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Limonoids from the fruits of *Swietenia macrophylla* with inhibitory activity against H_2O_2-induced apoptosis in HUVECs

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ABSTRACT

The fruits of *Swietenia macrophylla* (skyfruits) are commercially used as healthcare products to improve blood circulation. An investigation of active ingredients of skyfruits led to the isolation of four new limonoids, swietemacrolides A – D (**1**–**4**), together with ten known limonoids (**5**–**14**) and one proto-limonoid (**15**). Their structures were elucidated on the basis of MS and NMR data analysis. Swietemacrolide C (**3**) at the concentration of 10 μM showed significant protective effect on H_2O_2-induced apoptosis in human umbilical vascular endothelial cells (HUVECs), while swieteliacate D (**5**) displayed moderate anti-apoptotic activity.

1. Introduction

Cardiovascular diseases (CVDs) is the leading cause of death worldwide, which resulted in approximately 17.7 million people died in 2015, representing 31% of all global deaths [1]. Numbers of natural products from medicinal plants play a dominant role in the prevent-ment and treatment of CVDs through scavenging reactive oxygen species (ROS) and/or chelating metals [2]. The abnormal apoptosis of human umbilical vascular endothelial cells (HUVECs) might imply a wide array of pathophysiological processes, such as thrombosis, angiogenesis, atherosclerosis, diabetic vasculopathy, hypertension, and heart failure [3–5]. To protect HUVECs from ROS such as H_2O_2, hydroxyl radicals and superoxide radicals induced apoptosis, may be benefit for the prevention or treatment of CVDs.

The fruits of *Swietenia macrophylla* King (Meliaceae) are usually called “skyfruits” because they hang upwards from the tree to sky, which have been processed commercially to a wide range of healthcare products for improving blood circulation and alleviating many CVDs such as hypertension and hyperlipoidemia around the world especially in some countries which have high distribution of this species [6,7]. Previous studies disclosed numbers of ingredients of skyfruits, which exhibited various biological activities, including hypoglycemic, hypolipidemic, antiviral, anti-inflammatory, neuroprotective, antioxidant effect, *etc.* [8,9]. Although many investigations have been performed to characterize the anti-CVD related properties of skyfruits [8], the molecular mechanisms of how they function in organism remain unclear. Herein, we wish to describe the structure identification and anti-CVD activities of four new limonoids, named swietemacrolides A – D (**1**–**4**), along with a known compound, swieteliacate D (**5**), from skyfruits.

2. Materials and methods

2.1. Plant material

Skyfruits (fruits of *Swietenia macrophylla*) were purchased at Solomon Islands, in August 2014 and identified by Prof. C.-H. Tan, one of the authors. A voucher sample (No. 20140912) has been deposited to Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, P.R. China.

2.2. General experimental procedures

Optical rotations were measured by a PerkinElmer 341 polarimeter (PerkinElmer, Waltham, MA, USA). Infrared (IR) spectra were recorded on a Nicolet-Magna-750-FTIR spectrometer (ThermoFisher, Madison, WI, USA) with KBr disks. Nuclear magnetic resonance (NMR) spectra,

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including heteronuclear multiple-bond correlation (HMBC), and heteronuclear single-quantum coherence (HSQC) experiments, were recorded on a Bruker Advance III 500 NMR spectrometer (Bruker, Ettlingen, Germany) operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively, with chemical shifts (δ) given in ppm with tetramethylsilane as an internal standard. High-resolution electrospray ionization mass (HRESIMS) data were acquired using a Waters/Micromass Q-TOF-Ultima Global mass spectrometer (Waters, Milford, MA, USA). Semi-preparative high performance liquid chromatography (HPLC) was performed with a HPLC system (Waters 2545) with a detector (Waters 2489) and an Agilent ZORBAX SB-C18 column (5 μm, 250 mm × 9.4 mm). Silica gel (100–200 mesh, 200–300 mesh and HG/T2354–92, Qingdao Marine Chemical Plant, Qingdao, China), RP-C18 silica gel (ODS, 150–200 mesh, Fuji Silysia Chemical Ltd., Aichi, Japan) and Sephadex LH-20 (Phamacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). Pre-coated silica gel GF254 plates (Qingdao Marine Chemical Plant) were used for thin-layer chromatography (TLC), which were monitored by ultraviolet light at 254/365 nm or by heating after sprayed with vanillin-sulfuric acid. All chemical reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used without further purification.

