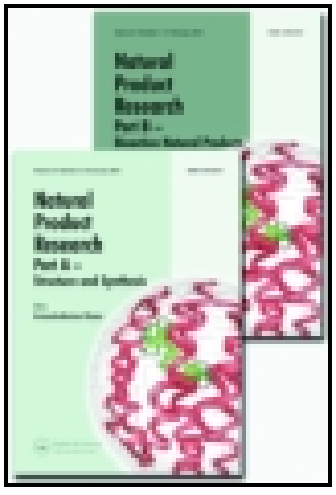

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A new aromatic glucoside from stem bark of Illicium difengpi K.I.B. et K.I.M.

Chuntong Li[ab], Zhijun Wu[b] & Wansheng Chen[b]
[a] Department of Pharmacy and Pharmacology, First Affiliated Hospital of PLA General Hospital, Beijing 100048, P.R. China
[b] Department of Pharmacy, Changzheng Hospital, Second Military Medical University, No. 415, Fengyang Rd, Shanghai 200003, P.R. China
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A new aromatic glucoside from stem bark of *Illicium difengpi* K.I.B. et K.I.M.

Chuntong Li[a,b], Zhijun Wu[b]* and Wansheng Chen[b]*

[a]*Department of Pharmacy and Pharmacology, First Affiliated Hospital of PLA General Hospital, Beijing 100048, P.R. China;* [b]*Department of Pharmacy, Changzheng Hospital, Second Military Medical University, No. 415, Fengyang Rd, Shanghai 200003, P.R. China*

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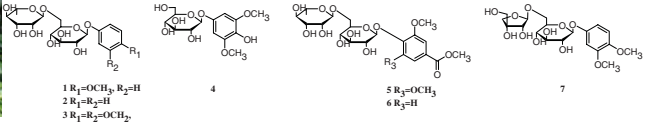

A new aromatic glucoside, namely 4-methoxyphenyl-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**1**), together with six known aromatic glucosides (**2**–**7**) were isolated from the stem bark of *Illicium difengpi*. The structures of these compounds were established by spectroscopic methods. The isolated aromatic glucosides were tested for anti-inflammatory activity. Compounds **1**, **3** and **6** showed significant inhibitory effect on nuclear factor kappa B (NF-κB) in RAW 264.7 macrophages induced by lipopolysaccharide.

Keywords: *Illicium difengpi*; anti-inflammatory; aromatic glucoside

1. Introduction

Illicium difengpi is a small shrub growing in Guangxi province in China, which belongs to the family Illiciaceae. The stem bark of *I. difengpi* was listed in Chinese Pharmacopoeia (Committee 2010). It is an important traditional Chinese medicine and mainly used as a treatment of rheumatic arthritics (RA). The alcoholature of stem bark of *I. difengpi* showed outstanding clinical efficacy and pharmacodynamics potency. Previous studies led to the isolation of phenylpropanoids, lignans, neolignans and triterpenoids from the extract of *I. difengpi* and monoterpenoids and sesquiterpenes from volatility oil of its fruit (Wang et al. 1994; Yao 1996; Huang et al. 1997; Fang et al. 2010; Chu et al. 2011; Li et al. 2013).

As part of an ongoing search for bioactive natural products from folk medicine, we have conducted a phytochemical investigation on BuOH fraction of 80% aqueous EtOH extract of the stem bark. This has led to the isolation of one new and six known aromatic glucosides, named as 4-methoxyphenyl-(6-deoxy-α-L-mannopyranosyl)- β-D-glucopyranoside (**1**), phenyl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**2**), 3,4-dimethoxyphenyl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**3**), 4-hydroxy-3,5-dimethoxyphenyl-β-D-glucopyranoside (**4**), methyl ester-4-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-3,5-dimethoxybenzoic acid (**5**), 4-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]

*Corresponding authors. Email: wuzhijun999@sina.com; chenws126@126.com

oxy]-3-methoxy-benzoic acid (**6**) and 3,4-dimethoxyphenyl-6-*O*-D-apio-β-D-furanosyl-β-D-glucopyranoside (**7**) (Figure 1). These compounds were evaluated for their anti-inflammatory activity. This paper reports the isolation, structure elucidation and anti-inflammatory activity of these aromatic glucosides.

2. Results and discussion

The 80% ethanol extract of stem bark of *I. difengpi* was suspended in water and extracted sequentially with petroleum ether, EtOAc and *n*-BuOH, respectively. The *n*-BuOH fraction was subjected to column chromatography (CC) to yield compound **1** and six known aromatic glucosides (**2**–**7**).

