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Two new sesquiterpenoid glycosides from the stems of *Zanthoxylum armatum* DC

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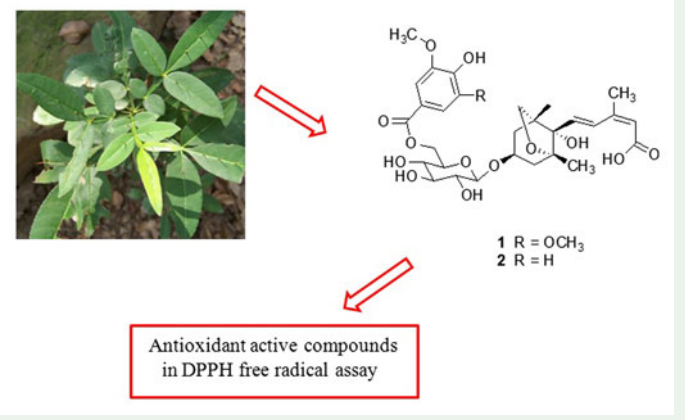
ABSTRACT

Two new sesquiterpenoid glycosides as dihydrophaseic acid 4′-*O*-[6″-*O*-(4‴-hydroxy-3‴, 5‴-dimethoxy) benzoyl)]-*β*-D-glucopyranoside (**1**) and dihydrophaseic acid 4′-*O*-[6″-*O*-(3‴-methoxy-4‴-hydroxy) benzoyl)]-β-D-glucopyranoside (**2**), were isolated from the stems of *Zanthoxylum armatum* in the study. The compound **1** and **2** showed moderate scavenging activity in DPPH free radical assay with IC_{50} values of 241 and 264 μM, respectively.



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Zanthoxylum armatum; sesquiterpenoid glycoside; scavenging activity in DPPH free radical

1. Introduction

Genus *Zanthoxylum* (Rutaceae) consists of about 250 species widely distributed in the tropical and subtropical regions of Asia, Africa, Oceania and North America. There are 39 species and 14 varieties in China, occurring nearly everywhere in the country

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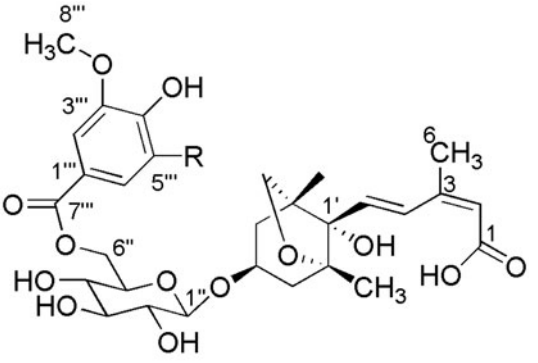

1 R = OCH_3
2 R = H

Figure 1. Chemical structures of compound **1** and **2**.

(Guo et al. 2012). *Zanthoxylum armatum* DC. is a common specie in the genus. Its root and stem were used as Traditional Chinese medicine, and its fruit was also often used as food condiment and aromatic preservatives (Guo et al. 2017). Zhuyejiao tablets as a Chinese patented drug are mainly made up of raw herb powder and ethanol extract from *Z. armatum* (Guo et al. 2011). Modern pharmacological studies have shown that *Z. armatum* possess xanthine oxidase inhibitory (Ranjana et al. 2019), antiasthmatic (Sharma et al. 2018), hypolipidemic and hypoglycemic (Alam et al. 2018), anti-inflammatory (Nooreen et al. 2017), anticancer (Alam et al. 2017), antidiabetic (Karki et al. 2014) and hepatoprotective (Verma and Khosa 2010) activities. Phytochemical investigations (Bhatt et al. 2015; Guo et al. 2012, 2015, 2017; Samad et al. 2014) have shown that *Z. armatum* contains a variety of chemical constituents, such as alkaloid, lignan, amide, flavone and phenol glycoside. As part of our systematic investigations on chemical constituents from *Z. armatum*, the present study investigated the water fraction of the ethanol extract of *Z. armatum*, resulting in isolation of two new compounds, 4′-*O*-[6″-*O*-(4‴-hydroxy-3‴,5‴-dimethoxy) benzoyl)]-β-D-glucopyranoside (**1**) and dihydrophaseic acid 4′-*O*-[6″-*O*-(3‴-methoxy-4‴-hydroxy) benzoyl)]-β-D-glucopyranoside (**2**) (Figure 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS showed quasimolecular ion at *m/z* 647.2295 $[M+Na]^+$ (calcd. for $C_{30}H_{40}O_{14}Na$, 647.2310), indicating a molecular weight of 624. According to the HR-ESI-MS, ^1H and ^{13}C-NMR spectral data, the molecular formula of **1** was determined to be $C_{30}H_{40}O_{14}$. In the low field of ^1H-NMR spectrum (Table **S1**), the signals at δ 7.18 (2H, s) defined a 1,3,4,5-substituted aromatic ring, which was further confirmed in the ^{13}C-NMR spectrum by four quaternary aromatic carbons at δ 119.9 (C-1‴), 147.2 (C-3‴, C-5‴), 139.6 (C-4‴) and two methine carbons at δ 107.1 (C-2‴, C-6‴). The signals at δ 7.47 (1H, d, $J = 15.9$ Hz) and 6.18 (1H, d, $J = 15.9$ Hz) indicated the *trans*-double bond, while 5.79 (1H, s)

