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A new glucopyranoside from the leaves of *Microsorium fortunei*

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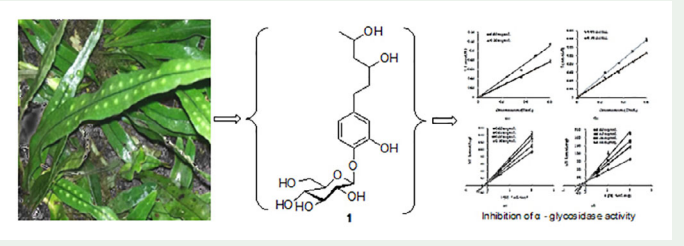
ABSTRACT

A new compound, 2-hydroxy-4-[3′,5′-dihydroxyhexyl]phenyl-β-D-glucopyranoside (**1**), together with five known compounds (**2**–**6**), were isolated from the leaves of *Microsorium fortunei*. Their structures were determined by spectroscopic techniques, especially 2D NMR and MS data analyses. All of these compounds are phenolic glycosides and were isolated from this plant for the first time. In addition, compound **1** showed moderate inhibitory activity against α-glucosidase with IC_{50} value at 0.111 ± 0.061 mg/mL.



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Microsorium fortunei; glucopyranoside; phenolic glycoside; α-glucosidase

1. Introduction

Microsorium fortunei (T. Moore) Ching (=*M. fortunei*), belonging to the family Polypodiaceae, grows in south of the Yangtze river (Sun and Cheng. 2004). The whole plant can be used to treat pain of body surface or internal organs and snake bite, scorpion venom, burns, etc. (Yao et al. 2005). It is also used for the treatment of stomach cold pain, bronchial asthma, rheumatic pain, gonococcal disease and dysentery by some ethnic minorities, such as Tujia, Miao, Mulao and Lisu nationality (Wei et al. 1999). Several biological activities have been reported for extracts of this plant such as cure jaundice, urinary tract infection and bruises (Wu et al. 2015). Some flavonoids and phenolic acids were found from this species in our previous phytochemical

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studies (Liang et al. 2017). Motivated by a search for bioactive metabolites from this plant, a reinvestigation of the chemical constituents was carried out. As a result, an unprecedented glucopyranoside (**1**) was isolated from this plant. In this paper, we report the isolation, structure elucidation, and biological activities of the new compound.

2. Results and discussion

The 75% ethanol extract of the leaves of *M. fortunei* was partitioned with EtOAc and n-BuOH, successively. The n-BuOH-soluble portion was separated by a combination of silica gel, repeated medium-pressure liquid chromatography and HPLC to afford a new glucopyranoside (**1**) and five known compounds (**2**–**6**) (Figure 1). The known compounds, compared with literature, were identified as 4-*O*-β-D-glucopyranosyl- caffeic acid (**2**) (Cui et al. 1990), baihuaqianhuoside (**3**) (Kong et al. 1993), isovitexin (**4**) (Calis et al. 2007), lispedin (**5**) (Fátima et al. 2003), 4,4′-dimethoxy-3′-hydroxy-7, 9′,7′,9-diepoxylignan-3-*O*-β-D-glucopyranoside (**6**) (Li et al. 2003).

Compound **1** was obtained as a yellow amorphous powder with $[\alpha]20\ D - 37.3$ (*c* 1.0, MeOH). Its molecular formula was established as $C_{18}H_{28}O_9$ by HRESIMS at *m/z* 411.1831 $[M + Na]^+$ (calcd for 411.1631), indicating five degrees of unsaturation. The IR spectrum showed absorption bands of hydroxy (3368 cm^{-1}) and benzene group (1508 and 1596 cm^{-1}). The ^{1}H-NMR spectrum (Figure S4) showed the presence of three aromatic protons at δ_H 6.61 (dd, $J = 8.2$, 2.1 Hz, 1 H), 6.69 (d, $J = 2.1$ Hz, 1 H) and 7.06 (d, $J = 8.2$ Hz, 1 H), one methyl at δ_H 1.15 (d, $J = 6.3$ Hz, 3 H), and an anomeric proton at δ_H 4.68 (d, $J = 7.2$ Hz, 1 H). The ^{13}C NMR and DEPT spectra (Figure S5) exhibited 18 carbon resonances, including one methyl carbon (δ_C 24.3), three methylene carbons (δ_C 47.3, 41.0, 32.4), one oxymethylene carbon (δ_C 62.4), seven oxymethine carbons (δ_C 104.7, 78.2, 77.6, 74.9, 71.3, 68.7, 65.5), six phenyl carbons (δ_C148.3, 144.9, 139.6, 120.9, 119.1, 117.2). These data indicated there was a benzene ring and a sugar unit.