Compound **1** was obtained as white amorphous powder and its molecular formula was determined to be $C_{19}H_{28}O_{11}$ by HR-ESI-MS data (*m/z* 477.1620 $[M + COOH]^-$, calc. for $C_{19}H_{28}O_{11}$ 432.1632), requiring six degrees of unsaturation. The ^{13}C NMR and DEPT spectra of **1** exhibited 17 carbon resonances, classified into 2 quaternary carbons, 12 methines, 1 methylenes, 1 methyl and 1 methoxyl. The ^{13}C NMR spectrum showed particularly intense signals at δ_C 119.2 and δ_C 115.4 accounting for two magnetically equivalent carbons each (C-3 + C-5 and C-2 + C-6). These aromatic carbons together with the quaternary carbons δ_C 153.1 (C-1) and δ_C 156.7 (C-4) gave evidence of a opposite-substituted benzene. The ^1H and ^{13}C NMR spectra indicated that **1** contained a methoxyl group (δ_H 3.70, 3H, s; δ_C 56.0) connected to the benzene group which was confirmed by HMBC correlation from OCH_3 (δ_H 3.70) to C-4 (δ_C 156.7). Comparison of the ^{13}C NMR spectrum of **1** with that of compound **2** (Feng et al. 2010) indicated that the structure of the aglycone moieties of **1** was identical to those of compound **2**. In the HMBC experiment (Figure S1), the correlation from H-1′ (δ_H 4.67) to C-1 (δ_C 153.1) was observed, indicating that a glucopyranosyl group was attached to C-1 of the benzene. The correlation from H-6′ (δ_H 3.55) to C-1″ (δ_C 102.2) indicated that the terminal mannopyranosyl group was connected to the glucose unit by 6′ → 1″. The structure of **1** was established on the basis of 1D and 2D NMR spectra (^1H, ^{13}C, DEPT, COSY, HSQC and HMBC). The absolute configurations of glucopyranosyl and mannopyranosyl were further verified by acid hydrolysis

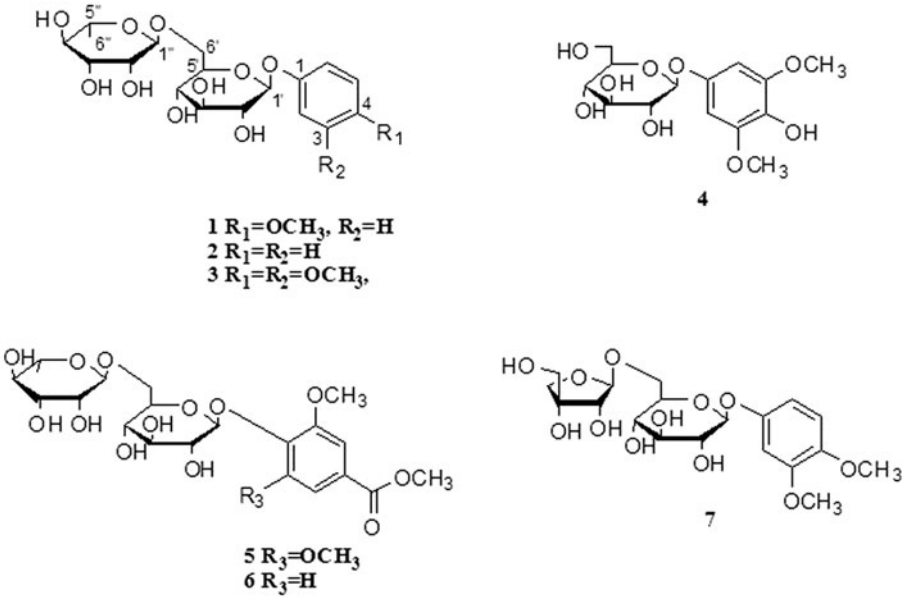

Figure 1. Structures of compounds **1**–**7**.

and chiral GC-MS analysis. Therefore, the structure of **1** is assigned as 4-methoxyphenyl-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside.

In addition to the new aromatic glucoside (**1**), six known aromatic glucosides phenyl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**2**) (Feng et al. 2010), 3,4-dimethoxyphenyl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**3**) (Graikou et al. 2005), 4-hydroxy-3,5-dimethoxyphenyl-β-D-glucopyranoside (**4**) (Rojas et al. 2000), methyl ester-4-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-3,5-dimethoxybenzoic acid (**5**) (Ma et al. 2011), 4-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-3-methoxybenzoic acid (**6**) (Ma et al. 2011) and 3,4-dimethoxyphenyl-6-*O*-D-apio-β-D-furanosyl-β-D-glucopyranoside (**7**) (Warashina et al. 2004) were isolated from *I. difengpi*. These compounds were identified by spectral analysis and compared with spectroscopic data reported in the literatures.