indicated another double bond. The high field of ^{1}H-NMR spectrum showed the presence of three methyl groups attached to the quaternary carbon at $\delta = 1.93$ (3H, s), 1.06 (3H, s), and 0.45 (3H, s). Further analysis of ^{1}H-^{1}H COSY, HMBC, and HMQC spectra of **1** established the ionone-type skeleton with two double bonds and one conjugated carboxyl group, substituted benzoic acid moiety, and a glucose moiety (Youn et al. 2011). The structure of ionone-type skeleton was established to be dihydrophaseic acid. In the sugar part of the ^{1}H-NMR spectrum, the anomeric proton δ 4.46 (1H, d, $J = 7.9$ Hz) indicated the presence of glucose, which was confirmed by acid hydrolysis and comparison with authentic sample. The stereochemistry of the anomeric carbon of glucose was determined as β according to the coupling constant of the anomeric proton and the chemical shift of C-1″ (102.1). The glycosidic site was established unambiguously by a HMBC experiment in which long-range correlation between H-1″ (δ 4.46) and C-4′ (δ 74.9) was observed. The down-field shift of hydroxymethylene signal C-6″ (δ 63.9) and long-range correlation between H-6″a, H-6″b (δ 4.66, 4.49) and C-7‴ (δ 167.4) indicated the esterification of hydroxymethyl. The relative configuration was deduced from the results of the NOESY spectrum, in which NOE signals were observed between H-4′ (δ 3.95) and H-5′α (δ 2.10), H-8′b (δ 3.20). As a steric assumption for the successful ether formation in ionone-type skeleton (Youn et al. 2011), the hydroxymethylene C-8′ and oxygen at C-2′ must have an axial orientation. The relative configuration at C-4′ was also determined to be β-stereochemistry by the coupling constant ($J_{4'\text{-}5'\beta} = 10.6$ and $J_{4'\text{-}5'\alpha} = 6.8$). Consequently, the structure of compound **1** was established as dihydrophaseic acid 4′-*O*-[6″-*O*-(4‴-hydroxy-3‴, 5‴-dimethoxy) benzoyl)]-β-D-glucopyranoside.

Compound **2** was obtained as a white amorphous powder. HR-ESI-MS showed quasimolecular ion at *m/z* 617.2197 $[M + Na]^{+}$ (calcd. for $C_{29}H_{38}O_{13}Na$, 617.2205). According to the HR-ESI-MS, ^{1}H and ^{13}C-NMR spectral data, the molecular formula of **2** was determined to be $C_{29}H_{38}O_{13}$. The molecular mass of **2** was 30 Da lower than that of **1**. Further analysis of ^{1}H-^{1}H COSY, HMBC, and HMQC spectra of **2** also established the ionone-type skeleton with two double bonds and one conjugated carboxyl group, substituted benzoic acid moiety, and a glucose moiety. The difference between **1** and **2** is the substituted benzoic acid moiety. Consequently, the structure of compound **2** was established as dihydrophaseic acid 4′-*O*-[6″-*O*-(3‴-methoxy-4‴-hydroxy) benzoyl)]-β-D-glucopyranoside.

The results of pharmacological experiments in *vitro* showed that compound **1** and **2** displayed moderate scavenging activity in DPPH free radical assay with IC_{50} values of 241 and 264 mM, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotation data were obtained on a Perkin-Elmer 241 automatic digital polarimeter. UV spectral data were obtained on a Shimadzu UV-260 instrument. IR spectral data were acquired on a Perkin–Elmer 599B instrument with KBr disks. Melting points (mp) were determined using an X-4 microscope melting point apparatus (Shanghai Precision Science Instrument Co., Ltd.) and were uncorrected. ^{1}H, ^{13}C NMR, ^{1}H-^{1}H

COSY, HMQC, HMBC and NOESY spectra were recorded on a Bruker DRX-400 spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz). The carbon multiplicities were obtained by DEPT experiment. HRESI-MS data were measured on a SCIEX QTOF-5600 with Triple Quad technology. Reversed-phase chromatography utilized TSK gel Toyopearl HW-40F (30–60 μm, Toso Co., Ltd.), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries Co., Ltd.) and Cosmosil 75 C_{18}-OPN (42–105 μm, Nacalai Tesque Inc.) columns. TLC was performed using precoated silica gel 60 F_{254} plates (0.2 mm, Merck).

3.2. Plant material

The stems of *Z. armatum* were collected from Nanning (located at 22.8° N and 108.3° E, Altitude: 400 m - 600 m), Guangxi Province of China in 2014 and identified by Prof. Ze-Hao Huang (Fujian University of Traditional Chinese Medicine). A voucher specimen of the plant (#1409-21) was deposited in the Herbarium of School of Pharmacy, Fudan University, Shanghai, China.

3.3. Extraction and isolation

Dried stems of *Z. armatum* (20 kg) were extracted with 95% ethanol (50 L × 3) at room temperature. The combined EtOH solvent was filtered and concentrated under reduced pressure to give 680 g of residue. The residue was then suspended in water (2.0 L) and extracted 3 times with petroleum ether. The remaining aqueous solution was concentrated to 1 L. And then, adjusted the pH of the aqueous solution to 2 ∼ 3 with 2% hydrochloric acid and extracted with ethyl acetate (EtOAc) (1 L) for three times to obtain ethyl acetate extract (9.4 g) and water part extract (83 g). The water-soluble extract (83 g) was purified by MCI gel CHP 20P (8 × 60 cm) and eluted with a mixture of MeOH and H_2O (0:10, 1:9, 3:7, 5:5, 7:3, 10:0, 600 mL for each step) to yield 2 fractions (Fr.A and Fr.B) . Fraction A was further chromatographed on Toyopearl HW-40F (6 × 60 cm, eluted with H_2O→10% MeOH) to yield Fr.A 1 and Fr.A 2. Fr.A 1 were purified using Cosmosil 75 C_{18}-OPN (4 × 30 cm, eluted with H_2O→20% MeOH) to yield compound **1** (13.2 mg) and **2** (11.9 mg).

3.4. Characterization

3.4.1. Compound 1

White amorphous powder; [α]20D -5.714° (*c* 0.10, H_2O); mp:135 °C; UV (MeOH): λ_{max} 256, 266 nm; IR ν_{max} (KBr): 3300, 2920, 1700, 1620, 1450 cm^{-1}. HR-ESI-MS *m/z* 647.2295 $[M + Na]^+$ (calcd. for $C_{30}H_{40}O_{14}Na$, 647.2310); ^{1}H and ^{13}C NMR: see Table **S1**.

3.4.2. Compound 2

White amorphous powder; [α]20D -8.333° (*c* 0.12 H_2O); mp:133 °C; UV (MeOH): λ_{max} 256, 267 nm; IR ν_{max} (KBr): 3310, 2918, 1710, 1625, 1455 cm^{-1}. HR-ESI-MS *m/z* 617.2197 $[M + Na]^+$ (calcd. for $C_{29}H_{38}O_{13}Na$, 617.2205); ^{1}H and ^{13}C NMR: see Table **S1**.

3.5. Acid hydrolysis

A solution of compound **1**–**2** (1 mg each) in 5% HCl (0.5 ml) was heated (90 °C) for 2 h. After removing HCl by evaporation in vacuum, the mixture was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 0.1 M NaOH and sugars were detected by TLC analysis with authentic sugar: TLC conditions: $CHCl_3$-MeOH-H_2O (14:6:1), R_f 0.13 (glucose); *n*-BuOH-pyridine-H_2O (6:4:3), R_f 0.37 (glucose). (Çalis and Kırmızıbekmez 2004)

3.6. DPPH radical-scavenging assay

DPPH free radical-scavenging method were adopted as previously described (Wang et al. 2014). The reaction mixtures containing 2.5mL of test samples solution and DPPH (0.025 mg/mL) were incubated for 30 min. Following that, the absorbance at 517 nm was measured and the inhibition (%) of DPPH radical formation was calculated. The concentration required for 50% inhibition (IC_{50}) of DPPH was determined graphically. Ascorbic acid was used as a positive control.

4. Conclusion

The chemical investigation of the stems of *Z. armatum* has resulted in the isolation of two new sesquiterpenoid glycosides, dihydrophaseic acid 4′-*O*-[6″-*O*-(4‴-hydroxy-3‴,5‴-dimethoxy) benzoyl)]- β-D-glucopyranoside (**1**) and dihydrophaseic acid 4′-*O*-[6″-*O*-(3‴-methoxy-4‴-hydroxy) benzoyl)]-β-D- glucopyranoside (**2**).

Disclosure statement

No potential conflict of interest was reported by the authors.

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