

Eucalmidins A–E, (+)-Oleuropeic Acid Derivatives from the Fresh Leaves of *Eucalyptus maideni*

Li-Wen Tian,^{†,‡} Ying-Jun Zhang,^{*,†} Yi-Fei Wang,[§] Chi-Choi Lai,[§] and Chong-Ren Yang^{†,⊥}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Graduate University of Chinese Academy of Sciences, Beijing 10049, People's Republic of China, Jinan University, Guangzhou 510632, People's Republic of China, and Weihe Biotech Research and Development Center, Yuxi 653101, People's Republic of China

Received May 14, 2009

Five new (+)-oleuropeic acid derivatives, eucalmidins A–E (**1–5**), together with 12 known compounds (**6–17**), were isolated from the fresh leaves of *Eucalyptus maideni*. Structures of the new compounds were determined on the basis of spectroscopic analyses (HSQC, HMBC, and ¹H–¹H COSY), chemical degradation, and enzymatic hydrolysis. Of the tested compounds, only quercetin showed slight anti-herpes simplex virus 1 (HSV-1) activity in vitro.

The genus *Eucalyptus* (Myrtaceae), containing about 945 species, mainly grows in the tropical and subtropical areas of the world, and most of them are native to Australia.¹ It was introduced into China in 1890, and currently about 300 species are distributed there. This genus is known to be rich sources of bioactive secondary metabolites. A number of unusual phloroglucinol-coupled terpenoids, named euglobals, macrocarpals,² and eucalyptals,³ have been isolated in the past decades. In addition, a series of phenolic glycosides conjugated with oleuropeic acid were also reported.^{4–6} Some of them displayed antitumor-promoting⁴ and antioxidant⁵ activities.

Eucalyptus maideni F. Muell is a tall timber tree growing widely in the southern parts of China.⁷ The trunks are commonly used in forestry, while its leaves are extracted for essential oil. Our detailed study led to the isolation of five new (+)-oleuropeic acid derivatives, together with 12 known compounds from the fresh leaves of this plant. Their structures were determined by extensive spectroscopic analyses, including HSQC, HMBC, ¹H–¹H COSY NMR, chemical methods, and enzymatic hydrolysis. In addition, the in vitro anti HSV-1 activity of the isolated compounds is also described.

Results and Discussion

The 80% aqueous acetone extract of the fresh leaves of *E. maideni* was partitioned between CHCl₃ and H₂O. The H₂O portion was subjected to repeated column chromatography over Diaion HP20SS, Sephadex LH-20, Toyopearl HW-40, and MCI-gel CHP-20P to yield 17 compounds (**1–17**). Compounds **6–17** were determined to be the known compounds (±)-oleuropeic acid (**6**),⁸ cypellocarpins A–C (**7–9**),⁴ cypellogin B (**10**),⁵ quercetin (**11**),⁹ quercetin 3-*O*-α-L-rhamnopyranoside (**12**),⁹ quercetin 3-*O*-β-D-glucopyranoside (**13**),⁹ quercetin 3-*O*-β-D-(6''-feruloyl)galactopyranoside (**14**),¹⁰ syringetin 3-*O*-β-D-glucopyranoside (**15**),¹¹ (–)-dihydrodehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside (**16**),¹² and (+)-isolariciresinol 3α-*O*-β-D-glucopyranoside (**17**),¹³ on the basis of detailed spectroscopic analyses, together with comparison of their spectroscopic and physical data with reported data. The known compounds **6** and **14–17** were reported from the genus *Eucalyptus* for the first time.

Eucalmidain A (**1**) was obtained as a pale, amorphous powder. Its molecular formula, C₁₆H₂₆O₈, was established on the basis of

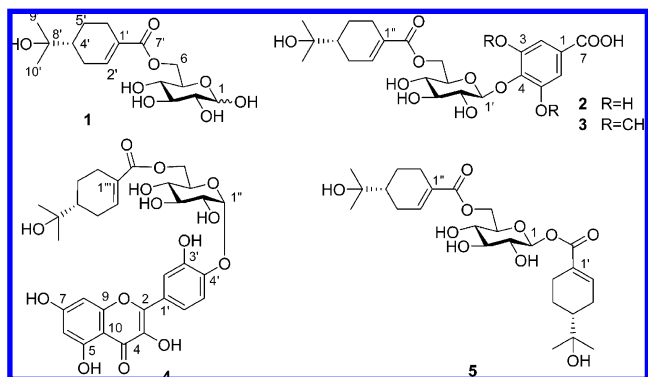


Figure 1. New compounds **1–5** isolated from *Eucalyptus maideni*.

