Phloroglucinol Glycosides from the Fresh Fruits of *Eucalyptus maideni*

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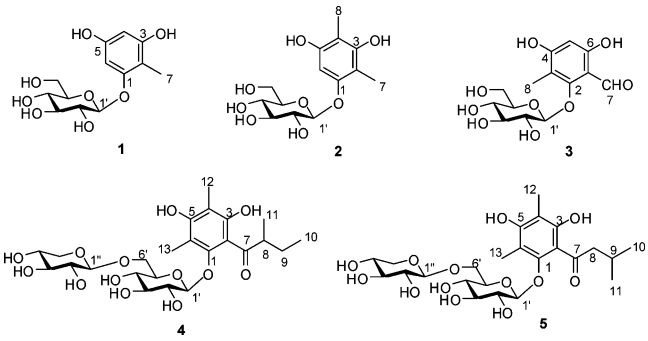
Five new phloroglucinol glycosides, eucalmainosides A−E (**1**−**5**), were isolated from the fresh fruits of *Eucalyptus maideni,* along with 15 flavonoids (**6**−**20**), seven (+)-oleuropeic acid derivatives (**15**, **16**, and **22**−**26**), three hydrolyzable tannins (**32**−**34**), and six simple phenolic compounds (**21**, **27**−**31**). Their structures were determined on the basis of spectroscopic analyses, including HSQC, HMBC, and acidic hydrolysis. The in vitro anti-herpes simplex virus 1 (HSV-1) assay indicated that the flavonols, myricetin (**6**) and quercetin (**7**), and the ellagitannin isocoriariin F (**33**) showed weak anti-HSV-1 activity with TIC values of 0.31, 0.33, and 0.12 mM, respectively.

The genus *Eucalyptus* (Myrtaceae) is known to be rich source of bioactive natural products. A series of terpenoids,[1] tannins,[2] flavonoids,[3] and phloroglucinol derivatives[4–7] with antiviral[6] and antibacterial[7] effects have been reported. Phloroglucinol derivatives, mainly obtained from the leaves or fruits, are representative active principals of the genus.

E. maideni F. Muell is a tall timber tree growing widely in the southern part of China.[8] The trunks are commonly used in forestry, while its leaves are extracted for essential oil. Recently, we have reported five new (+)-oleuropeic acid derivatives, eucalmaidins A−E, from the fresh leaves of this plant.[9] Further investigation on the fresh fruits of the same plant resulted in the isolation of five new phloroglucinol glycosides, eucalmainosides A−E (**1**−**5**), together with 29 known compounds (**6**−**34**). Their structures were determined by spectroscopic analyses and acidic hydrolysis. In addition, the in vitro anti HSV-1 activities of several of the isolated compounds are reported.



Results and Discussion

The 80% aqueous acetone extract of the fresh fruits of *E. maideni* was suspended in H_2O and partitioned successively with petroleum ether and EtOAc. The EtOAc and H_2O layers were separately subjected to various column chromatographies to yield five new compounds (**1**−**5**). In addition, 29 known compounds were identified to be myricetin (**6**),[10] quercetin (**7**),[11] tricetin (**8**),[11] luteolin (**9**),[10] quercetin 4′-*O*-*β*-D-glucopyranoside (**10**),[11] myricetin 3′-*O*-*β*-D-glucopyranoside (**11**),[10] quercetin 3-*O*-*β*-D-glucopyranoside (**12**),[11] tricetin 3′-*O*-*β*-D-glucopyranoside (**13**),[10] luteolin 3′-*O*-*β*-D-glucopyranoside (**14**),[11] cypellogins A and B (**15**, **16**),[12] quercetin 3-*O*-sambubioside (**17**),[13] quercetin 3-*O*-sophoroside (**18**),[14] gossypetin 3-*O*-glucuropyranoside (**19**),[15] quercetin 3-*O*-*β*-D-glucuropyranoside (**20**),[16] gallic acid (**21**),[16] cypellocarpin A (**22**),[17] eucalmaidin B (**23**),[9] eucaglobulin (**24**),[18] (±)-oleuropeic acid (**25**),[9] eucalmaidin E (**26**),[9] 3-*O*-methylellagic acid 3′-*O*-α-L-rhamnopyranoside (**27**),[19] (+)-isolariciresinol 3α-*O*-*β*-D-glucopyranoside (**28**),[20] *threo*-syringoylglycerol (**29**),[21] (1′*R*,2′*R*)-guaiacylglycerol (**30**),[22] tachioside (**31**),[23] 6-*O*-galloylglucose (**32**),[24] isocoriariin F (**33**),[25] and oenthein C (**34**)[25] on the basis of spectroscopic analyses. Compounds **13**, **17**−**19**, and **29**−**34** were isolated from the genus *Eucalyptus* for the first time.

