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Benzophenone glycosides and epicatechin derivatives from *Malania oleifera*

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ABSTRACT

A new benzophenone C-glycoside, malaferin A (**1**), and two new epicatechin derivatives, malaferin B (**2**) and malaferin C (**3**), together with five known compounds were isolated from *Malania oleifera*. In addition, (−)-epicatechin-3-*O*-benzoate (**6**) was isolated for the first time from a natural resource. Structures of **1**–**3** were determined on the basis of their spectroscopic methods, including 1D and 2D NMR techniques. All of the compounds were evaluated for anti-HIV activities.

1. Introduction

Malania oleifera Chun et S. Lee, the only species of the genus *Malania* (Olacaceae), is an endemic plant growing in the rocky mountains in the west of Guangxi Province and the southeast of Yunnan Province, China [1]. The seeds of this plant have been used for making edible oils and consumed widely by local people. Previous chemical investigation of the seeds of *M. oleifera* evidenced the presence of lipids [2]. Among them, *cis*-tetracos-15-enoic was an important synthetic material of muscone [3]. However, there are no phytochemical and biological study on other parts of this species. As part of our research for naturally occurring bioactive metabolites from the monotypic genus species endemic to China [4–6], we investigated the chemical constituents of the twigs and leaves of *M. oleifera*, which led to the isolation of a new benzophenone C-glycoside, malaferin A (**1**), and two new epicatechin derivatives, malaferin B (**2**) and malaferin C (**3**), together with five known compounds (**4**–**8**) (Fig. 1). Herein, we described the isolation, structural elucidation, and anti-HIV activities evaluation of the isolates.

2. Experimental

2.1. General

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained by a Tensor 27 spectrophotometer with KBr pellets. 1D and 2D spectra were run on a Bruker AM-400 or DRX 500 spectrometer with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS were recorded on an API QSTAR Pulsar i spectrometer. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden) were used for Column chromatography (CC). MPLC was performed on a Lisui EZ Purify III System including pump manager P03, detector modules P02, and fraction collector

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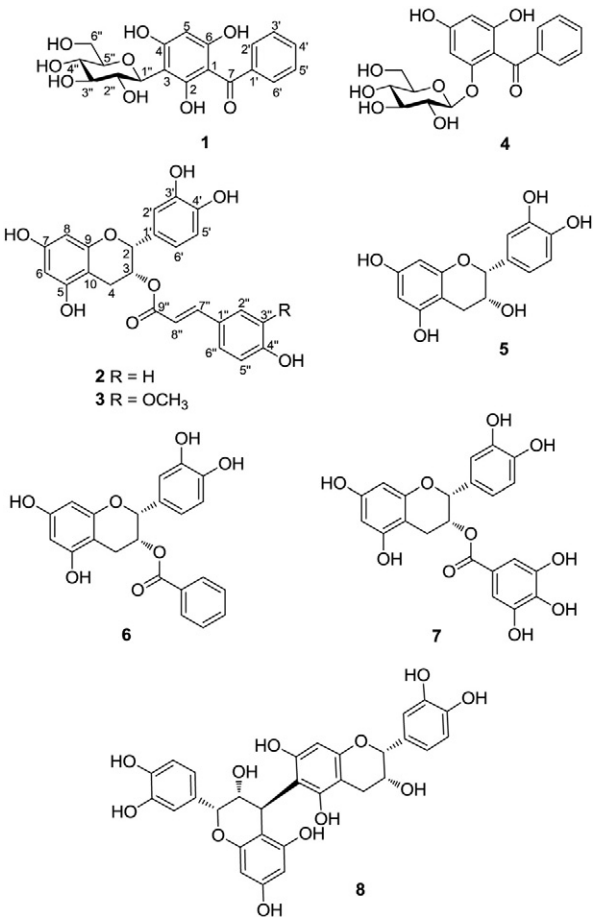

Fig. 1. Chemical structure of compounds **1**–**8**.

