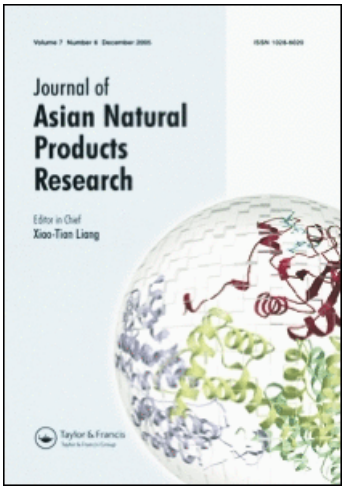

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A new antibacterial compound from Luo Han Kuo fruit extract (*Siraitia grosvenori*)

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Luo Han Kuo fruit (*Siraitia grosvenori* Swingle) has been used in China for centuries as a sweetening agent, and also used to treat sore throat and cough. In our recent study, a new bioactive compound, (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*cis*-5,3′-bimethoxy-7-(*trans*-2-propenal)-3,4-flavandiol (**1**), named siraitiflavandiol was obtained. The structure has been determined on the basis of spectroscopic studies including 1D and 2D NMR (^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY), CD, EI-MS, and HR-EI-MS spectra. The new compound was evaluated *in vitro* for its inhibitory ability against the growth of oral bacterial species *Streptococcus mutans*, *Porphyromonas gingivalis*, and yeast *Candida albicans*. The minimum inhibitory concentrations were 6, 24, and 6 μg/ml, respectively.

Keywords: *Siraitia grosvenori* Swingle; (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*cis*-5,3′-bimethoxy-7-(*trans*-2-propenal)-3,4-flavandiol; *Streptococcus mutans*; *Porphyromonas gingivalis*; *Candida albicans*

1. Introduction

Siraitia grosvenori Swingle (known as Luo Han Kuo in China), a medicinal and an edible plant, is cultivated in southern China. The fruit has been used for thousands of years as a traditional Chinese medicine and health food for the treatment of dry cough, sore throat, dire thirst, and constipation [1,2]. The fruit extract mainly composed of triterpene glycosides, also known as mogrosides, has high-intensity sweetness, low calories, and can serve as a substitute for sugar for obese and diabetic patients [1]. It is also reported that mogrosides possess a wide range of pharmacologic and health-promoting properties including anti-tumor, anti-inflammation, and anti-oxidative activity [1,3–5].

Besides Luo Han Kuo extract being used as an alternative sweetening agent to sugar, a recent study demonstrated that the Luo Han Kuo extract exhibited potent antibacterial activity against oral bacteria, such as *Streptococcus mutans* [6]. Dental caries is the predominant cause of tooth loss in children and young adults. Caries is a bacterial infection caused by a specific bacteria, albeit, this is not limited to a monospecific pathogen [7]. The presence of 'mutans streptococci' and lactobacilli have been associated with caries development. *S. mutans* metabolize sugar into lactic acid and the produced acid erodes the enamel on the teeth. In the investigation on the bioactive components from the Luo Han Kuo extract, a new flavandiol

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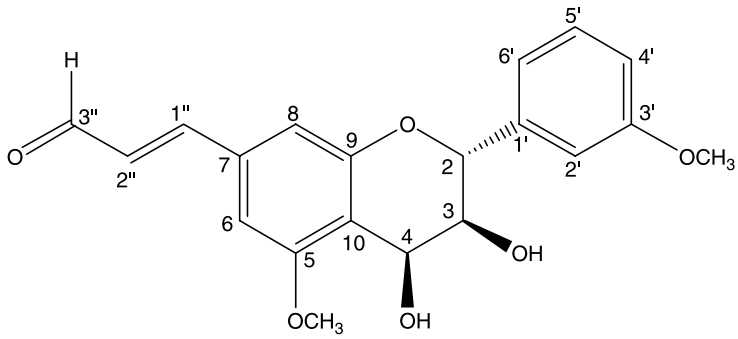

Figure 1. Structure of siraitiflavandiol (**1**).

was obtained (Figure 1), and the structural elucidation and antibacterial evaluation are reported herein.