The anti-inflammatory activities of compounds **1**–**7** were determined by measuring luciferase in RAW 264.7 cells stimulated with lipopolysaccharide (LPS) *in vitro* (Li et al. 2013; Huang et al. 2014). Tripterygium tablets (TRT) and total glucosides of paenia (TGP) were used as positive controls. The cytotoxic effects of tested compounds on LPS-stimulated RAW 264.7 cells were determined initially by MTT assay. The results showed that compounds **1**–**7** did not affect cell viability at concentrations up to 25 μg/mL. As shown in Figure 2, the concentrations of NF-κB in the RAW 264.7 cells pretreated with compounds **1**, **3** and **6** at 10 μg/mL were reduced by 64%, 59% and 46%, respectively, compared to LPS stimulated RAW 264.7 cells, while the inhibitory rate (IR) of two positive controls TRT and TGP were 82% and 61%, respectively (Figure 2). The results demonstrated that compounds **1**, **3** and **6** showed a significant inhibition of NF-κB production in LPS-stimulated RAW 264.7 cells (Figure 2).

3. Experimental

3.1. *General experimental procedures*

CC was performed on Sephadex LH-20 gel (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), *YMC*-GRL ODS-A (50 μm; *YMC*, MA, USA) and silica gel H (100–200 and 200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China). TLC analyses were

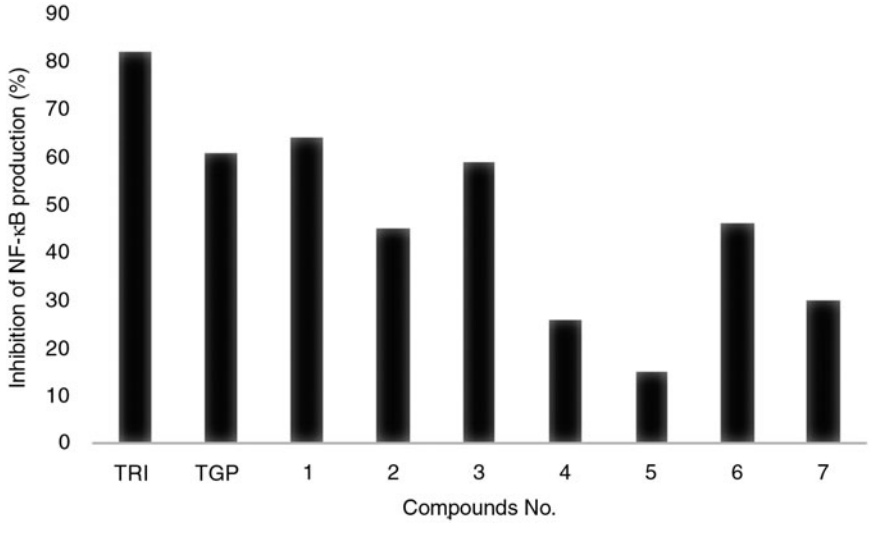

Figure 2. IR of NF-κB production (%) from LPS stimulated RAW 264.7 cells by compounds **1**–**7** at a concentration of 10 μg/mL.

performed on Si 60 GF_{254} plates and visualised under UV light or by heating after spraying with 10% H_2SO_4/EtOH solution. 1D and 2D NMR spectra were recorded on an American Varian Mercury Plus 400 NMR spectrometers (Agilent Technologies LDA UK Limited, Stockport, Cheshire, UK). HR-ESI-MS were acquired on an Agilent 6220 TOF LC-MS (Agilent Technologies Singapore (Sales) Pte Ltd, Singapore) instrument. GC-MS was conducted on an Thermo Finnigan Trace GC (Thermo Technologies Co., San Jose, CA, USA) apparatus using an L-Chirasil-Val column (25 m × 0.32 mm).

3.2. *Plant material*

The stem barks of *I. difengpi* were purchased from Caitongde Pharmacy, Shanghai, China, in January 2010. Plant material was authenticated by Professor Lianna Sun, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University. A voucher specimen (NO. 20100110) has been deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University.