HRESIMS (m/z 381.1326 [$M + Cl$][–], calcd 381.1316). The ¹³C NMR spectrum of **1** showed 10 carbon signals comprising one carboxylic (δ 169.0), one trisubstituted double bond (δ 141.3, 131.3), two methyl (δ 27.0, 26.6), three methylene (δ 28.5, 24.6, 26.3), one methine (δ 45.6), and one oxygen-bearing quaternary carbon (δ 72.8). These observations suggested the presence of an oleuropeic acid unit, also found in cypellocarpins A–C from *E. cypellocarpa*.⁴ The ¹H NMR spectrum also showed typical signals of an olefinic proton at δ 7.02 (m) and two tertiary methyls at δ 1.19 and 1.16 (each 3H, s). In addition, the complex proton signal patterns arising from the sugar moiety, as well as the appearance of the anomeric proton signals at δ 5.10 (1/2 H, d, $J = 3.7$ Hz) (α -form) and 4.47 (1/2 H, d, $J = 7.8$ Hz) (β -form) in the ratio of 1:1 and the anomeric carbon signals at δ 94.0 and 98.3, indicated that **1** is an anomeric mixture. Acidic hydrolysis of **1** liberated D-glucose, which was determined by GC analysis of its corresponding trimethylsilylated L-cysteine adduct. The presence of the (+)-oleuropeic acid moiety was confirmed by the methanolysis of **1** with NaOMe in MeOH, which gave the (+)-oleuropeic acid methyl ester [$\alpha_D^{20} +60$ (CHCl₃)].³ The linkage of the (+)-oleuropeoyl moiety at C-6 of the D-glucose unit was established by an HMBC experiment, which showed correlations of H-6 of glucose [δ 4.43, 4.27 (β -form), δ 4.38, 4.23 (α -form)] with the carboxylic carbon at δ 169.0 (C-7') of the oleuropeoyl moiety. On the basis of the above evidence, the structure of **1** was determined to be 6-*O*-oleuropeoyl-D-glucopyranose and named eucalmidain A.

Eucalmidain B (**2**), a pale, amorphous powder, had a molecular formula of C₂₃H₃₀O₁₂ deduced from the HRESIMS (m/z 497.1675 [$M - H$][–], calcd 497.1659), which was the same as that of cypellocarpin A (**7**) (see Supporting Information). Comparison of

* To whom correspondence should be addressed. Tel: + 86-871-522-3235. Fax: + 86-871-515-0124. E-mail: zhangyj@mail.kib.ac.cn.

[†] Kunming Institute of Botany.

[‡] Graduate University of the Chinese Academy of Sciences.

[§] Jinan University.

[⊥] Weihe Biotech Research and Development Center.

Table 1. ¹H and ¹³C NMR Data of Compounds **1** and **5** in Methanol-*d*₄ (δ values; *J* in Hz, in parentheses)

position	1				5	
	δ _H		δ _C		δ _H	δ _C
	β	α	β	α		
Glc-1	4.47 d (7.8)	5.10 d (3.7)	98.3	94.0	5.49 d (7.5)	95.7
2	3.13 t (8.4)	3.33 m	76.3	73.9	3.40 m	73.9
3	3.33 m	3.66 t (9.2)	78.1	74.9	3.45 t (8.9)	77.9
4	3.34 m	3.33 m	71.8	72.1	3.38 t (8.5)	71.3
5	3.49 m	3.98 m	75.6	70.9	3.60 m	76.2
6	4.43 m	4.38 m	64.8	64.9	4.40 dd (2, 11)	64.3
	4.27 m	4.23 m			4.24 dd (6, 11)	
oleuropeoyl-1'			131.3			131.1
2'	7.02 m		141.3		7.15 m	143.1
3',3''	2.02 m		28.5		2.34 m	28.7 ^a
	2.31 m				2.02 m	28.6 ^a
4',4''	1.52 m		45.6		1.53 m	45.5
5',5''	1.99 m		24.6		2.02 m	24.5
	1.21 m				1.21 m	
6', 6''	2.14 m		26.3		2.50 m	26.4 ^b
	2.49 m				2.16 m	26.2 ^b
7'			169.0			167.2
8',8''			72.8			72.8
9',9''	1.19 s		27.0		1.17 s	27.0
10',10''	1.16 s		26.6		1.17 s	26.5
oleuropeoyl-1''						130.6
2''					7.02 m	141.6
7''						168.8

