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SHORT REPORT

A new isoflavone from the ethyl acetate extracts of *Apios fortunei* Maxim and anti-inflammatory activity

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Abstract: A new isoflavone apiosisoflavone A (**1**) and five known isoflavones (**2**–**6**) were isolated from the ethanol extracts of *Apios fortunei* Maxim. The structures were elucidated by spectroscopic data analysis of NMR, HR-ESI-MS, and experimental and calculated ECD. All compounds were evaluated on the anti-inflammatory activity against RAW264.7 cell lines. The levels of NO and TNF-α released by RAW264.7 cells were detected by the kit. All the compounds displayed moderate anti-inflammatory activity.

Keywords: *Apios fortunei* Maxim, isoflavone, structure elucidation, anti-inflammatory

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1 Plant Source

The roots of *Apios fortunei* Maxim (*A. fortunei*) were purchased from Hunan Heran Biotechnology Development Company for use in this study. It was authenticated by Prof. Hao Liu (Hunan Academy of Chinese Medicine). The specimen of *A. fortunei* (No. 201700908) preserved in the plant specimen chamber was deposited in Institute of Chinese Materia Medica, Hunan Academy of Chinese Medicine.

2 Previous Studies

A. fortunei belongs to Leguminosae Apios Fabr, and is widely distributed in the east asia-north america region and southern area of China. Many species of this genus are rich in starch and contain a variety of vitamins and minerals essential to the human body, and its roots are used as traditional crops for medicine and food. According to the "Compendium of Materia Medica", plants of this genus are also used to sore throat, whooping cough, etc. Previous extensive phytochemical and pharmacological research on this genus, flavonoids, phenylpropanoids, glycosides have been isolated, some of which exhibit bioactivities including anti-inflammatory, anti-tumour, anti-oxidant, hypotensive and hypolipidemic (Kouzuma et al., 2014; Kim & Jin, 2019; Chu et al., 2019b; Ichige et al., 2013; Okubo et al., 1994; Lin et al., 2016; Kim et al., 2017; Kaneta et al., 2016; Chu et al., 2019c, 2019a; Kim et al., 2018; Yan et al., 2017; Sohn et al., 2015; Zhang et al., 2011; Iwai & Matsue, 2007). Currently, the research on this genus was mainly focused on *A. Americana* Medik and *A. taiwaniana* Hosokawa, with less on *A. fortunei*.

3 Present Study

The roots of *A. fortune* (10.0 kg) were exhaustively pulverised and extracted twice with 75% EtOH/H_2O under reflux (100 L × 2h). The combined extracts were concentrated under reduced pressure to yield crude extract (0.8 kg). This extract was suspended in water and sequentially partitioned with ethyl acetate and *n*-butanol. The EtOAc extract (60.0 g) was fractionated by silica gel column chromatography to afford twelve fractions (A–L), eluted with a gradient system of CH_2Cl_2/MeOH (from 100:0 to 0:100). The fraction B was further purified by Sephadex (HW-40C, 80% MeOH/H_2O) and preparative HPLC (3 mL/min, 35% ACN/H_2O, SilGreen C_{18}, 250 mm × 10 mm, 5 μM) to afford compounds **1** (1.5 mg) and **2** (2.1 mg). Fraction E was consecutively subjected on Sephadex HW-40C column chromatography and eluted with MeOH/H_2O (from 40% to 100%, v/v) to afford twenty-one fractions (E1–E21). Fraction E16 was further purified by preparative HPLC (3 mL/min, 35% ACN/H_2O, SilGreen C_{18}, 250 mm × 10 mm, 5 μM) to obtain compound **3** (4.3 mg). Fractions E5 was purified with preparative HPLC (3 mL/min,

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Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** in CD_3OD

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
2	7.95 s	156.0	3′	6.51 d (2.3)	100.3
3		122.5	4′		160.7
4		182.6	5′	6.44 dd (8.2, 2.3)	107.2
5		157.9	6′	7.06 d (8.2)	133.2
6		109.9	1″	2.95 dd (9.6, 15.6)	31.4
				3.36 dd (7.3, 15.6)	
7		167.8	2″	5.39 dd (7.9, 8.8)	89.2
8	6.43 s	89.5	3″		145.1
9		160.0	4″	4.94 brs, 5.10 brs	112.5
10		107.2	5″	1.78 s	16.9
1′		112.0	-OCH_3	3.75 s	55.7
2′		160.3			