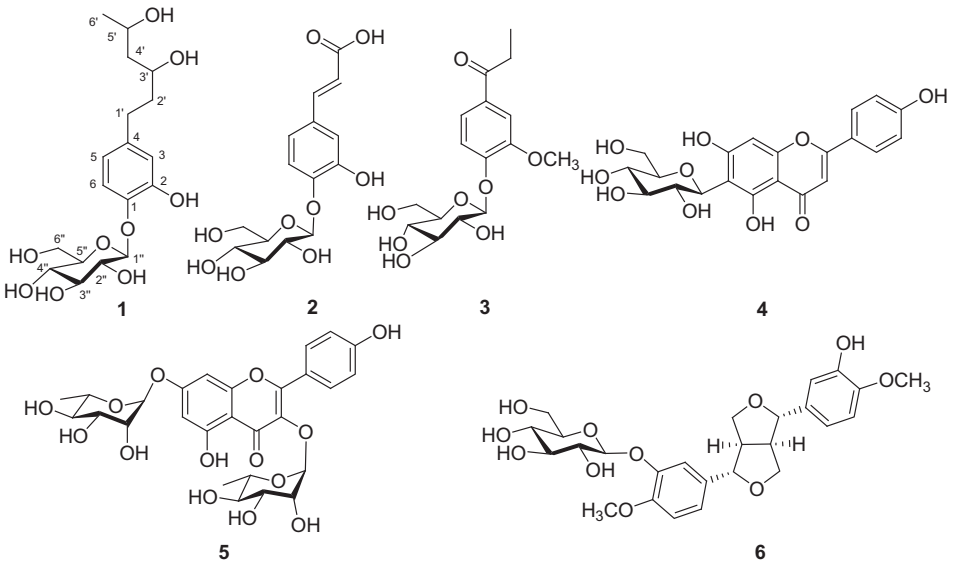

Figure 1. Structures of compounds **1**-**6** from *M. fortunei*.

The signals of the three aromatic protons at δ_H6.61 (1 H, dd, $J = 8.2$, 2.1 Hz, H-5), 6.69 (1 H, d, $J = 2.1$ Hz, H-3) and 7.06 (1 H, d, $J = 8.2$ Hz, H-6) constituteda classical ABX system, indicated the presence of a 1,3,4-trisubstituted aromatic ring. The aromatic ring was also established through the HMBC correlations (Figure S1) of H-3 to C-1 (δ_C 144.9), C-2 (δ_C 148.3) and C-5 (δ_C 120.9), H-5 to C-1 (δ_C 144.9) and C-3 (δ_C 117.2) and H-6 to C-1 (δ_C 144.9), C-2 (δ_C 148.3) and C-4 (δ_C 139.6). The sugar unit was confirmed to be D-glucose based on acid hydrolysis of compound **1** and HPLC analysis of its derivative (Supporting information). The configuration of the D-glucose was determined to be β from its anomeric proton coupling constant at δ_H 4.68 (1 H, d, $J = 7.2$ Hz, H-1′′) (Agrawal 1992). And the HMBC correlations of H-1′′ with C-1 (δ_C144.9) indicated the sugar unit was located at C-1.

Extensive analysis of the NMR spectroscopic data of **1** showed a close resemblance with 2-hydroxy-4-[(3S)−3-hydroxybutyl]phenyl-β-D-glucopyranoside (Shimoda et al. 2007). The most prominent differences of them were the branched chains attached to the aromatic ring. The correlations of H_2-1′/H_2-2′/H-3′/H_2-4′/H-5′/H_3-6′ in the ^{1}H-^{1}H COSY spectrum (Figure S1), indicated that the branched chain of **1** was a 3′,5′-dioxyhexylmoiety. The HMBC correlations of H_2-1′ [δ_H 2.64 (m, 1 H) and δ_H 2.52 (m, 1 H)] with C-3 (δ_C 117.2), C-4 (δ_C 139.6) and C-5 (δ_C 120.9), indicated the 3′,5′-dioxyhexyl moiety was located at C-4.

Considering the molecular formula of compound **1**, in addition to one hydroxyl group attached to C-2 on the benzene ring, there should be two other hydroxyl groups which attached to C-3′ and C-5′, respectively. On the basis of the above analysis, the structure of compound **1** was determined to be 2-hydroxy-4-[3′,5′-dihydroxyhexyl] phenyl-β-D-glucopyranoside.