P01 (Shanghai Li Sui Chemical Engineering Co., Ltd., China) and columns packed with RP-18 silica gel (40–63 μm, Merck, Darmstadt, Germany). Preparative and semipreparative HPLC was performed on Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column and Agilent 1100 apparatus equipped with a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column, respectively. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

2.2. Plant material

The twigs and leaves of *M. oleifera* were collected from Guangnan County, Yunnan Province, China, in September 2010, and identified by Prof. X. Gong, Kunming Institute of Botany. A voucher specimen (KIB20100912m) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered twigs and leaves of *M. oleifera* (10 kg) were extracted three times with 95% EtOH at room temperature and concentrated under vacuum to give a residue (800 g). The residue was subjected to silica gel CC and eluted with $CHCl_3$–MeOH (from 1:0 to 0:1) to afford fractions A–E. Fraction B (20 g) was separated into four subfractions, B1–B4, by silica gel CC with petroleum ether–Me_2CO (from 8:2 to 1:0) as the eluent. Subfraction B2 (1.2 g) was further purified over a silica gel CC, eluted with $CHCl_3$–MeOH (9:1) to obtain (−)-epicatechin-3-*O*-benzoate (100 mg). Fraction C (45 g) was chromatographed by MPLC eluting with MeOH–H_2O (from 5:95 to 100:0) to provide five subfractions, C1–C5. (−)-epicatechin-3-*O*-gallate (2.8 g) was obtained from subfraction C1 by repeated silica gel CC, eluted with $CHCl_3$–MeOH (from 8:2 to 6:4). Subfraction C2 was subjected to silica gel CC ($CHCl_3$–MeOH, 8:2), followed by Sephadex LH-20 (MeOH) to yield (−)-epicatechin (5.2 g) and garcimangosone D (8.4 mg). Subfraction C3 further chromatographed over silica gel eluted with $CHCl_3$–MeOH (8:2) and then purified by semipreparative HPLC (MeOH–H_2O, 38:62) to afford **2** (8.7 mg) and **3** (10.5 mg). Fraction D was subjected to silica gel CC eluted with $CHCl_3$–MeOH (from 8:2 to 6:4), followed by Sephadex LH-20 (MeOH), and further purified by preparative HPLC (MeOH–H_2O, 26:64) to afford **1** (11.7 mg) and procyanidin B5 (83.5 mg).

2.4. Spectroscopic data

Malaferin A (**1**): colorless oil; $[\alpha]_D^{22.7}$ +48.01 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε): 307 (4.05), 210 (4.47) nm; IR (KBr) ν_{max} 3424, 2927, 1699, 1626, 1559, 1541, 1507, 1455, 1419, 1293, 1077, 737, 702, 625 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; negative ESIMS: *m/z* 391 $[M-H]^-$; negative HRESIMS $[M-H]^-$ *m/z* 391.1032 (calcd for $C_{19}H_{19}O_9$, 391.1029).

Malaferin B (**2**): brown amorphous powder; $[\alpha]_D^{22.8}$ −224.59 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε): 313 (3.81), 208 (4.82) nm; IR (KBr) ν_{max} 3423, 2932, 1732, 1698, 1684, 1626, 1605, 1558, 1516, 1457, 1362, 1285, 1262, 1168, 1016, 830, 782, 668 cm^{-1}; ^1H and ^{13}C NMR

Table 1
^1H (400 MHz) and ^{13}C (100 MHz) NMR data of **1** in CD_3OD.

Pos.	δ_H (*J* in Hz)	δ_C mult.
1		104.6 s
2		163.3 s
3		106.2 s
4		164.5 s
5	5.97 (s)	96.2 d
6		162.3 s
7		200.9 s
1′		142.8 s
2′	7.61 (m)	129.4 d
3′	7.39 (t, 7.5)	128.7 d
4′	7.49 (t, 7.5)	132.3 d
5′	7.39 (t, 7.5)	128.7 d
6′	7.61 (m)	129.4 d
1″	4.89 (d, 9.9)	76.2 d
2″	3.47 (m)	71.5 d
3″	3.49 (m)	79.9 d
4″	3.99 (m)	73.3 d
5″	3.40 (m)	82.5 d
6″	3.75 (dd, 12.1, 4.9)	62.5 t
	3.87 (dd, 12.1, 1.8)	

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **2** and **3** in CD_3OD.