2. Results and discussion

Siraitiflavandiol was obtained as an orange gum and had the molecular formula $C_{20}H_{20}O_6$ by HR-EI-MS at *m/z* 356.1271 $[M]^+$. The ^{1}H NMR spectrum showed a set of signals of a 5,7,3′-trisubstituted 3,4-dihydroxyl flavan [δ 5.62 (d, 1H, $J = 7.2$ Hz, H-2); 3.65 (dd, 1H, $J = 7.2$, 2.0 Hz, H-3); 3.93 (d, 1H, $J = 2.0$ Hz, H-4); 7.11 (d, 1H, $J = 1.2$ Hz, H-6); 7.02 (d, 1H, $J = 1.2$ Hz, H-8); 6.88 (d, 1H, $J = 0.8$ Hz, H-2′); 6.90–6.87 (m, 3H, H-4′, 5′, 6′)] [8,9], and two aromatic methoxyl singlets at δ 3.85 and 3.91 (s each, 3H each, 5-OMe and 3′-OMe). A *trans*-2-propenal group [δ 7.40 (d, 1H, $J = 15.6$ Hz, H-1″); 6.59 (dd, 1H, $J = 7.6$, 15.6 Hz, H-2″); 9.63 (d, 1H, $J = 7.6$ Hz, H-3″)] [10–12] was observed as well. The ^{13}C NMR and HSQC spectra confirmed the presence of a flavan moiety by displaying 12 aromatic carbons, 3 oxygenated carbons (δ 89.2, C-2; 58.7, C-3; 64.2, C-4), and 2 aromatic methoxyl carbons (δ 56.4, 5-OMe; 56.3, 3′-OMe) [13]. Additionally, the *trans*-2-propenal group (δ 153.2, C-1″; 126.7, C-2″; 193.7, C-3″) was observed. Correlations from H-2″ (δ 6.59) to C-7 (δ 128.4), and from H-1″ (δ 7.40) to C-6 (δ 118.3) and C-8 (δ 112.5) in the HMBC spectrum (Figure 2) suggested that the *trans*-2-propenal group was located at C-7, which was further elucidated by the NOESY

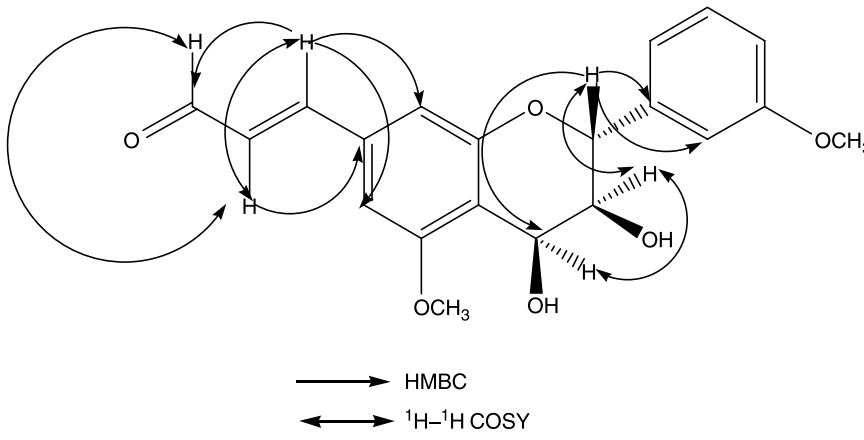

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations for siraitiflavandiol (**1**).

