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One new flavonoid from *Oroxylum indicum*

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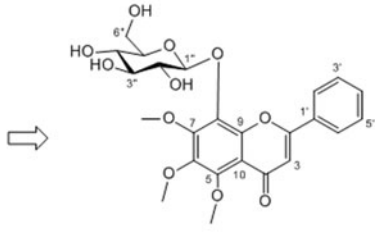

One new flavonoid, 5,6,7-trimethoxyflavone-8-*O*-*β*-D-glucopyranoside (**1**), along with six known compounds **2**–**7**, was isolated from *Oroxylum indicum*. Their structures were determined on the basis of spectral data. The antibacterial activities of compounds **1**–**4** were studied. Compounds **1** and **3** showed medium antibacterial activity against *Staphylococcus aureus* with MIC/MBC at 32–128 μg/ml.

Keywords: *Oroxylum indicum*; flavonoid; antibacterial activity

1. Introduction

Oroxylum indicum is a valuable traditional Chinese herbal medicine and Dai medicine, which distributes in Yunnan and Guangxi provinces of China (Flora of China Editorial Committee 1990). The dry seeds and bark of *O. indicum* were used to treat acute cough, sore throat and so on. *O. indicum* has drawn considerable research interest because of its many observed biological activities: anti-inflammatory, anti-pyretic and anti-hypersensitivity effects, inhibited *in vitro* proliferation of HL-60 cells (Kumar Roy et al. 2007; Siriwatanametanon et al. 2010). In the previous studies, flavonoids aglycones (mainly chrysin and baicalein) and certain glucosides of the flavonoids (e.g. baicalin) have been found in this plant (Subramanian & Nair 1972; Chen et al. 2003; Dinda et al. 2007, 2012).

As a part of our ongoing search for bioactive secondary metabolites from Chinese tropical medicinal plants, a careful investigation on the chemical constituents of the barks of *O. indicum* led to the isolation and identification of one new compound, 5,6,7-trimethoxyflavone-8-*O*-β-D-glucopyranoside (**1**), together with six known compounds(see Figure S1), Oroxylin A-7-*O*-β-D-glucuronide butyl ester (**2**) (Chen et al. 2012), 6-methoxy-baicalein (**3**) (Collado et al. 1985),

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oroxylin-A-7-*O*-glucoside (**4**) (Nicollier et al. 1981), 5,7-dihydroxy–flavone (**5**) (Chauhan et al. 1984), uracil (**6**) (Wu et al. 2011) and baicalein 6-methoxy-7-glucuronide (**7**) (Flamini et al. 2002). The details of the isolation and structural elucidation of the new compound **1** from *O. indicum* are presented in this article. Moreover, we report the antibacterial activities of compounds **1**–**4**.

2. Results and discussion

Compound (**1**) was obtained as a yellow amorphous powder. The molecular formula was determined as $C_{24}H_{26}O_{11}$ on the basis of the $[M]^+$ peak in the HR-ESI-MS spectrum at *m/z* 490.1490. The UV spectrum showed absorption maxima at 304.40, 269.80 and 204.00 nm, while the IR spectrum suggested the presence of OH groups (3441.71 cm^{-1}) and α,β-unsaturated C═C group (1632.09 cm^{-1}), and a conjugated olefinic bond (1592.98 cm^{-1}).

The ^{1}H NMR spectra of **1** showed three ABX coupling aromatic H-atoms at 7.52–7.59 (*m*, 3H, H-3′, 4′, 5′), which suggested a typical flavonoid nucleus with an unsubstituted B ring. A single signal coupled with one proton was visible at 6.86. This situation is typical of a flavonoid nucleus with an unsubstituted C ring. The ^{1}H NMR spectra of **2** also showed five H-atoms in the range of δ (H) 3.38–4.95, and three methoxy group at δ (H) 3.80, 3.85and 4.02.