Eucalmainoiside A (**1**) was obtained as a pale, amorphous powder. Its molecular formula, $C_{13}H_{18}O_8$, was established on the basis of HRESIMS (*m*/*z* 337.0696 $[M + Cl]^-$, calcd 337.0690). The ^{1}H NMR spectrum of **1** displayed two *m*-coupled aromatic protons [*δ* 6.04, 6.18 (each 1H, d, 2.2 Hz, H-4, H-6)], an anomeric proton [*δ* 4.77 (1H, d, 7.5 Hz, H-1′)], and methyl protons [*δ* 1.91, (3H, s, H-7)]. In the ^{13}C NMR spectrum of **1**, six characteristic aromatic carbons at *δ* 157.5 (C), 156.7 (C), 156.1 (C), 105.3 (C), 97.3 (CH), and 95.4 (CH) indicated the presence of an unsymmetrically substituted phloroglucinol unit, the typical principal within the genus *Eucalyptus*. In addition, a shielded carbon at *δ* 8.1, which suggested its linkage with a benzene ring, was observed, together with a set of signals arising from a glucose moiety [*δ* 101.6 (CH, C-1′), 73.9 (CH, C-2′), 76.9 (CH, C-3′), 70.5 (CH, C-4′), 76.9 (CH, C-5′), and 61.8 (CH_2, C-6′)]. Acid hydrolysis of **1** yielded D-glucose, the absolute configuration of which was determined by GC analysis of its corresponding trimethylsilylated L-cysteine adduct. The linkages of the glucosyl and methyl groups with the phloroglucinol unit were deteremined by 2D NMR experiments. In the HMBC spectrum of **1**, the methyl protons (*δ* 1.91) and the anomeric proton (*δ* 4.77) were correlated with C-2 (*δ* 105.3) and C-1 (*δ* 157.5), respectively, revealing the location of the methyl group on the benzene ring and the glucosyl moiety attached to one of the phenolic hydroxy groups of the phloroglucinol unit. Furthermore, the ROESY correlation of the anomeric proton (*δ* 4.77) with H-6 (*δ* 6.18) and the unsymmetrically substituted phloroglucinol unit confirmed the structure of **1**. Compound **1** was therefore determined to be 2-methylphloroglucinol-*O*-*β*-D- glucopyranoside and named eucalmainoside A.

Eucalmainoside B (**2**), a pale, amorphous powder, had a molecular formula $C_{14}H_{20}O_8$ deduced from the HRESIMS (*m*/*z* 351.0842 $[M + Cl]^-$, calcd 351.0846), which is 14 mass units more than that of **1**. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1**

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Table 1. ^{1}H NMR Data of Compounds **1**−**5**

	1[a]	**2**[a]	**3**[b]	**4**[c]	**5**[c]
4	6.04 d (2.2)				
5			6.15 s		
6	6.18 d (2.2)	6.26 s			
7	1.91 s	1.93 s	10.1 s		
8		1.88 s	2.03 s	4.00 br. q (7.1)	3.23 dd (7.5, 15.8) 2.89 dd (6.9, 15.8)
9				1.91 m 1.37 m	2.12 m
10				0.95 t (7.5)	0.87 d (6.7)
11				1.00 d (7.1)	0.85 d (6.7)
12				2.04 s	2.02 s
13				2.18 s	2.16 s
1′	4.77 d (7.5)	4.70 d (7.5)	4.56 d (7.7)	4.80 d (7.6)	4.46 d (7.8)
2′	3.46 m	3.42 m	3.30 t (8.5)	3.49 dd (7.6, 9.2)	3.49 t (8.5)
3′	3.50 t (8.8)	3.47 m	3.22 t (9.0)	3.39 m	3.37 t (9.0)
4′	3.23 t (8.8)	3.34 t (9.0)	3.14 t (9.0)	3.24 m	3.32 m
5′	3.43 m	3.40 m	3.07 m	3.24 m	3.24 m
6′	3.84 dd (1.9, 12.0)	3.80 dd (1.9, 12.0)	3.84 m	3.93 dd (2.0, 11.4)	3.88 dd (2.0, 11.0)
	3.64 dd (5.6, 12.0)	3.61 dd (5.8, 12.0)	3.39 dd (5.5, 11.5)	3.57 dd (6.0, 11.4)	3.60 dd (6.0, 11.0)
1″				4.10 d (7.2)	4.08 d (7.3)
2″				3.06 t (8.0)	3.09 t (8.5)
3″				3.24 m	3.24 m
4″				3.42 m	3.43 m
5″				3.77 dd (4.2, 11.2)	3.77 dd (5.0, 11.5)
				3.13 dd (9.0, 11.2)	3.13 dd (9.0, 11.5)