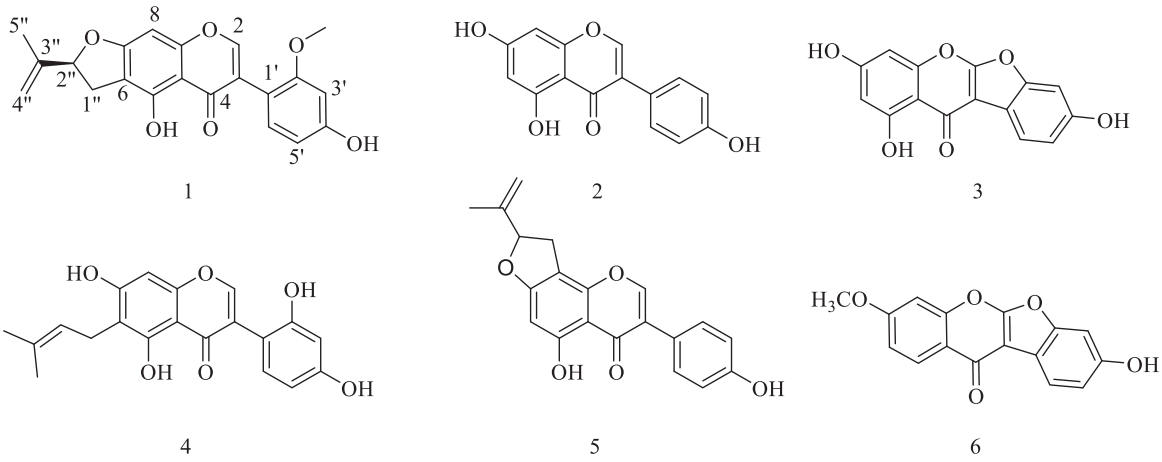

Figure 1. Structure of compounds **1**–**6**

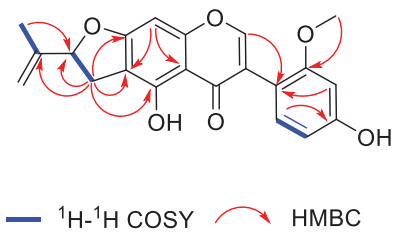

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**

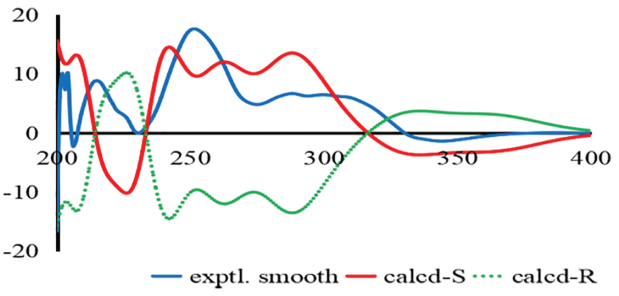

Figure 3. Calculated and experimental CD spectra of **1**

35% ACN/H_2O, v/v) to afford compound **4** (2.0 mg), compound **5** (2.3 mg) and compound **6** (4.1 mg).

Compound **1** was obtained as a white amorphous powder and the molecular formula was determined as $C_{21}H_{18}O_6$ by negative HRESIMS *m/z* 365.1023 $[M\text{-}H]^-$ (calcd for $C_{21}H_{17}O_6$, 365.1025). The ^{1}H-NMR spectrum of **1** (Table 1) exhibited characteristic signals for 1, 2, 4-trisubstituted aromatic proton signals [δ_H 6.51 (1H, d, *J* = 2.3, H-3′), 6.44 (1H, dd, *J* = 2.3, 8.2, H-5′) and 7.06 (1H, d, *J* = 8.2, H-6′)], a aromatic signal [δ_H 6.43 (1H, s, H-8)], three olfinic protons [δ_H 7.95 (1H, s, H-2), 5.10 (1H, brs, H-4″) and 4.94 (1H, brs, H-4″)], a methylene signal [δ_H 2.95 (1H, dd, *J* = 9.6, 15.6, H-1″) and 3.36 (1H, dd, J = 7.3, 15.6, H-1″)], a substituted methyl signal [δ_H 5.39 (1H, dd, *J* = 7.9, 8.8, H-2″)], a methyl signal [δ_H 1.78 (3H, s, H-5″)], and a methoxy signal [δ_H 3.75 (3H, s, 2′-OCH_3)]. The ^{13}C-NMR and and DEPT spectrums of **1** (Table 1) revealed 21 carbon signals, including a isoflavone skeleton, three olefinic, one methylene, one substituted methyl, one methyl and one methoxyl carbon signals. Those 1D NMR data of **1** was similar to that of lupinisoflavone A. The main difference in **1** was the hydroxyl at C-2 was substituted by