2.3. Extraction and isolation

The dried fruits of *S. macrophylla* were removed the pericarps and seed coats to obtain the cotyledon parts (1.5 kg), which were powdered then refluxed with methanol to give a crude extract. After defatted by petroleum ether (PE), this extract was partitioned between EtOAc and H_2O (1:1) to get EtOAc-soluble layer (136 g), which was subjected to a CC of silica gel (100–200 mesh), eluting with gradient PE − acetone (20:1 to 0:1) to yield fractions A − F. Fr. C (40 g) was separated into frs. C1–C4 by CC of ODS (MeCN − H_2O, 40:60 to 60:40). Isolation of Fr. C1 (266 mg) by CC of silica gel (PE − EtOAc, 3:2, *v/v*) furnished **6** (18 mg); Fr. C2 (1.1 g) was isolated by a silica gel column ($CHCl_3$ − MeOH, 200:1) and semi-preparative HPLC (4 ml/min, MeCN − H_2O, 60:40) to afford **7** (42 mg), **9** (35 mg), **10** (23 mg), **11** (14 mg) and **14** (8 mg). Fr. C3 (9.6 g) was purified by CCs of silica gel (PE − EtOAc, 4:1; $CHCl_3$ − MeOH, 200:1; $CHCl_3$ − acetone, 40:1) and finally semi-preparative HPLC (4 ml/min, MeCN − H_2O, 55:45) to give **1** (69 mg), **2** (4 mg), **12** (28 mg), **13** (16 mg) and **15** (7 mg). Fr. D (10.5 g) was isolated by CC of silica gel (PE − acetone, 4:1) to give fr. D1 − D5. Fr. D2 (1.6 g) was isolated by an ODS column (acetone − H_2O, 45:55 to 55:45) to afford subfractions D2a − D2e. Fr. D2a (250 mg) was purified through a silica gel column (CH_2Cl_2 − MeOH, 25:1 to 50:1) and finally by semi-preparative HPLC (4 ml/min, MeOH − H_2O, 1:1 or 32:68) to give **3** (18 mg) and **5** (10 mg), respectively. By the similar procedure, **4** (4 mg) and **8** (15 mg) was obtained from fr. D2c.

Swietemacrolide A (**1**). White amorphous powders; $[\alpha]_D^{20}$ −127 (*c* 0.10, MeOH); IR (KBr) ν_{max} 2979, 1730, 1504, 1047, 875, 758, 603 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; HRESIMS *m/z* 621.2665 [M + Na]$^+$ (calcd for $C_{33}H_{42}O_{10}Na$, 621.2676).

Swietemacrolide B (**2**). White amorphous powders; $[\alpha]_D^{20}$ −117 (*c* 0.10, MeOH); IR (KBr) ν_{max} 2951, 1726, 1637, 1127, 875, 757, 603 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; HRESIMS *m/z* 619.2527 [M + Na]$^+$ (calcd for $C_{33}H_{40}O_{10}Na$, 619.2519).

Swietemacrolide C (**3**). White amorphous powders; $[\alpha]_D^{20}$ −84 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3413, 2982, 1747, 1715, 1439, 1076, 1026, 875, 758, 602 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 2; HRESIMS *m/z* 503.2283 [M + H]$^+$ (calcd for $C_{27}H_{35}O_9$, 503.2276).

Swietemacrolide D (**4**). White amorphous powders; $[\alpha]_D^{20}$ −6.7 (*c* 0.04, MeOH); IR (KBr) ν_{max} 3450, 2954, 1731, 1675, 1467, 1027, 875, 766, 601 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 2; HRESIMS *m/z* 505.2425 [M + H]$^+$ (calcd for $C_{27}H_{37}O_9$, 505.2432).

Table 1
NMR data of compounds **1–2** ($CDCl_3$).