The ^{13}C NMR spectra of **1** were similar to **2** in rings B and C. The ^{13}C NMR spectrum showed 24 signals, sorted by DEPT experiments into 3 CH_3, 1 CH_2, 11 CH and 9 quaternary C. The quaternary C signals δ (C) 151.63, 147.80, 143.82 and 134.13 were typical of (C-7), (C-5), (C-6) and (C-8), respectively, of a 5,6,7,8-*O*-substituted flavonoid moiety. Based on comparison of the NMR spectral data with those reported in the literature, the signals of six O-bearing C-atoms at 103.47 (C-1″), 77.29 (C-5″), 76.32 (C-3″), 74.14 (C-2″), 70.05 (C-4″) and 61.87 (C-6″) were consistent with the β-D-glucuronide group. The HMBC from the signal δ (H) 4.95 (*d*, $J = 7.7$ Hz, 1H-1″) to that of δ (C) 134.13 (C-8) indicated that the β-D-glucuronide methyl ester group was linked to the baicalein group through C(1″)–O–C(7). Moreover, the HMBC showed the correlations from the signal δ (H) 4.02 (*s*, 3H, C7—OCH_3) to δ (C) 151.63 (C-7), from δ (H) 3.84 (*s*, 3H, C6—OCH_3) to δ (C) 143.82 (C-6) and from δ (H) 3.80 (*s*, 3H, C5—OCH_3) to δ (C) 147.80 (C-5) (Figure S2). Thus, the structure of **1** was determined as 5,6,7-trimethoxyflavone-8-*O*-β-D-glucopyranoside.

Compound **2** was isolated as a yellow amorphous powder. The molecular formula was determined as $C_{26}H_{28}O_{11}$ on the basis of the $[M]^+$ peak in the HR-ESI-MS spectrum at *m/z* 516.1639. The UV spectrum showed absorption maxima at 276.50 and 213.00 nm, while the IR spectrum suggested the presence of OH groups (3442.56 cm^{-1}), an ester CO group (1739.13 cm^{-1}) and α,β-unsaturated C═C group (1656.64 cm^{-1}), and a conjugated olefinic bond (1618.80 cm^{-1}).

The ^{1}H NMR spectra of **2** showed three ABX coupling aromatic protons at 7.66–7.58 (m, 3H, H-3′, 4′, 5′), five protons in the range of δ (H) 3.38–5.35, four chain protons in the range of δ (H) 0.78–4.10 and one methoxy group at δ (H) 3.76. A single signal coupled with one proton was visible at δ (H) 7.06. This situation is typical of a flavonoid nucleus with an unsubstituted C ring.

The ^{13}C NMR spectra of **2** were similar to those of baicalein-6-methylether-7-*O*-β-galactopyranuronoside (Flamini et al. 2002). A major difference was in the signals of 64.32 (C-1‴), 29.96 (C-2‴), 18.41 (C-3‴) and 13.41 (C-4‴). The ^{13}C NMR spectrum showed 26 signals, sorted by DEPT experiments into 2 CH_3, 3 CH_2, 12 CH and 9 quaternary C. The ^{13}C NMR spectrum confirmed the identification of baicalein skeleton. The quaternary C signals δ (C) 156.15, 152.24, 132.63 and 94.22 were typical of (C-7), (C-5), (C-6) and (C-8) of a 5,6,7-O-substituted flavonoid moiety. Based on comparison of the NMR spectral data with those reported in the literature, the signals of seven O-bearing C at 168.63 (C-6″), 99.50 (C-1″), 75.73 (C-5″),