In addition, there is a pair of intersite hydroxyl groups in 3′,5′-dihydroxyhexyl moiety, the relative configuration of them still could not be determined by ROESY, Mosher's, induced CD, and dimolybdenum tetraacetate methods (Li and Cui. 2015). Thus, the structure still require solid evidence, such as X-ray analysis, or comparison with synthetic compounds, for unequivocal assignment. Unfortunately, we could not obtain crystal for X-ray analysis, and synthesize the corresponding compounds for the comparison.

The α-glucosidase inhibitory effect of compound **1** was evaluated along with the clinical α-glucosidase inhibitor acarbose. The results showed the inhibition effect of compound **1** was found to be dose-dependent like as acarbose (Figure S2a) with IC_{50} value of 0.111 ± 0.061 mg/mL (acarbose = 0.235 ± 0.022 mg/mL). According to in Supplementary information Figure S2b, when the mass concentration of compound **1** was 0.5 mg/mL, the enzyme activity could be rapidly inhibited within 1 min, and the inhibition rate can reach a maximum, and then decreased. In addition, in the enzyme kinetics experiment, the results showed that compound **1** is a reversible inhibitor, because the increment in compound **1** concentration resulted in lowering the slope of the lines (Figure S3a) (Wei et al. 2017). According to Figure S3c, the inhibition of compound **1** on the enzyme showed a non-competitive inhibition type (Wei et al. 2017) with a K_m of 6.570 mg/mL.

3. Experimental

3.1. General experimental procedures

IR was measured by BRUKER TENSOR27 infrared spectrometer, KBr compression; UV was determined by Shimadzu UV2401PC UV spectrometer; LC-Quadrupole Mass

Spectrometer (LC-MS8030, Shimadzu, Japan); HR-ESI-MS spectrum was determined on Liquid Chromatography Mass Spectrometry (Exavtie, Thermo Fisher Scientific); 1D and 2D NMR spectra were performed on Bruker DRX-500 MHz and avance III 600 MHz; CombiFlash Rf (RF200, Teledyne Isco, Inc., USA); Agilent Technologies (Agilent LC1260 infinity, Agilent); Rotary Evaporator (RE-52A, Shanghai Yarong Biochemical Instrument Factory); Electronic Balance (BS400S, Beijing Sartorius Co., Ltd.; Mettler Toledo XS105 DualRange, USA); Silica gel (100–200 mesh, Qingdao Marine Chemical Inc., China) were used for column chromatography (CC); TLC was conducted with glass precoated with silica gel GF254 (Qingdao Marine Chemical Inc., China); Inverting filler (RP-18, Merk). MCI filler (MCI-gel CHP-20P, Mitsubishi, Japan); RP column (Zorbax SB-C18, 5 μm, 9.4 × 250 mm, Agilent, USA); D-glucose and 1-Phenyl-3-methyl-5-pyrazolone (PMP) (Aladdin Co., Shanghai, China); Chloroform, petroleum ether, ethyl acetate, methanol (AR, all purchased from Xilong Scientific Co., Ltd., China); α-Glycosidase (G0660-750UN, SIGMA, Germany); Acarbose (109A032, solarbio, Beijing Solarbio Technology Co., Ltd.); 4-pNPG (N0493, Tokyo Chemical Industry Co., Ltd.).

3.2. Plant material

Dried leaves of *M. fortunei* were collected from Ziyuan country of Guangxi Zhuang Autonomous Region, China and identified by professor Yunqiu Li (Guilin Medical University, College of Pharmacy). The voucher specimen (No. 2016101201) has been deposited in the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Guilin Medical University (Guilin, China).

3.3. Extraction and isolation

The power-dried leaves of *M. fortunei* (40 kg) were heated for reflux extractions with 75% ethanol three times, each time for 1 hour. The extract was concentrated in vacuo to afford a brown reside. Then it was suspended in H_2O, and partitiomed with EtOAc and n-BuOH, succsssively. The n-BuOH extraction was applied to silica gel (100-200 mesh) column chromatography, eluting with a $CHCl_3$-CH_3OH gradient system (v/v, 1:0, 9:1, 8:2, 2:1, 1:1, 0:1), to yiled six fractions (Fr.A ∼ Fr.F) based on the TLC analysis. The fraction B was subjected by CombiFlash Rf 200 (flow rate 10.0 mL/min), eluted with CH_3OH-H_2O gradient system 10% ∼ 50%, yielded fraction B1 ∼ B5. The further separation of Fr.B3 was separated by Agilent LC1260 HPLC (flow rate 1.0 mL/min, 17% CH_3OH-H_2O) to afford compound **3** (16.8 mg). The Fr.B5 was subjected by Agilent LC1260 HPLC (flow rate 1.0 mL/min, 13% CH_3CN-H_2O) to afford compound **6** (18.2 mg). The fraction C was subjected by silica gel conlumn chromatography, eluted with $CHCl_3$-CH_3OH gradient system (v/v, 5:1), yielded forty major fractions (Fr.C1 ∼ Fr.C40). The further separation of Fr.C13 was subjected by Agilent LC1260 (flow rate 1.0 mL/min, 25% CH_3OH-H_2O) to afford compound **4** (16.1 mg). And after merged together by analyzing TLC characteristics, the Fr.C30 ∼ Fr.C34 were subjected by Agilent LC1260 HPLC (flow rate 1.0 mL/min, 20% CH_3CN-H_2O) to afford compound **1** (20.8 mg) and **2** (16.6 mg).

3.3.1. 2-hydroxy-4-[3′,5′-dihydroxyhexyl]phenyl-β-D-glucopyranoside

Obtained as yellow powder with [α]20 D − 37.3 (*c* 1.0, MeOH); UV (MeOH), λ_{max} (log ε) 203 (4.47) nm; IR (KBr) V_{max} 3368, 2925, 1596, 1508, 1384, 1277, 1072, 868, 803, 617 cm^{-1}; ^{1}H-NMR (500 MHz, MeOD) δ_H: 7.06 (1 H, d, $J = 8.2$ Hz, H-6), 6.69 (1 H, d, $J = 2.1$ Hz, H-3), 6.61 (1 H, dd, $J = 8.2$, 2.1 Hz, H-5), 4.68 (1 H, d, $J = 7.2$ Hz, H-1′′), 3.96 (1 H, m, H-5′), 3.86 (1 H, dd, $J = 12.1$, 1.7 Hz, H-6′′a), 3.76 (1 H, m, H-3′), 3.70 (1 H, dd, $J = 12.1$, 4.5 Hz, H-6′′b), 3.47 (1 H, m, H-3′′), 3.44 (1 H, m, H-2′′), 3.37 (2 H, m, H-4′′, H-5′′), 2.64 (1 H, m, H-1′a), 2.52 (1 H, m, H-1′b), 1.66 (2 H, m, H-2′), 1.49 (2 H, m, H-4′), 1.15 (3 H, d, $J = 6.3$ Hz, H-6′)。 ^{13}C-NMR (125 MHz, MeOD) δ_C: 144.9 (C-1), 148.3 (C-2), 117.2 (C-3), 139.6 (C-4), 120.9 (C-5), 119.1 (C-6), 32.4 (C-1′), 41.0 (C-2′), 68.7 (C-3′), 47.3 (C-4′), 65.5 (C-5′), 24.3 (C-6′), 104.7 (C-1′′), 77.6 (C-2′′), 74.9 (C-3′′), 71.3 (C-4′′), 78.2 (C-5′′), 62.4 (C-6′′). ESI-MS *m/z* 411 $[M + Na]^+$; HR-ESI-MS *m/z* 411.1831 $[M + Na]^+$ (calcd for $C_{18}H_{28}O_9Na$, 411.1631).

3.4. Acid hydrolysis and HPLC analysis of compound 1

The absolute configuration of the sugar unit was determined by the method of pre-column derivatization with PMP-High Performance Liquid Chromatography (HPLC) with slight modifications (Li et al. 2013). Compound **1** afforded D-glucose ($t_R = 34.25$ min).

3.5. Bioactivity assay

The assay was performed as reported method (Didem et al. 2017) with the positive control of acarbose. Briefly, a mixture of 50 μL of different concentrations (0.001, 0.01, 0.05, 0.1, 0.2, 0.5 mg/mL) of the test samples and 100 μL of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (0.5 U/mL) was incubated in 96-well plates at 25 °C for 15 min, then 50 μL of 5 mM 4-pNPG was added into the mixture and interact at 25 °C for another 10 min. The absorbance was recorded at 405 nm (1, 5, 10, 15, 20 min) by a microplatereader (Infinite M200 Pro, Tecan Corp., Switzerland). α-glucosidase inhibition (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$. Where $A_{control}$ is the activity of enzyme without compound/positive and A_{sample} is the activity of enzyme with compound/positive at different concentrations. The inhibitory mechanism assay was applied with varying the concentration of the enzyme in the reaction mixture, and the inhibition type was then assayed by the Lineweaver–Burk plot.

4. Conclusion

In summary, comparing with the positive control acarbose, we found that the new compound of 2-hydroxy-4-[3′,5′-dihydroxyhexyl]phenyl-β-D-glucopyranoside isolated from the leaves of *M. fortunei* possessed potent α-glucosidase inhibitory activity with an IC_{50} value of 0.111 ± 0.061 mg/mL. This compound reversibly inhibited the enzyme in a non-competitive manner. Hence, the new compound identified in this work may promise candidates for developing as novel anti-diabetic agents.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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