^{a,b} Assignments for the same corresponding position of different moieties may be interchanged.

the 1D NMR data with those of cypellocarpin A suggested that the structure of **2** was composed of an oleuropeoyl, a glucosyl, and a gallic acid moiety. However, instead of the two *m*-coupled protons at δ 7.32, 7.22 (d, 2.6 Hz) from the gallic acid moiety of **7**, compound **2** showed one two-proton singlet at δ 7.05 (2H, s) arising from a symmetrically substituted gallic acid moiety. This suggested C-4 substitution of the gallic acid moiety in **2**, relative to C-3 substitution in **7**. In the HMBC spectrum of **2**, the anomeric proton [δ 4.64 (d, 7.7 Hz)] of glucose was correlated with the C-4 (δ 135.7) of gallic acid, confirming the linkage between the gallic acid and glucosyl moieties. In addition, HMBC correlation of the glucosyl H-6' (δ 4.54, 4.24) with the oleuropeoyl carboxylic carbon at δ 168.8 was also observed. Methanolysis of **2** with NaOMe in MeOH gave (+)-oleuropeic acid methyl ester {[α]_D +56.7 (CHCl₃)}. Acidic hydrolysis of **2** gave D-glucose as a sugar residue. Consequently, the structure of eucalmaidin B was assigned as shown in **2**.

Eucalmaidin C (**3**) was obtained as a pale, amorphous powder. Its molecular formula C₂₅H₃₄O₁₂ was elucidated from the HRESIMS (*m/z* 525.1978 [M - H]⁻, calcd 525.1972). The ¹H and ¹³C NMR spectra of **3** were similar to those of **2**, except for the appearance of two methoxy groups [δ_H 3.91 (6H, s) and δ_C 56.9 (2 × C)], suggesting that the C-3 and C-5 hydroxy groups in **2** were methylated. This was further confirmed by the HMBC correlation of the *O*-methyl protons (δ 3.91) with C-3 and C-5 of the gallic acid moiety at δ 153.7. Correlations of H-1' [δ 4.88, (d, *J* = 7.7 Hz)] with C-4 (δ 137.6) and H-6' (δ 4.42, 4.27 m) with C-7'' (δ 168.7) were also observed in the HMBC experiment. Methanolysis of **3** with NaOMe gave (+)-oleuropeic acid methyl ester {[α]_D +42.5 (CHCl₃)}, while acidic hydrolysis of **3** yielded D-glucose as a sugar residue. On the basis of the above evidence, the structure of compound **3** was determined to be 3,5-dimethyleucalmaidin B and named eucalmaidin C.

Eucalmaidin D (**4**), a yellowish, amorphous powder, showed a quasi-molecular ion peak at *m/z* 629.1852 in the HRESIMS, corresponding to the molecular formula C₃₁H₃₄O₁₄. The ¹H and ¹³C NMR spectra showed the presence of an oleuropeoyl and a glucosyl moiety. The small coupling constant (3.7 Hz) as well as the ¹³C NMR data assigned an α configuration of the glucosyl anomeric center.¹⁴ Acidic hydrolysis of **4** liberated D-glucose, which was determined by GC analysis of its corresponding trimethylsilylated