Pos.	**2**		**3**	
	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)	δ_C mult.
2	4.99 (br s)	78.5 d	4.99 (br s)	78.4 d
3	5.46 (m)	69.8 d	5.46 (m)	69.8 d
4	2.85 (dd, 17.4, 2.0)	26.8 t	2.85 (dd, 17.4, 1.9)	26.8 t
	2.98 (dd, 17.4, 4.6)		2.98 (dd, 17.4, 4.4)	
5		157.9 s		157.9 s
6	5.97 (d, 2.2)	96.5 d	5.96 (d, 2.2)	96.5 d
7		157.9 s		157.9 s
8	5.95 (d, 2.2)	95.8 d	5.98 (d, 2.2)	95.8 d
9		157.2 s		157.2 s
10		99.3 s		99.4 s
1′		131.4 s		131.4 s
2′	6.96 (d, 1.7)	115.1 d	6.97 (d, 1.7)	115.1 d
3′		146.0 s		146.0 s
4′		146.0 s		146.0 s
5′	6.78 (d, 8.1)	115.9 d	6.78 (d, 8.1)	116.0 d
6′	6.73 (dd, 8.1, 1.7)	119.3 d	6.73 (dd, 8.1, 1.7)	119.3 d
1″		127.2 s		127.8 s
2″	7.39 (d, 8.6)	131.3 d	7.09 (d, 1.8)	111.5 d
3″	6.76 (d, 8.6)	116.7 d		149.2 s
4″		161.2 s		150.5 s
5″	6.76 (d, 8.6)	116.7 d	6.98 (d, 8.2)	116.3 d
6″	7.39 (d, 8.6)	131.3 d	6.76 (dd, 8.2, 1.8)	124.3 d
7″	7.48 (d, 15.9)	146.8 d	7.46 (d, 15.8)	147.1 d
8″	6.24 (d, 15.9)	115.1 d	6.26 (d, 15.8)	115.3 d
9″		168.6 s		168.6 s
OCH_3			3.82 (s)	56.4 q

data, see Table 2; negative ESIMS: m/z 435 $[M-H]^-$; negative HRESIMS $[M-H]^-$ m/z 435.1075 (calcd for $C_{24}H_{19}O_8$, 435.1079).

Malaferin C (**3**): brown amorphous powder; $[\alpha]_D^{22.5}$ −175.55 (c 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 327 (4.26), 207 (4.79) nm; IR (KBr) ν_{max} 3429, 2924, 1771, 1698, 1684, 1634, 1558, 1540, 1517, 1457, 1436, 1339, 1283, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; negative ESIMS: m/z 465 $[M-H]^-$; negative HRESIMS $[M-H]^-$ m/z 465.1184 (calcd for $C_{25}H_{21}O_9$, 465.1185).

2.5. Anti-HIV-1 assay

Cytotoxicity was measured by the MTT method as described previously [7]. Briefly, C8166 cells were seeded in the absence or presence of various concentrations of compounds in triplicate for 3–7 days. The percentage of viable cells was quantified on a Bio-Tek ELx-800 ELISA reader at 595/630 nm ($A_{595/630}$). The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined from a dose–response curve. The cytopathic effect in the same cell lines was measured by counting the number of syncytia (multinucleated giant cells) in each well under an inverted microscope [8]. Various concentrations of compound were added in a 96-well microtiter plate. C8166 cells were infected with HIV-1_{IIIB} at 1300 $TCID_{50}$ HIV-1 and then incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 3 days. AZT was used as a positive control. The number of syncytia in each well was counted under an inverted microscope. The percentage inhibition of syncytial cell formation was calculated by percentage of syncytial cell numbers in compound-treated cultures to that of infected control culture. The concentration of the antiviral sample reducing HIV-1 replication by 50% (EC_{50}) was determined from the dose–response curve. The therapy index (TI) was calculated from the ratio of CC_{50}/EC_{50}.