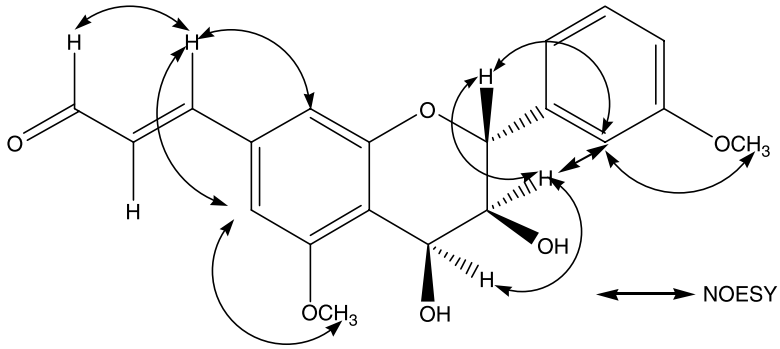

Figure 3. NOESY correlations for siraitiflavandiol (**1**).

spectrum (Figure 3). Crossing peaks among 5-OCH_3 to H-6 and 3′-OCH_3 to H-2′ from the NOESY spectrum explained that the methoxyl groups were located at C-5 and C-3′, respectively (Figure 3). In the H–H COSY spectrum, the correlations between H-2 and H-3 and between H-3 and H-4 were shown (Figure 2), and the presence of a positive Cotton effect at 236 nm and a negative Cotton effect at 290 nm in the CD spectrum suggested the 2*R*, 3*S*, 4*S* absolute configuration [14,15]. So the structure of **1** was elucidated as (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*cis*-5,3′-bimethoxy-7-(*trans*-2-propenal)-3,4-flavandiol, named siraitiflavandiol.

Siraitiflavandiol was evaluated *in vitro* for its inhibitory ability against the growth of bacteria *S. mutans*, *P. gingivalis*, and yeast *Candida albicans*, which are the three major oral pathogens. It showed antibacterial and anti-yeast activities against all of them with minimum inhibitory concentrations (MICs) of 6, 24, and 6 μg/ml for *S. mutans*, *P. gingivalis*, and *C. albicans*, respectively.

3. Experimental

3.1 General experimental procedures

The UV spectrum was obtained on a USB4000 miniature fiber optic spectrometer (Ocean Optics, Inc., Dunedin, FL, USA) with MeOH as the solvent. The IR spectrum was recorded on a Nicolet iS10 FT-IR spectrometer (Thermo Scientific, Inc., Waltham, MA, USA). Mass spectra were determined on a ThermoFinnigan PolarisQ (for EI-MS) and a JEOL JMS-700T MStation spectrometer (for HR-MS). 1D and 2D NMR spectra were recorded on a Varian INOVA-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) using chloroform-*d* as the solvent and TMS as the internal standard. Circular dichroism spectrum was performed on a Jasco 810 spectropolarimeter. Column chromatography was carried out using silica gel 60 (70–230 mesh, EMD Chemicals, Inc., Darmstadt, Germany; 230–400 mesh, Fisher Scientific, Pittsburgh, PA, USA) as the stationary phase. TLC was conducted on silica gel GF254 (Merck KGaA, Darmstadt, Germany). PTLC was performed on silica gel 60 (500 μm in thickness; Merck KGaA). All the solvents were ACS grade (Fisher Scientific). Bacterial species *S. mutans* ATCC 25175, *P. gingivalis* ATCC 33277, and yeast *C. albicans* ATCC 2091 were obtained from ATCC (Manassas, VA, USA). TSBYE and YPD media were purchased from Oxoid Ltd (Hampshire, UK), and blood agar plates were obtained from Remel (Lenexa, KS, USA).

3.2 Plant material

The *S. grosvenori* Swingle extract was purchased from China Natural Products, Inc. (Hunan, China, www.chinanaturalproducts.com), and a voucher sample

(No. 20060612) has been deposited in the Oral Health Research and Development Center, University of Kentucky.