Table 2. ¹H NMR Data of Compounds **2** and **3** in Methanol-*d*₄ (δ values; *J* in Hz, in parentheses)

position	2		3	
	δ _H	δ _C	δ _H	δ _C
aglycone-1		136.5		135.7
2,6	7.05 s	110.1	7.40 s	108.3
3,5		150.8		153.7
4		135.7		137.6
7		168.8		174.3
OMe			3.91 s	56.9
Glc-1'	4.64 d (7.7)	107.4	4.88 d (7.7)	104.6
2'	3.52 dd (8.0, 8.9)	74.9	3.48 m	75.6
3'	3.46 t (8.8)	77.4	3.52 t (9.0)	77.9
4'	3.40 t (9.3)	71.5	3.45 t (9.3)	72.0
5'	3.62 m	76.3	3.58 m	75.7
6'	4.54 m	64.4	4.42 m	64.6
	4.24 m		4.27 m	
oleuropeoyl-1''		130.9		131.1
2''	7.03 m	142.1	6.89 m	141.5
3''	2.01 m	28.6	2.02 m	28.6
	2.32 m		2.35 m	
4''	1.54 m	45.5	1.55 m	45.5
5''	1.98 m	24.5	1.20 m	24.5
	2.01 m		2.05 m	
6''	2.15 m	26.2	2.10 m	26.3
	2.49 m		2.42 m	
7''		168.8		168.7
8''		72.9		72.9
9''	1.18 s	27.1	1.24 s	27.1
10''	1.17 s	26.4	1.23 s	26.5

L-cysteine adduct. In addition, the 15 aromatic carbons (Table 3) and five typical aromatic proton signals [δ 6.18, 6.43 (each 1H, s, H-6, 8), 7.68 (brs, H-2'), 7.17 (1H, d, *J* = 8.7 Hz, H-5'), 7.55 (1H, d, *J* = 8.7 Hz, H-6')] revealed the existence of a quercetin moiety. The connectivity of the glucosyl unit with oleuropeoyl and quercetin units was further confirmed by the HMBC spectrum, in which correlations of the anomeric proton (δ 5.07) with C-4' (δ 145.4) and the glucosyl H-6'' (δ 4.42, 4.04) with the oleuropeoyl C-7'' (δ 165.2) were observed. Thus, the structure of compound **4** was determined as quercetin 4'-*O*-(6-*O*-oleuropeoyl)-α-D-glucopyranoside and named eucalmaidin D.

Table 3. ¹H and ¹³C NMR Data of Compound **4** in DMSO-*d*₆ (δ values; *J* in Hz, in parentheses)

position	δ _H	δ _C	position	δ _H	δ _C
aglycone-2		145.5	4''	3.20 m	69.3
3		135.5	5''	3.70 m	72.9
4		175.0	6''	4.42 m	62.6
5		159.7		4.04 m	
6	6.18 s	97.3	oleuropeoyl-1'''		128.5
7		163.1	2'''	6.93 m	139.3
8	6.43 s	92.3	3'''	2.20 m	26.1
9		155.2		1.95 m	
10		102.1	4'''	1.37 m	42.7
1'		124.1	5'''	1.90 m	
2'	7.68 s	114.1		1.07 m	21.9
3'		144.7	6'''	2.37 m	24.0
4'		145.4		2.04 m	
5'	7.17 d (8.7)	114.6	7'''		165.2
6'	7.55 d (8.7)	118.3	8'''		69.4
Glc-1''	5.07 d (3.7)	99.6	9'''	0.98 s	25.9
2''	3.36 m	72.2	10'''	0.96 s	25.3
3''	3.36 m	74.8			

Eucalmaindin E (**5**) had a molecular formula of C₂₆H₄₀O₁₀, which was established from the HRESIMS (*m/z* 547.2321 [M + Cl]⁻, calcd 547.2310). The additional 166 mass units compared to **1** corresponded to the presence of a second oleuropeoyl unit. The 1D NMR spectra of **5** were similar to those of **1**. However, instead of an anomeric free glucose moiety in **1**, the ¹H NMR spectrum of compound **5** showed the presence of a β-glucosyl unit [δ 5.49 (d, *J* = 7.5 Hz)]. In addition, the ¹H and ¹³C NMR spectra displayed two sets of signals assignable to two oleuropeoyl moieties [δ 7.15, 7.02 (each 1H, m, H-2', 2''), 1.17 (12H, s, CH₃-9', 9'', 10', 10'')]. Connectivities of the two oleuropeoyl moieties with the glucosyl unit were revealed by the HMBC experiment, in which the glucosyl anomeric proton (δ 5.49) and H-6 (δ 4.40 and 4.24) were correlated with C-7' (δ 167.2) and C-7'' (δ 168.8), respectively. Enzymatic hydrolysis of **5** yielded (+)-oleuropeic acid and eucalmaindin A. Thus, the structure of eucalmaindin E was constructed as shown in **5**.