3. Results and discussion

3.1. Chemistry

Malaferin A (**1**), obtained as colorless oil, has the molecular formula of $C_{19}H_{20}O_9$ based on HRESIMS at m/z 391.1032 $[M-H]^-$ (calcd m/z 391.1029), indicating 10 degrees of unsaturation. Its IR spectrum showed absorptions due to hydroxy (3424 cm^{-1}) and conjugated carbonyl (1626 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 1) of **1** exhibited a typical monosubstituted benzene ring at δ_H 7.61 (2H, m, H-2′ and H-6′), 7.39 (2H, t, J = 7.5 Hz, H-3′ and H-5′), and 7.49 (1H, t, J = 7.5 Hz, H-4′), and a singlet of one additional aromatic proton at δ_H 5.97 (1H, s, H-5). The ^{13}C NMR spectrum of **1** (Table 1) showed resonances for 12 aromatic carbons, belonging to the two aromatic rings, and a characteristic of conjugated carbonyl group at δ_C 200.9 (s, C-7). In addition, six aliphatic carbon signals at δ_C 76.2 (d, C-1″), 71.5 (d, C-2″), 79.9 (d, C-3″), 73.3 (d, C-4″), 82.5 (d, C-5″), and 62.5 (t, C-6″) and a characteristic doublet signal of an anomeric proton with a large coupling constant (J = 9.9 Hz) at δ_H 4.89 indicated the presence of a *C*-β-D-glucopyranosyl unit [9,10]. The above spectroscopic data suggested that compound **1** was a benzophenone C-glycoside, having a monosubstituted benzene and pentasubstituted benzene rings. The position of substituents on the pentasubstituted benzene ring was elucidated on the basis of chemical shift consideration, coupled with the 2D NMR experiment (Fig. 2). The ^{13}C NMR and HSQC spectra displayed six characteristic aromatic carbon signals at δ_C 104.6 (s, C-1), 163.3 (s, C-2), 106.2 (s, C-3), 164.5 (s, C-4), 96.2 (d, C-5), and 162.3 (s, C-6), three of which were highly deshielded, implying that these carbons were attached to electron-withdrawing groups. The pentasubstituted benzene ring of these signals suggested a phloroglucinol unit [11]. In the HMBC spectrum, correlations from the aromatic proton at δ_H 5.97 to C-1, C-2, C-4, C-5, and C-7, and from the anomeric proton to C-2, C-3, and C-4, confirmed the position of hydroxyl groups and the connection between the anomeric carbon and C-4 through C-linkage. Therefore, the structure of compound **1** was assigned as 2,4,6-trihydroxybenzophenone 3-*C*-β-D-glucopyranoside.

Malaferin B (**2**) was obtained as a brown amorphous powder and had a molecular formula $C_{24}H_{20}O_8$, as deduced from HRESIMS at m/z 435.1075 $[M-H]^-$ (calcd m/z 435.1079), requiring 15 degrees of unsaturation. Its IR

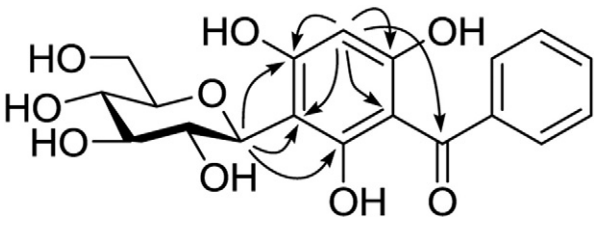

Fig. 2. Selected HMBC (H→C) correlations of **1**.