3.3 Extraction and isolation

The Luo Han Kuo extract (96 g) was chromatographed on a silica gel column, and eluted with $CHCl_3$–MeOH–H_2O (40:1:0.02, 20:1:0.02, 10:1:0.02, 4:1:0.1, 2:1:0.1, v/v), gradiently, to afford 25 fractions. Fraction 6 was subjected to a silica gel column again, eluted with $CHCl_3$–acetone–MeOH (1:1:0, 1:2:0, 1:2:0.25, 1:2:1, v/v), successively, and followed by further purification with repeatedly PTLC ($CHCl_3$–MeOH–H_2O, 7:1:0.1) to yield siraitiflavandiol (6.6 mg).

3.3.1 Siraitiflavandiol

An orange gum. UV (MeOH) λ_{max} (log ε): 230 (4.60), 280 (4.49), 332 (4.19) nm; IR (KBr) ν_{max} (cm^{-1}): 3421, 1672, 1625, 1474, 1466, 1459, 1449; CD ($c = 0.45 \times 10^{-3}$, MeOH) $\Delta\varepsilon_{236} = +1.62$, $\Delta\varepsilon_{272} = -1.08$, $\Delta\varepsilon_{290} = -1.38$; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.63 (d, 1H, $J = 7.6$ Hz, H-3″), 7.40 (d, 1H, $J = 15.6$ Hz, H-1″), 7.11 (d, 1H, $J = 1.2$ Hz, H-6), 7.02 (d, 1H, $J = 1.2$ Hz, H-8), 6.90–6.87 (m, 3H, H-4′, 5′, 6′), 6.88 (d, 1H, $J = 0.8$ Hz, H-2′), 6.59 (dd, 1H, $J = 7.6$, 15.6 Hz, H-2″), 5.62 (d, 1H, $J = 7.2$ Hz, H-2), 3.93 (d, 1H, $J = 2.0$ Hz, H-4), 3.91 (s, 3H, 3′-OMe), 3.85 (s, 3H, 5-OMe), 3.65 (dd, 1H, $J = 7.2$, 2.0 Hz, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 193.7 (C-3″), 153.2 (C-1″), 151.8 (C-9), 147.0 (C-5), 146.2 (C-3′), 129.3 (C-1′), 128.4 (C-7), 126.7 (C-2″), 119.7 (C-2′), 119.7 (C-4′), 118.3 (C-6), 114.5 (C-5′), 112.5 (C-8), 108.9 (C-6′), 108.7 (C-10), 89.2 (C-2), 64.2 (C-4), 58.7 (C-3), 56.4 (C-5-OMe), 56.3 (C-3′-OMe); EI-MS *m/z*: 356 $[M]^+$, 338, 323, 306, 277, 263, 235, 165, 151; positive HR-EI-MS *m/z*: 356.1271 $[M]^+$ (calcd for $C_{20}H_{20}O_6$, 356.1260).

3.4 Bacterial growth inhibition assay

S. mutans and *P. gingivalis* were grown in TSBYE media (trypticase soy broth yeast extract), and *C. albicans*, in YPD media (yeast extract, peptone, and glucose) [6,16,17]. Growth conditions were at 37°C under anaerobic conditions (85% N_2, 10% H_2, and 5% CO_2) for *S. mutans* and *P. gingivalis* and aerobic conditions for *C. albicans*. The new compound was tested for bioactivity using 5 μl of a twofold dilution series from 96 μg/ml to individual wells of a 96-well plate with wells containing 200 μl of TSBYE or YPD media and a 10% inoculum of bacteria or yeast from an overnight culture [18]. The plates were incubated for 16–18 h. After incubation and 25,000 times dilution, 10 μl of the culture were plated onto blood agar plates and incubated at 37°C under anaerobic or aerobic conditions in the presence of various concentrations of the isolated flavandiol. The MICs against different bacteria were determined by counting CFUs compared with those of the untreated bacterial suspension as a negative control. All the data were replicated three times.

Acknowledgements

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