Eucalmaindin D (**4**) represents a rare example of an α-configured glucoside in nature. Instead of the quercetin C-3 substitution with the 6-*O*-oleuropeoyl-β-D-glucosyl moiety in cypellogins,⁵ eucalmaindin D (**4**) has an 6-*O*-oleuropeoyl-α-D-glucosyl moiety linked at C-4' of the quercetin moiety.

Compounds **1–3**, **5–9**, **11**, **12**, **16**, and **17** were evaluated for their in vitro anti-herpes simplex virus type 1 (HSV-1) activity using a cytopathic effect (CPE) assay and cytotoxicity on African green monkey kidney cells (Vero cells) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. The maximal noncytotoxic concentrations (MNCC) against Vero cells and total inhibitory concentrations (TIC) against HSV-1 of compounds **1–3**, **5–9**, **11**, **12**, **16**, and **17** are shown in Table 4. Of all the tested compounds, only quercetin (**11**) showed slight anti-HSV-1 activity. Among the (+)-oleuropeic acid derivatives, compounds **2**, **3**, **5**, and **7–9** showed stronger cytotoxicity than 6-*O*-oleuropeoyl-D-glucopyranose (**1**).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were measured in methanol-*d*₄ or DMSO-*d*₆ solution on a Bruker DRX-500 instrument (500 MHz for ¹H NMR and 125 MHz for ¹³C NMR) at 25 °C, using TMS as an internal standard. FABMS were recorded on a VG Auto Spec-3000 mass spectrometer using glycerol as matrix. ESIMS and HRESIMS were recorded on an API QSTAR Pular-1 mass spectrometer (for compounds **1** and **5**, one drop of 0.01% aqueous NaCl was added while measuring the HRESIMS). The GC was performed on an HP5890 gas chromatograph (Agilent, America) with a quartz capillary column (30 mm × 0.32 mm × 0.25 μm); detection, FID.

Table 4. Anti-HSV-1 Activity of Compounds **1–3**, **5–9**, **11**, **12**, **16**, and **17**

compound	MNCC (mM) ^a	TIC (mM) ^b
1	>0.58	— ^d
2	0.40	—
3	0.38	—
5	0.20	—
6	>1.09	—
7	0.03	—
8	0.02	—
9	>0.38	—
11	<0.02	0.33
12	0.01	—
16	0.39	—
17	0.38	—
aciclovir	1.11	0.0043 ^c

^a MNCC: maximal noncytotoxic concentration against Vero cells. ^b TIC: total inhibitory concentration against HSV-1. ^c IC₅₀ value (concentration required to reduce 50% of cytopathic effect). ^d —: no activity.

Column chromatography (CC) was performed on Diaion HP20SS (Mitsubishi Chemical Co.), MCI-gel CHP-20P (75–150 μm, Mitsubishi Chemical Co.), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co.), RP-8 (40–63 μm, Merck), and Toyopearl HW-40 (fine grade) (TOSOH, Japan). Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done by spraying the plates with anisaldehyde-sulfuric acid, followed by heating. Sustainable medium used for assays was Dulbecco's modified Eagle's medium with 2% fetal bovine serum, whose pH value was adjusted to 7.2 by 0.75% NaHCO₃ and Hepes buffer (47.6 g of Hepes was dissolved in H₂O (200 mL) and its pH adjusted to 7.5–8.0 by 1 N NaOH).