3.3. *Extraction and isolation*

The air-dried stem bark of *I. difengpi* (40 kg) was powdered and extracted three times with 80% ethanol under reflux. The solvent was concentrated to obtain a crude extract (1200 g), which was suspended in water (10 L) and extracted with petroleum ether, EtOAc and BuOH, respectively. The Fr 3 of BuOH section (40 g) was subjected to silica gel CC gradient with CH_2Cl_2/MeOH (100:1 to 0:100) to give four subfractions (Fr_{3-1}–Fr_{3-4}). Fr_{3-1} (6 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (9:1:0.25) to afford compound **4** (1.0 mg). Fr_{3-2} (8 g) was applied to ODS CC eluted gradient with MeOH/H_2O (50:50 to 100:0) to afford four subfractions (Fr_{3-2-1}–Fr_{3-2-4}). Fr_{3-2-3} (2.6 g) was purified by Sephadex LH-20 CC eluted with MeOH/H_2O (50:50) to yield compound **5** (28.9 mg) and **7** (18 mg). Fr_{3-3} (8.6 g) was subjected to Sephadex LH-20 eluted with MeOH/H_2O (50:50) to yield a mix of **2** and **3**, which was separated with ODS CC eluted with MeOH/H_2O (50:50 to 75:25) to give **2** (34 mg) and **3** (34 mg). Fr_{3-4} (7.4 g) was subjected to Sephadex LH-20 eluted with MeOH/H_2O (50:50) to afford four subfractions (Fr_{3-4-1}–Fr_{3-4-4}), then Fr_{3-4-3} (2.2 g) was subjected to ODS CC eluted with MeOH/H_2O (40:60 to 75:25) to afford **6** (34.2 mg) and **1** (33.5 mg).

3.4. *Acid hydrolysis of compounds*

Compound **1** was heated in 2 M HCl (1 mL) at 120°C for 1.0 h. The mixture was concentrated and the residue was dissolved in 1-trimethylsilyl imidazole and pyridine (0.2 mL), and the solution was stirred at 60°C for 5 min. After drying the solution, the residue was partitioned between CH_2Cl_2 and H_2O (1 mL, 1:1 v/v). The organic phase was submitted to GC-MS analysis using an L-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200°C for both. A temperature gradient system was used for the oven, starting at 100°C for 1 min and increasing up to 180°C at a rate of 5°C/min. Retention times for authentic samples after being treated in the same manner with 1-trimethylsilyl imidazole in pyridine. D-glucose and L-rhamnose were identified by comparing the retention time with those of authentic D-glucose and L-rhamnose after treatment using the same method.

3.5. *Characterisation of compounds*

4-Methoxyphenyl-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**1**): amorphous powder; $[\alpha]_D^{25} + 8.9$ ($c = 0.20$, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): δ: 1.17 (3H, d, $J = 6.2$ Hz, H-6″), 3.30 (1H, m, H-5′), 3.33 (1H, m, H-3′), 3.36 (1H, m, H-4″), 3.38 (1H, m, H-4′), 3.46 (1H, m, H-

2′), 3.55 (1H, dd, $J = 11.0$ and 6.6 Hz, H-6′a), 3.60 (1H, m, H-5″), 3.65 (1H, dd, $J = 9.5$ and 3.5 Hz, H-2″), 3.70 (3H, s, 4-OCH_3), 3.79 (1H, dd, $J = 3.3$ and 1.7 Hz, H-3″), 3.96 (1H, d, $J = 11.0$ and 1.6 Hz, H-6′b), 4.66 (1H, d, $J = 1.2$ Hz, H-1″), 4.67 (1H, d, $J = 7.2$ Hz, H-1′), 6.80 (2H, d, $J = 9.6$ Hz, H-2,6), 6.98 (2H, d, $J = 9.6$ Hz, H-3,5); ^{13}C-NMR (CD_3OD, 100 MHz): δ: 153.1 (C-1), 115.4 (C-2,6), 119.2 (C-3,5), 156.7 (C-4), 103.4 (C-1′), 74.0 (C-2′), 76.8 (C-3′), 71.6 (C-4′), 78.0 (C-5′), 67.9 (C-6′), 102.2 (C-1″), 72.4 (C-2″), 72.2 (C-3″), 74.9 (C-4″), 69.8 (C-5″), 18.0 (C-6″), 56.0 (4-OCH_3). HR-ESI-MS, *m/z* 477.1620 $[M + COOH]^-$ (calc. for $C_{19}H_{28}O_{11}$ 432.1632).

4. Conclusion

A new aromatic glucoside, 4-methoxyphenyl-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**1**), together with six known aromatic glucosides (**2**–**7**) was obtained from the stem bark of *I. difengpi*. Compounds **1**, **3** and **6** showed significant inhibitory effect on nuclear factor kappa B (NF-κB) in RAW 264. 7 macrophages induced by LPS with inhibitory rates of 64%, 59% and 46%, respectively. The observed potential anti-inflammatory activity warrants further investigations.

Supplementary meterial

Supplementary data (Figure S1, 1D, 2D NMR spectra and HR-ESI-MS data of compound **1**) associated with this article are available online.

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