75.26 (C-3″), 72.80 (C-2″), 71.10 (C-4″) and 64.32 (C-1‴), 29.96 (C-2‴), 18.41 (C-3‴), 13.41 (C-4‴) were consistent with the β-D-glucuronide butyl ester group (Woo and Piao 2004; Yan et al. 2014). The HMBC from the signal δ (H) 5.35 (*d*, $J = 7.1$ Hz, 1H-1″) to δ (C) 156.15 (C-7) indicated that the β-D-glucuronide methyl ester group was linked to the baicalein group through C(1″)—O—C(7). The HMBC showed that the signal δ (H) 3.76 (*s*, 3H, C6—OCH_3) was related to δ (C) 132.63 (C-6). Moreover, the ROESY showed that the signal δ (H) 5.35 (*d*, $J = 7.1$ Hz, H—C(1″)) was related to δ (H) 7.10 (*s*, H—C(8)) (Figure S2). Thus, the structure of **2** was determined as Oroxylin A-7-*O*-β-D-glucuronide butyl ester. Although **2** was already known, the structure data of **2** were useful for compound **1**.

The compounds **1**–**4** were individually tested against four microbial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC Y0109), which were provided by the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, China). The minimal inhibitory concentration (MICs) and minimal bactericidal concentrations (MBCs) or minimal fungicidal concentrations (MFCs) were determined by standardised broth microdilution techniques with starting inoculums of 5×10^5 cfu/ml according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2006; Meng et al. 2009; National Committee for Clinical Laboratory Standards 1999; Zuo et al. 2008). Compounds **1** and **3** showed medium antibacterial activity against the *S. aureus* strain (Table S2).

3. Experimental

3.1. *General experimental procedures*

Optical rotations were taken on a Jasco DIP-370 digital polarimeter. UV spectra were measured on a Shimadzu-UV-2401A spectrophotometer with methanol as the solvent. Infrared (IR) spectra were recorded on a Bio-Rad-FTS-135 spectrometer in KBr pellets. 1D and 2D NMR spectra were obtained on a Bruker-DRX-500 spectrometer with chemical shifts recorded in δ (ppm) using tetramethylsilane (TMS) as the internal standard, while the coupling constants (*J*) were given in hertz. Mass spectra were obtained on a MS Waters AutoSpec Premier P776 mass spectrometer (EI-MS) and a Micro Q-TOF MS (HERSIMS). Column chromatography was run on silica gel (200–300 mesh; 10–40 mm) (Qingdao Marine Chemical Inc., P.R. China), Lichroprep RP-18 gel (40–63 mm) (Merck) and Sephadex LH-20 (Pharmacia). Fractions were monitored by thin-layer chromatography (TLC) and spots were visualised by heating silica gel plates sprayed with 10% H_2SO_4/H_2O.

3.2. *Plant material*

The bark of *O. indicum* were collected from Xishuangbanna, Yunnan Province, P.R. China, in August 2012, and authenticated by Professor Qi-Shi Song, Xishuangbanna Tropical Botanical Garden. A voucher specimen (no. 20120811) was deposited at the Research Group on Ethnomedicine of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

3.3. *Extraction and isolation*

The air-dried and powdered bark of *O. indicum* (10 kg) were extracted with 95% aqueous ethanol and filtered at room temperature. The filtrate was concentrated and extracted with petroleum ether, then extracted by $CHCl_3$ and BuOH. The BuOH extract (361.7 g) was subjected to silica gel column chromatography eluted with a MeOH/$CHCl_3$ (1:30–1:1) gradient system to furnish seven fractions, G1–G7. All fractions were collected and combined by TLC monitoring. Fraction G4 (61 g) was further chromatographed over silica gel using MeOH/$CHCl_3$ (1:30) and

Sephadex LH-20 (MeOH) to yield compound **1** (254 mg). Fraction G3 (74 g) was further chromatographed over silica gel using MeOH/$CHCl_3$ (1:30) to give **2** (350 mg). Compounds **3**–**7** were obtained from G5–G7 in a similar fashion.