Plant Material. The fresh leaves of *E. maideni* were collected in the Botanical Garden of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China, during May 2007. A voucher specimen (KIB-ZL2007001) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The fresh leaves of *E. maideni* (3.5 kg) were extracted with 80% aqueous acetone at room temperature (3 × 10 L, each 1 week). The extracts were concentrated under reduced pressure and then partitioned with CHCl₃ (6 × 2 L) after filtration of the precipitate. The water portion was concentrated to a small volume (200 mL) and subjected to Diaion HP20SS column chromatography (CC), eluting with MeOH–H₂O (0:1–1:0) to afford seven fractions. Fraction 4 (22 g) was chromatographed over Sephadex LH-20 (MeOH–H₂O, 0:1–1:0) to yield five subfractions (A–E). Subfraction A was chromatographed over MCI-gel CHP-20P (0–80% MeOH) to yield **3** (21 mg) and **6** (25 mg). Subfraction B was subjected to CC over MCI-gel CHP-20P (30%–100% MeOH) and silica gel (CHCl₃–MeOH–H₂O, 8.5:1.5:0.1–8:2:0.2) to yield **16** (7 mg) and **17** (18 mg). Subfraction C was applied to MCI-gel CHP-20P (20%–100%, MeOH) and Rp-8 (50%–100%, MeOH) CC, as well as preparative TLC of silica gel (C₆H₆–HCOOEt–HCOOH, 2:7:1), to yield **1** (91 mg), **2** (30 mg), and **7** (98 mg). Subfraction E was chromatographed over silica gel (CHCl₃–MeOH–H₂O, 8.5:1.5:0.1–7:3:0.5) and MCI-gel CHP-20P (20%–100% MeOH) to afford **12** (18 mg), **13** (7 mg), and **15** (33 mg). Fraction 5 (4 g) was successively chromatographed over Sephadex LH-20 (0–60% MeOH), MCI-gel CHP-20P (40%–100% MeOH), and Toyopearl HW-40 (0–40% MeOH) to yield **1** (16 mg) and **5** (12 mg). Fraction 6 (1.92 g) was subjected to CC over Sephadex LH-20 (0–100%, MeOH), MCI-gel CHP-20P (40%–100% MeOH), and silica gel (CHCl₃–MeOH–H₂O, 9:1:0.1–8:2:0.2) to yield **4** (10 mg), **8** (18 mg), **9** (70 mg), **10** (6 mg), **11** (70 mg), and **14** (7 mg).

Eucalmaindin A (1): pale, amorphous powder; [α]_D²⁰ +26.7 (c 0.3, MeOH); UV (MeOH), λ_{max} (log ε) 225 (3.54) nm; IR (KBr) ν_{max} 3413, 2930, 2886, 1696, 1258, 1073, 1059, 1035 cm⁻¹; ¹H NMR (methanol-*d*₄, 500 MHz), see Table 1; ¹³C NMR (methanol-*d*₄, 125 MHz), see Table 1; FABMS (negative ion mode) *m/z* 345 [M – H]⁻; HRESIMS *m/z* 381.1326 [M + Cl]⁻ (calcd for C₁₆H₂₆O₈Cl, 381.1316).

Eucalmaindin B (2): pale, amorphous powder; [α]_D²⁰ –17.9 (c 1.2, MeOH); UV (MeOH), λ_{max} (log ε) 209 (3.83) nm; IR (KBr) ν_{max} 3417, 2926, 2887, 1703, 1562, 1394, 1259, 1062 cm⁻¹; ¹H NMR (methanol-*d*₄, 500 MHz), see Table 2; ¹³C NMR (methanol-*d*₄, 125 MHz), see

Table 2; FABMS (negative ion mode) m/z 497 [M - H]⁻; HRESIMS m/z 497.1675 [M - H]⁻ (calcd for C₂₃H₂₉O₁₂, 497.1659).

Eucalmaidin C (3): pale, amorphous powder; $[\alpha]_D^{27}$ -27.5 (c 0.2, MeOH); UV (MeOH), λ_{max} (log ϵ) 218 (3.03), 265 (2.85) nm; IR (KBr) ν_{max} 3425, 2938, 2880, 1703, 1566, 1385, 1123, 1070 cm⁻¹; ¹H NMR (methanol-*d*₄, 500 MHz), see Table 2; ¹³C NMR (methanol-*d*₄, 125 MHz), see Table 2; ESIMS (negative ion mode) m/z 525 [M - H]⁻; HRESIMS m/z 525.1978 [M - H]⁻ (calcd for C₂₅H₃₃O₁₂, 525.1972).

Eucalmaidin D (4): yellowish, amorphous powder; $[\alpha]_D^{20}$ -58.0 (c 0.5, MeOH); UV (MeOH), λ_{max} (log ϵ) 251 (4.91), 367 (4.01) nm; IR (KBr) ν_{max} 3422, 2967, 2924, 1696, 1650, 1601, 1506, 1253, 1067 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz), see Table 3; ¹³C NMR (DMSO-*d*₆, 125 MHz), see Table 3; FABMS (negative ion mode) m/z 629 [M - H]⁻, 301 [M - H - Glc - oleuropeoyl]⁻; HRESIMS m/z 629.1852 [M - H]⁻ (calcd for C₃₁H₃₃O₁₄, 629.1870).

Eucalmaidin E (5): pale, amorphous powder; $[\alpha]_D^{27}$ -1.3 (c 0.8, MeOH); UV (MeOH), λ_{max} (log ϵ) 225 (3.42) nm; IR (KBr) ν_{max} 3471, 2924, 2853, 1673, 1641, 1433, 1286 cm⁻¹; ¹H NMR (methanol-*d*₄, 500 MHz), see Table 1; ¹³C NMR (methanol-*d*₄, 125 MHz), see Table 1; FABMS (negative ion mode) m/z 511 [M - H]⁻; HRESIMS m/z 547.2321 [M + Cl]⁻ (calcd for C₂₆H₄₀O₁₅Cl, 547.2310).

Acid Hydrolysis of Compounds 1–4. Compounds 1–4 (each 6 mg, 4 mg for compound 4) were hydrolyzed with 1.5 N HCl (2 mL) at 80 °C for 5 h. The mixture was neutralized with NaOH (1 N). The mixture was passed through MCI-gel CHP-20P (1.5 × 14 cm), developing with H₂O. The H₂O eluate was evaporated to dryness. The dry powders were dissolved in pyridine (2 mL), L-cysteine methyl ester hydrochloride (1.5 mg) was added, and the mixture was heated at 60 °C for 1 h. Trimethylsilylimidazole (1.5 mL) was added, and the mixture was heated at 60 °C for another 30 min. An aliquot (4 μ L) of the supernatant was removed and directly subjected to GC analysis under the following conditions: column temp 180–280 °C at 3 deg/min, carrier gas N₂ (1 mL/min), injector and detector temp 250 °C, split ratio 1:50. The configurations of D-glucose for compounds 1–4 were determined by comparison of the retentions times of the corresponding derivatives with standard D-glucose (retention time: 19.208 min), respectively.

Methanolysis of 1–3. A solution of 1 (6 mg) in 0.02 M NaOMe–MeOH (1 mL) was kept standing at room temperature for 12 h. The solution was then subjected to column chromatography over MCI-gel CHP-20P (1.5 × 14 cm), eluting with H₂O, 60% and 100% MeOH to give (+)-oleuropeic acid methyl ester (1a) (3 mg): colorless oil; $[\alpha]_D^{+60}$ (c 0.2, CHCl₃); ¹H NMR (CDCl₃) δ 6.99 (1H, m, H-2), 2.33, 2.00 (m, H-3), 1.55 (m, H-4), 2.03, 1.23 (m, H-5), 2.54, 2.17 (m, H-6), 1.20 (3H, s, H-9), 1.21 (3H, s, H-10), 3.72 (3H, s, OCH₃); ¹³C NMR (CDCl₃) δ 130.1 (C-1), 139.5 (C-2), 27.4 (C-3), 44.1 (C-4), 23.3 (C-5), 25.1 (C-6), 167.8 (C-7), 72.4 (C-8), 27.3 (C-9), 26.6 (C-10), 51.6 (OCH₃).⁴ Similar methanolysis of 2 and 3 also gave 1a $\{[\alpha]_D^{+56.7}$ (c 0.25, CHCl₃) and $[\alpha]_D^{+42.5}$ (c 0.2, CHCl₃), respectively}.

Enzymatic Hydrolysis of 5. A mixture of 5 (10 mg) and β -glucosidase (8 mg, Sigma) in H₂O (1.5 mL) was kept in a water bath at 37 °C for 8 days. The mixture was subjected to MCI-gel CHP-20P (50% and 100% MeOH) column chromatography. The 50% and 100% MeOH eluates were separately chromatographed over silica gel (CHCl₃–MeOH–H₂O, 9:1:0.1–8:2:0.2, and CHCl₃–MeOH, 15: 1) to give (+)-oleuropeic acid (2 mg) $\{[\alpha]_D^{+13.3}$ (c 0.1, MeOH)} and 1 (4 mg) $\{[\alpha]_D^{+10.0}$ (c 0.2, MeOH)}, respectively. Oleuropeic acid and eucalmaidin A were identified by co-chromatography in TLC with authentic samples.

