups were connected to the nucleus. Compared with the ursoic acid saponins (Tsutomu et al., 1993), the carbon signals of echinocaulisaponin C on C-12 and C-13 did not have double bond and the hydrogen at positions C-16 and C-20 were replaced by hydroxy groups. They all had antioxidant activity, but the antioxidant activity of echinocaulisaponin A was stronger than echinocaulisaponin C. So the relationship between structure and antioxidant activity still needs further study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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