5,6,7-Trimethoxyflavone-8-*O*-β-D-glucopyranoside (= 2-phenyl-5,6,7-trimethoxy-8-(6-β-D-glucopyranoside)-4*H*-1-benzopyran-4-one) (**1**): Yellow amorphous powder. UV λ_{max} (MeOH) (log ε): 304.40 (2.43), 269.80 (2.95), 204.80 (3.23) nm. IR (neat)max: 3441.71, 2938.23, 1632.09, 1463.19, 1369.63, 1072.92 cm^{-1}. For ^{1}H NMR (600 MHz, DMSO) and ^{13}C NMR (125 MHz, DMSO), see Table S1. HRESI [M] $^{+}$: 490.1490 *m/z* for $C_{24}H_{26}O_{11}$ (calcd. 490.1475).

Oroxylin A-7-*O*-β-D-glucuronide butyl ester (= 2-phenyl-5-hydroxy-6-methoxy-7-[(6-butyl-β-D-glucopyranuronosyl)oxy]-4*H*-1-benzopyran-4-one) (**2**): Yellow amorphous powder. UV λ_{max} (MeOH) (log ε): 276.50 (2.80), 213.00 (3.10) nm. IR (neat)max: 3442.56, 2047.75, 1739.13, 1656.64, 1618.80 cm^{-1}. For ^{1}H NMR (600 MHz, DMSO) and ^{13}C NMR (150 MHz, DMSO), see Table S1. HRESI [M] $^{+}$: 516.1639 *m/z* for $C_{26}H_{28}O_{11}$ (calcd. 516.1632).

3.4. *Antibacterial assays*

3.4.1. *Bacterial strains*

Four microbial strains: *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *C. albicans* (ATCC Y0109), were provided by the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, China). Vancomycin (VAN) (Eli Lilly Japan K. K., Seishin Laboratories) was used as the positive control agent.

3.3.2. *Media*

Standard Mueller–Hinton agar and broth (MHA and MHB, Tianhe Microbial Agents Co., Hangzhou, China) were used as bacterial culture media. MHB was used for all susceptibility testing and time–kill experiments. Colony counts were determined using MHA plates.

3.3.3. *Assay of MIC and MBC*

The minimal inhibitory concentrations (MICs)/minimal bactericidal concentrations (MBCs) or minimum fungicidal concentrations (MFCs) were determined by standardised broth microdilution techniques with starting inoculums of 1×10^{6} cfu/ml or 1.0×10^{4} cfu/ml according to CLSI guidelines. Briefly, stock solutions (30 mg/ml in 50% DMSO) of all extracts were passed through a pyrogenic filter to sterilise the solution and serially diluted to arrive at concentrations between 30 mg/ml and 0.1 mg/ml (100 μg/ml and 0.2 μg/ml for vancomycin). The 96-well plates were prepared by dispensing into each well 100 μl each of an appropriate medium, test compounds and 20 μl of the inoculum. A standard nutrient MHB was employed for the bacterial assays. The growth of the micro-organisms was determined by turbidity. Clear wells indicated the absence of bacterial growth. For every experiment, a sterility check (50% DMSO and medium), negative control (50% DMSO, medium and inoculum) and positive control (50% DMSO, medium, inoculum and vancomycin) were included. The microtitre plates were incubated at 35°C for 24 h and were examined for growth in daylight. The MIC of the preparations was the lowest concentration in the medium that completely inhibited the visible growth. The solvent value was deducted accordingly to get the final results of activity. All experiments were performed in triplicate.

4. Conclusions

O. indicum is a valuable Dai medicine; one new flavonoid, 5,6,7-trimethoxyflavone-8-*O*-β-D-glucopyranoside (**1**), along with six known compounds **2**–**7**, was isolated from *O. indicum*.

Compounds **1** and **3** showed medium antibacterial activity against *S. aureus* with MIC/MBC at 32–128 μg/ml. We conclude that the findings may offer new evidences that why *O. indicum* was used widely in Dai medicine.

Supplementary material

Supplementary material relating to this article is available online, alongside Tables S1–S2, Figures S1–2 and the original spectra of compound **1**.

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