No.	**1**			**2**		
	δ_H	δ_C, mult.		δ_H	δ_C, mult.	
1	–	215.9	s	–	215.8	s
2	3.50 dd (9.6, 7.2)	48.7	d	3.53 t-like (8.2)	48.7	d
3	4.66 d (9.6)	77.8	d	4.69 d (9.5)	78.3	d
4	–	39.0	s	–	39.0	s
5	3.63 br s	44.6	d	3.69 s	44.5	d
6	5.57 br s	72.7	d	5.58 s	72.7	d
7	–	171.1	s	–	171.2	s
8	–	138.4	s	–	138.7	s
9	2.29 dd (12.2, 3.3)	57.4	d	2.27 dd (13.0, 4.3)	57.4	d
10	–	50.2	s	–	50.2	s
11	1.81 m 2.18 m	21.1	t	1.79 m 2.17 m	21.1	t
12	1.44 td (13.9, 4.2) 1.75 dt (14.3, 3.0)	34.5	t	1.42 td (14.3, 3.9) 1.71 dt (14.3, 3.9)	34.5	t
13	–	36.7	s	–	36.7	s
14	2.25 br d (5.2)	45.2	d	2.22 d (6.5)	45.2	d
15	2.81 br d (18.6) 2.90 dd (18.6, 5.2)	29.7	t	2.76 d (19.0) 2.85 dd (19.0, 6.0)	29.6	t
16	–	169.2	s	–	168.8	s
17	5.63 s	77.1	d	5.56 s	77.0	d
18	1.05 s	21.7	q	1.03 s	21.6	q
19	1.19 s	15.6	q	1.19 s	15.6	q
20	–	120.8	s	–	120.8	s
21	7.68 br s	141.3	d	7.67 br s	141.3	d
22	6.44 br s	109.5	d	6.42 br s	109.5	d
23	7.44 br s	143.1	d	7.43 t (1.7)	143.2	d
28	0.94 s	22.6	q	0.97 s	22.78	q
29	1.10 s	23.0	q	1.11 s	22.84	q
30	5.37 br d (7.2)	122.9	d	5.34 d (7.3)	122.9	d
7-OMe	3.72 s	53.2	q	3.72 s	53.2	q
Ac	–	169.7	s	–	169.7	s
	2.19 s	21.0	q	2.19 s	21.0	q
1′	–	176.3	s	–	166.4	s
2′	2.62 sept (7.0)	33.9	d	–	135.1	s
3′	1.16 d (6.9)	18.4	q	5.64 br s; 6.14 br s	127.1	t
4′	1.17 d (6.9)	18.9	q	1.94 s	17.9	q

2.4. Cell culture and drug treatment

HUVECs obtained from Royo (Nanchang Biotech Co., Ltd., China) were digested and suspended, and then seeded into 96-well plates, at a density of 1 × 10^4 cells/well in a humidified atmosphere supplemented with phosphate buffer saline (PBS). The cells were incubated at 37 °C under 5% CO_2 for 24 h for the following experiments. To investigate the cytotoxicity of H_2O_2 and limonoids on HUVECs, cells were cultured with various concentrations of limonoids (0.1, 1, 10, 100 and 1000 μM) and H_2O_2 (10, 50, 100, 300, and 500 μM) for 24 h. To explore the inhibitory effect of limonoids on H_2O_2-induced apoptosis, cells were pretreated with different concentrations of limonoids for 3 h, then exposed to H_2O_2 (300 μM) for 24 h. After 24 h, cellular images were taken at 100 × magnification. Each experiment was duplicated for three times.

2.5. Determination of cell viability

Cell viability analysis was conducted based on the MTT (=3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*- tetrazolium bromide) assay. After treated with different medium conditions, 10 μL of MTT (Sigma) was added to each well and cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 6 h. Then the MTT solution was removed, 100 μL of dimethylsulfoxide per well of the plates was added for the solubilization of colored formazan crystals and gently shaken for 10 min. The optical density was measured at 492 nm with an enzyme-labeled instrument DNM-9602A (Perlong Medical, Beijing, China).