[a] In acetone-d_6 + D_2O. [b] In DMSO-d_6. [c] In methanol-d_4.

indicated that **2** had one more methyl group [δ_H 1.88 (3H, s), δ_C 8.4], relative to **1**. In the HMBC spectrum of **2**, the hydrogens of two methyl protons (δ 1.93 and 1.88) were correlated with two aromatic carbons at δ 106.2 and 106.3, respectively, while the anomeric proton (δ 4.70) was correlated with an aromatic carbon at δ 153.9. These observations confirmed the two methyl groups on the benzene ring and the glucosyl unit attached to one of the phenolic hydroxy groups of the phloroglucinol unit. The ROESY correlation of the anomeric proton (δ 4.70) with H-6 (δ 6.26) and the unsymmetrically substituted phloroglucinol unit determined the locations of two methyl groups in **2**. Consequently, the structure of eucalmaidin B was assigned as shown in **2**.

Eucalmainoside C (**3**) was obtained as a pink, amorphous powder. Its molecular formula, $C_{14}H_{18}O_9$, was elucidated from the HRESIMS (*m*/*z* 329.0878 [M − H]$^-$, calcd 329.0872). The ^{1}H and ^{13}C NMR spectra of **3** were closely related to that of **2**, except that one of the methyl signals in **2** was replaced by a formyl hydrogen [δ_H 10.06 (1H, s), δ_C 194.5] in **3**. Acid hydrolysis of **3** produced D-glucose, the absolute configuration of which was determined by GC analysis of its corresponding trimethylsilylated L-cysteine adduct. The relative locations of the formyl, methyl, and β-D-glucosyl moieties were established from the HMBC spectrum, in which correlations of the formyl proton (δ 10.06) with C-1 (δ 108.9) and C-6 (δ 162.1), the residual aromatic proton (δ 6.15) with C-1 (δ 108.9), C-3 (δ 110.9), C-4 (δ 156.6), and C-6 (δ 162.1), the methyl protons (δ 2.03) with C-2 (δ 158.7), C-3 (δ 110.9), and C-4 (δ 156.6), and the anomeric proton (δ 4.56) with C-2 (δ 158.7) were observed. Thus, the structure of compound **3** was determined as 2,4,6-trihydroxy-3-methylbenzaldehyde 2-*O*-β-D-glucopyranoside, namely, eucalmainoside C.

Eucalmainoside D (**4**) had the molecular formula $C_{26}H_{36}O_{13}$, which was established from the HRESIMS (*m*/*z* 531.2064 [M − H]$^-$, calcd 531.2077). The ^{1}H and ^{13}C NMR spectra of **4** showed the existence of one unsymmetrically fully substituted phloroglucinol unit [δ 161.5, 160.3, 154.2, 111.5, 111.3, and 109.4 (each C)], with two methyl groups [δ_H 2.18, 2.04 (each 3H, s), δ_C 9.9, 8.4] and a disaccharide unit [anomeric protons at δ 4.80 (d, 7.6 Hz, H-1′) and 4.10 (d, 7.2 Hz, H-1″)] attached to it. In addition, one carbonyl [δ 213.5 (C)], one methine [δ 47.0, δ_H 4.00 (br q, 7.1 Hz)], one methylene [δ 26.0, δ_H 1.91, 1.37 (each 1H, m)], and two methyl [δ 12.6 and 18.6, δ_H 1.00 (d, 7.1 Hz) and 0.95 (t, 7.5 Hz)] carbons arising from a 2-methylbutyryl group, which is a common substituent within phloroglucinol derivatives in the genus *Eucalyptus,* were observed.[4] Acidic hydrolysis of **4** produced D-xylose and D-glucose, whose absolute configurations were determined by GC analysis of their corresponding trimethylsilylated L-cysteine adducts. Connectivities of the methyl and sugar moieties with the phloroglucinol unit were confirmed by a 2D NMR experiment. The methyl proton (δ 2.04, H-12) was correlated with C-3 (δ 160.3), C-4 (δ 109.4), and C-5 (δ 161.5), while the methyl proton (δ 2.18, H-13) was correlated with C-1 (δ 154.2), C-5 (δ 161.5), and C-6 (δ 111.3) in the HMBC experiment. The HMBC correlations of H-1′ (δ 4.80) with C-1 (δ 154.2) and of H-1″ (δ 4.10) with C-6′ (δ 69.5) revealed that the xylosyl unit was attached at C-6′ of the glucosyl unit, which was linked to C-1 of the aglycone. On the basis of the above evidence, the planar structure of eucalmainoside D was constructed as shown in **4**. