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Original Article

One New Phloroglucinol Derivative with *A*-Glucosidase Inhibitory Activity from *Syzygium Austroyunnanense*

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Abstract:
Type 2 diabetes mellitus (T2DM) represents a substantial global public health burden, highlighting an urgent demand to discover new, safe and effective anti diabetic therapeutic agents. This study reports the isolation of one new phloroglucinol derivative (1) isolated from Syzygium austroyunnanense and its α-glucosidase inhibitory activity with quercetin as the positive control. It showed moderate α-glucosidase inhibitory activity with inhibition rate of 51.46±0.49 % at 50 μM.
Keywords: Syzygium austroyunnanense, phloroglucinol, α-glucosidase.

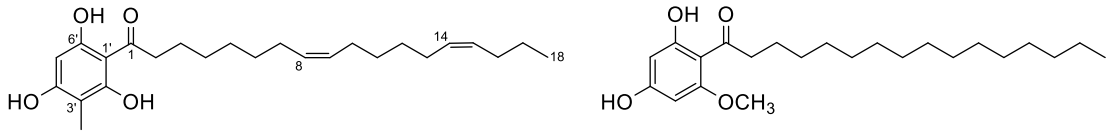
Introduction

TYPE 2 DIABETES MELLITUS (T2DM) is a chronic metabolic disorder characterized by insulin resistance and impaired insulin secretion, leading to elevated blood glucose levels[1]. Globally, around 529 million people had diabetes in 2021, and over 80% of these cases were T2DM. Although medical therapies have improved, T2DM is difficult to manage because of its complicated pathogenesis. Conventional synthetic drugs frequently cause adverse reactions and show poor long-term therapeutic effects.[2,3]. Such limitations underscore the need to investigate safer, multi- targeted natural alternatives. Natural compounds derived from botanical sources have attracted considerable attention for their potential therapeutic effects, attributed to their bioactive components and multi-targeted mechanisms of action[4–6]. The genus *Syzygium* (Myrtaceae) comprises 1200–1800 species worldwide[7]. Plants of this genus have long been used as edible medicines for diabetes treatment in Southeast Asia[8]. Meanwhile, multiple classes of compounds isolated from various *Syzygium* species have been documented to possess antidiabetic activity[9]. *S. austroyunnanense*, called “Bajiamiao” in the Dai Nation, is a fruit tree that originated in Xishuang Banna Prefecture and Guangxi Province in China[10]. Our previous research on the chemical constituents of *S. austroyunnanense* presented six new antidiabetic phloroglucinol derivatives[7,11]. In our ongoing to search for more potential antidiabetic agents, the EtOAc extract of *S. austroyunnanense* were chemically reinvestigated and one new phloroglucinol derivative was obtained. Its *α*-glucosidase inhibitory activity was also assessed.

Results and Discussion

Structural identification

Compound **1**, a red gum, the molecular formula of $C_{25}H_{38}O_4$, was decided by ESI-MS m/z 401 $[M - H]^-$. The ^1H NMR spectrum disclosed a four-proton olefinic signal at δ 5.36 (4H, m) corresponding to the presence of two disubstituted double bonds, a terminal methyl signal (δ 0.88, t, J = 7.5 Hz), a methyl signal attaching to benzene ring (δ 2.08, s). The ^{13}C NMR spectra (Table 1) gave 25 resonances covering one ketonic carbon (δ 206.1), four olefinic carbons (δ 130.8, 130.5 × 2, 130.3), six aromatic carbons (δ 164.75, 162.6, 160.2, 104.9, 103.2, 94.7), twelve methylenes (δ 23.5–44.5) and two methyls (δ 14.0, 7.4). Furthermore, the NMR and MS data revealed the presence of a side chain of octadecanoyl in **1**. These evidence suggested that **1** was a phloroglucinol derivative with an octadecanoyl side chain connected at C-1′ and had isolated double bonds[7,11]. Detailed comparison of spectroscopic data discovered that **1** closely resembled the known compound austroyunone G[7] with the exception of one hydroxyl at 2′ in **1** instead of methoxyl in austroyunone G. This



1 **2**

Figure 1. Chemical structures of compounds 1 and 2

hypothese was supported on the basis of 2D NMR (HSQC and HMBC) correlations from H-2 (δ 3.10) to C-1 (δ 206.1), from H-6 (δ 1.30) to C-8 (δ 130.8), from H-7 (δ 1.39) to C-9 (δ 130.8), from H-13 (δ 2.06) and H-17 (δ 1.35) to C-15 (δ 30.3), from H-18 (δ 0.88) to C-16 (δ 30.6) and C-17 (δ 23.5), and from CH_3 (δ 2.08) to C-3′ (δ 103.2). Further COSY cross-peaks (Fig. 2) confirmed the aforementioned conclusion. The location of the two double bonds was speculated to be at C-8 and C-14 based on the chemical shift of two double allylic methylenes (C-7 δ 27.8, C-10 δ 27.4, and C-13 δ 27.4), and of C-16 (δ 30.6) in consistent with the austroyunone G. The configurations of the olefinic bonds were assigned as *Z* by three characteristic double allylic methylenes (C-7 δ 27.8, H-7 δ 2.06; C-10 δ 27.4, H-10 δ 2.06 and C-13 δ 27.4, H-13 δ 2.06)[7]. Consequently, compound **1** was elucidated as (*Z*,*Z*)-1-(3-methyl-2,4,6-trihydroxyphenyl)-octadeca-8,14-dien-1-one.

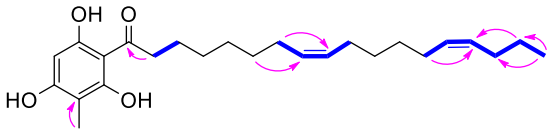


Fig. 2. Key 2D NMR correlations of 1.

Table 1 NMR Data of compound 1 (δ in ppm, J in Hz)[a]

No.	δ_H	δ_C	No.	δ_H	δ_C
1		206.1	14	5.36 overlap	130.5 [b]
2	3.10 t (6.7)	44.5	15	5.36 overlap	130.3 [b]
3	1.65 m	25.6	16	2.06 overlap	30.6 [b]
4	1.28−1.40	30.1 [b]	17	1.35 m	23.5

5	1.28−1.40	30.2 [b]	18	0.88 t (7.5)	14.0
6	1.28−1.40	30.2 [b]	1′		104.9
7	2.06 m	27.8	2′		160.2
8	5.36 overlap	130.8 [b]	3′		103.2
9	5.36 overlap	130.5 [b]	4′		164.7
10	2.06 overlap	27.4	5′	5.94 s [c]	94.7
11	1.28−1.40	30.4 [b]	6′		162.6
12	1.28−1.40	30.6 [b]	CH_3	2.08 s	7.4
13	2.06 overlap	27.4			

Chemical shifts are given in ppm; assignments were confirmed by 2D NMR experiments.

[a]Data measured in $(CD_3)_2CO$ at 500 Hz. [b] Indicate that the assignments may be intermixed.

[c] Chemical shift is predicted.

The known compound was characterized as 1-(2,4-dihydroxy-6-methoxyphenyl)-1-hexadecanone (**2**)[12].

α-Glucosidase inhibitory activity

Compound **1** was tested for their inhibitory effects against *α*-glucosidase with quercetin as positive control and exhibited moderate inhibitory effects with inhibitory rate of 51.46 ± 0.49 % at 50 *μ*M.

Materials and Methods

Plant Material

Syzygium austroyunnanense were collected in October 2014 from Xishuang Banna Prefecture, Yunnan Province, People's Republic of China, and authenticated by Mr. Yu Chen, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. Chen 20141012) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered twigs and leaves of *S. austroyunnanense* (4.0 kg) were extracted with MeOH at room temperature. The extract was then partitioned with petroleum ether (PE), EtOAc, and n-BuOH, sequentially.The EtOAc extract (8.3 g) was subjected to silica gel column chromatograph (CC) ($CHCl_3$– MeOH, 100:0 to 0:100) to give three fractions A–C. Fraction B (1.9 g) was decolorized by MCI gel (CHP 20P) CC using a gradient system of CH_3OH–H_2O (95:5), then subjected to silica gel CC (PE–Me_2CO, 20:1 to 1:1) to give three subfractions B1–B3. Subfraction B1 was separated by Sephadex LH-20 CC ($CHCl_3$–MeOH, 3:2) to get three subfractions B1-1–B1-3. Subfraction B1-1 was further separated by semipreparative HPLC (MeOH–H_2O, 90:10) to get compound **2** (2.5 mg, t_R = 18.3 min). Part D (1.6 g) was separated by the same procedure and final purification was achieved by semipreparative HPLC (MeOH–H_2O, 90:10) to yield compound **1** (1.7 mg, t_R = 32.5 min).

α-Glucosidase inhibitory activity assay

The *α*-glucosidase inhibitory assay was performed by referring to the method reported previously[13]. A mixture containing 20 *μ*L of test samples at five different concentrations, 10 *μ*L of *α*-glucosidase solution (0.025 U/mL), 10 *μ*L of 0.1 M phosphate buffer (pH 6.9), and 20 *μ*L of 1 mM *p*-nitrophenyl *α*-D-glucopyranoside solution was incubated in 96 well plates at 37 °C for 50 min. After incubation, the optical density (OD) at 405 nm was measured using a microplate reader (SpectraMax M2, Molecular Devices Corp., operated with SoftmaxPro v.4.6 software, Sunnyvale, CA, USA). The OD values were compared with that of the positive control (quercetin), where 20 *μ*L of buffer solution replaced the test sample. The *α*- glucosidase inhibitory activity was expressed as percent

inhibition, calculated as described below. The IC_{50} (concentration required for 50% inhibition) was determined by the Reed–Muench method[14].

$$\% \text{ inhibition} = \frac{\Delta Abs_{control} - \Delta Abs_{sample}}{\Delta Abs_{control}} \times 100$$

Conclusions

Phytochemical reinvestigation of the EtOAc extract of twigs and leaves of *S. austroyunnanense* lead to the isolation of two phloroglucinol derivatives. Compound **1** was identified as a new natural α-glucosidase inhibitors with inhibitory rate of 51.46±0.49 % at 50 μM. Our findings further enrich the chemical knowledge of *S. austroyunnanense* but also provide further scientific basis for its development as a potential antidiabetic agent. Quite surprisingly, the aromatic proton H-5′ showed no corresponding signals both in ^{1}H and 2D NMR spectra. However, ^{13}C NMR, MS, and literature comparison with known compound clearly disclosed the existence of H-5′. Hence, its chemical shift was merely predicted based on literature reference[7].

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Conflicts of Interest: The authors declare no conflict of interest.

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