spectrum showed absorption bands at 3423 and 1684 cm^{-1}, suggesting the presence of hydroxy and carbonyl groups. The ^1H and ^{13}C NMR spectra (Table 2) of **2** showed one typical AB-spin-system with meta-coupling at δ_H 5.97 (1H, d, J = 2.2 Hz, H-6) and 5.95 (1H, d, J = 2.2 Hz, H-8), one ABX coupling system at δ_H 6.96 (1H, J = 1.7 Hz, H-2′), 6.78 (1H, d, J = 8.1 Hz, H-5′), and 6.73 (1H, dd, J = 8.1, 1.7 Hz, H-6′), and three aliphatic carbon signal at δ_C 78.5 (d, C-2), 69.8 (d, C-3), 26.8 (t, C-4). These finding revealed that compound **2** was a flavan-3-ol derivative with a 3,5,7,3′,4′-pentasubstitution pattern. The 1D NMR data of **2** showed similarities with the analogous values for (−)-epicatechin (**5**) [12]. However, the H-3 multiplet showed a strong downfield shift at δ_H 5.49, suggesting a substitution with an acyl moiety at C-3. Additionally, the ^1H NMR signals due to aromatic and olefinic protons at δ_H 7.39 (2H, d, J = 8.6 Hz, H-2″ and H-6″), 6.76 (2H, d, J = 8.6 Hz, H-3″ and H-5″), 7.48 (1H, d, J = 15.9 Hz, H-7″), and 6.24 (1H, d, J = 15.9 Hz, H-8″), as well as one ester carbonyl carbon at δ_C 168.6, implied the presences of a (*E*)-coumaroyl moiety. A long-range correlation between H-3 (5.46, m) and the ester carbonyl carbon in the HMBC spectrum indicated the (*E*)-coumaroyl unit was located at C-3. The 2,3-*cis* configuration of **2** was determined on the basis of a broad singlet at δ_H 4.99 for H-2 and the upfield shift at δ_C 78.5 of C-2 [13,14]. The optical rotation value of **2** {$[\alpha]_D^{22.8}$ −224.59 (*c* 0.24, MeOH)} was found to be similar to those of (−)-epicatechin-3-*O*-gallate {$[\alpha]_D^{22.8}$ −157.00 (*c* 0.30, acetone)} [15]. Thus, the structure of compound **2** was determined to be (−)-epicatechin-3-*O*-(*E*)-coumarate.

Malaferin C (**3**), brown amorphous powder, gave a molecular formula $C_{25}H_{22}O_9$, as deduced from HRESIMS at *m/z* 465.1184 $[M-H]^-$ (calcd 465.1185). Detailed analysis the ^1H and ^{13}C NMR data (Table 2) of **3** with those of **2** indicated they were analogues. The only difference was that the (*E*)-coumaroyl moiety in **2** was replaced by an (*E*)-feruloyl moiety [δ_H 7.09 (1H, d, J = 1.8 Hz, H-2″), 6.98 (1H, d, J = 8.2 Hz, H-5″), 6.76 (1H, dd, J = 8.2, 1.8 Hz, H-6″), 7.46 (1H, d, J = 15.8 Hz, H-7″), 6.26 (1H, d, J = 15.8 Hz, H-8″), and 3.82 (s, OCH_3)] in **3**. This deduction was confirmed by the HMBC correlation from H-3 to the ester carbonyl carbon at δ_C 168.6 of the (*E*)-feruloyl group. The configuration of **3** was determined to be same as **2** according to the NMR data and optical rotation experiment. Hence, compound **3** was elucidated as (−)-epicatechin-3-*O*-(*E*)-ferulate.

The known compounds were readily identified as garcimangosone D (**4**) [16], (−)-epicatechin (**5**) [12], (-)-epicatechin-3-*O*-benzoate (**6**) [17], (−)-epicatechin-3-*O*-gallate (**7**) [18], and procyanidin B5 (**8**) [19] by analysis of their NMR spectra and by comparison with the data reported in literature.

3.2. Biological activity

Considering some phenolics, including benzophenone and epicatechin derivatives, have been reported to have modest or strong anti-HIV activities [20,21]. Thus, the isolated compounds **1**–**8** were tested for their ability to prevent the cytopathic effects of HIV-1 in C8166, and their cytotoxicity was measured in parallel with the determination of antiviral activity, using AZT as a positive control. The results showed that garcimangosone D (**4**) exhibited moderate anti-HIV-1 activity with EC_{50} values of 18.06 μg/mL and TI (therapy index) value of more than 11.07. The other compounds showed weak bioactivity (Table 3).

Table 3
Anti-HIV-1 activities of compounds **1**–**8**.

Compounds	cytotoxicity CC_{50} (μg/mL)	Anti-HIV-1 activity EC_{50} (μg/mL)	Therapy index (TI) CC_{50}/EC_{50}
1	>200	131.70	>1.52
2	24.57	18.07	1.36
3	20.46	15.85	1.29
4	>200	18.06	>11.07
5	>200	52.31	>3.82
6	22.90	17.01	1.35
7	185.42	83.83	2.21
8	>200	31.41	>6.37
AZT	870.00	4.25×10^{-3}	204706

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