HSV-1 Inhibition Activity. HSV-1 inhibition activity was assayed with the plaque reduction assay,¹⁵ with acyclovir as positive control. The Vero cells were seeded into 24-well culture plates. After 24 h of incubation, the cells were infected with 30 PFU HSV-1 in the presence of samples of different concentrations (samples were diluted with cell sustainable medium), while the dilution medium without samples was

used as the control. Then each well was overlaid with medium containing 1% of methylcellulose, and the plate was incubated for 3 days. Thereafter, the cell monolayer was fixed and stained with formalin and crystal violet, respectively. The viral plaques were counted under a binocular microscope. The concentration reducing plaque formation by 100% relative to control was estimated from graphic plots and defined as 100% inhibitory concentration.

Cytotoxicity Assays. Cytotoxic activity was performed using the MTT reduction assay.¹⁶ Vero cells were seeded into a 96-well plate. Different concentrations of samples (100 μ L), diluted with cell sustainable medium, were applied to the wells of a 96-well plate containing confluent cell monolayer in triplicate, while a dilution medium without sample was used as the control. After 3 days of incubation, 12 μ L of the MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well. The plate was further incubated for 4 h to allow for MTT formazan formation. After removing the medium, 100 μ L of DMSO was added to dissolve the formazan crystals. The content in the wells was homogenized on a microplate shake 30 min later. The OD (optical density) was then read on a microplate spectrophotometer at double wavelengths of 540 and 630 nm. The maximal nontoxic concentration was defined as the maximal concentration of the sample that did not exert a cytotoxic effect evaluated from the OD values of nonviable cells.

Acknowledgment. We are grateful to the members of the Analytical Group in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the measurements of all spectra. This work was supported by the NSFC U0632010 and the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences (P2008-ZZ08).

Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Zhang, R. G.; Li, S. G.; Jiang, Y. D. *Xibu Linze Kexue* **2007**, *36*, 97–102.
- Singh, I. P.; Bharate, S. B. *Nat. Prod. Rep.* **2006**, *23*, 558–591.
- Yin, S.; Xue, J. J.; Fan, C. Q.; Miao, Z. H.; Ding, J.; Yue, J. M. *Org. Lett.* **2007**, *9*, 5549–5552.
- Ito, H.; Koreishi, M.; Tokuda, H.; Nishino, H.; Yoshida, T. *J. Nat. Prod.* **2000**, *63*, 1253–1257.
- Kasajima, N.; Ito, H.; Hatano, T.; Yoshida, T.; Kaneda, M. *Chem. Pharm. Bull.* **2005**, *53*, 1345–1347.
- Hasegawa, T.; Takano, F.; Takata, T.; Niyama, M.; Ohta, T. *Phytochemistry* **2008**, *69*, 747–753.
- Institutum Botanicum Kunmingense Academiae Sinicae Edita. *Flora Yunnanica*; Science Press: Beijing, 1997; Vol. 7, p 102.
- Nakanishi, T.; Iida, N.; Inatomi, Y.; Murata, H.; Inada, A.; Murata, J.; Lang, F. A.; Inuma, M.; Tanaka, T.; Sakagami, Y. *Chem. Pharm. Bull.* **2005**, *53*, 783–787.
- Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389–1397.
- Braca, A.; Bilia, A. R.; Mendez, J.; Morelli, I. *Fitoterapia* **2001**, *72*, 182–185.
- Moon, H. I.; Lee, J.; Zee, O. P.; Chung, J. H. *J. Ethnopharmacol.* **2005**, *101*, 176–179.
- Abe, F.; Yamauchi, T. *Chem. Pharm. Bull.* **1986**, *34*, 4340–4345.
- Achenbach, H.; Lowel, M.; Waibel, R.; Gupta, M.; Solis, P. *Planta Med.* **1992**, *58*, 270–272.
- Gorgin, P. A. J.; Mazurek, M. *Can. J. Chem.* **1975**, *53*, 1212–1223.
- Cheng, H. Y.; Lin, T. C.; Yang, C. M.; Wang, K. C.; Lin, C. C. *Microbes Infect.* **2004**, *6*, 738–744.
- Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

NP900290S