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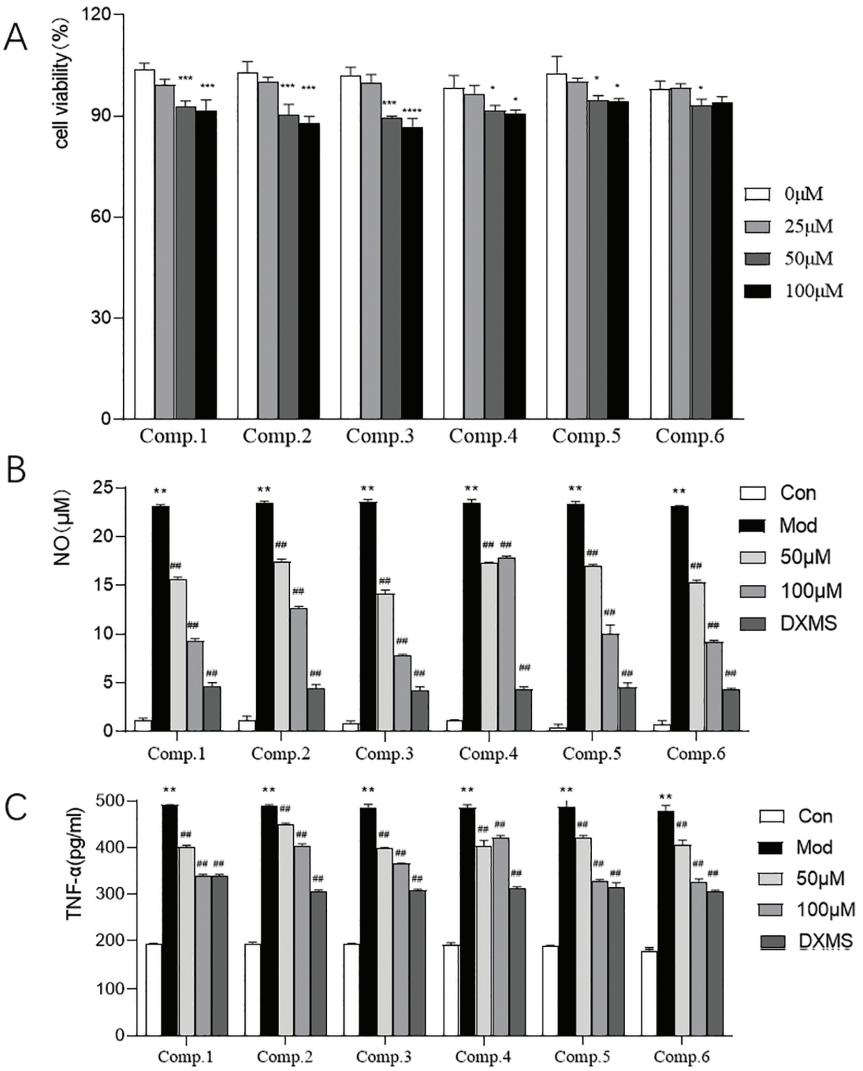

Figure 4. The effect of isoflavonoids on the proliferation activity of RAW264.7 cells and the release levels of NO and TNF-α. (Figure A comparison between each group and the normal group; Figures B and C show the comparison between model group and normal group, as well as the comparison between the administration group and model group. $^{*}p < 0.05$; $^{**}p < 0.01$)

methoxyl group in lupinisoflavone A (Tahara et al., 1984). The existence confirmed by the HMBC correlation between 2′-OCH_3 [δ_H 3.75 (3H, s, 2′-OCH_3)] and C-2′ [δ_c 160.3 (C-2′)] (Figure 2). Additionally, The *S* and *R* enantionmers of **1** were calculated for ECD spectra. By comparing the calculated ECD spectra and experimental data of **1** (Figure 3), the absolute configurations of **1** was determined as *S*-configuration at C-2″. Thus, the structure of **1** was elucidated to be (*S*) 4-hydroxy-6-(4-hydroxy-2-methoxyphenyl)-2-(prop-1-en-2-yl)-2, 3-dihydro-5*H*-furo[3,2-g] chromen-5-one, and named as apiosisoflavone A.

Apiosisoflavone A (**1**): white amorphous powder; $[\alpha]_D^{25}$ + 28.7 (*c* 0.13, MeOH); HPLC-UV (ACN/H_2O) λ_{nm} 261 nM, 285 nM; HRESIMS (negative) *m/z* 365.1023 $[M\text{-}H]^-$, (calculated for $C_{21}H_{17}O_6$, 365.1025); CD (*c* 0.37 mM, MeOH) 250 (+17.68), 350 (−1.14); ^{1}H-NMR (CD_3OD, 600 MHz) and ^{13}C-NMR (CD_3OD, 150 MHz) see Table 1.

The five known compounds (**2**–**6**) were identified by comparing with the reported literature data as genistein (Tahara et al., 1984), lupinalbin A (Yin et al., 2008), luteone (Tahara et al., 1984), vigvexin A (Lin et al., 2016), 7-methoxylupinalbin (Miller, Collini & Harris, 2003), respectively (Figure 1).

The cytotoxicity of the compounds against RAW264.7 cells was assayed using the CCK-8. The effects of each compound on the contents of NO and TNF-α in cells were detected by using the reagent kit. All the compounds displayed moderate anti-inflammatory activity (Figure 4).

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Author Contributions

Jie Xiao and Duzhun Zou: Original draft preparation, performing the experiments. Hongliang Zeng and Yibing Hu:

data analysis. Shuihan Zhang and Fei Cheng: review and editing, funding acquisition, supervision. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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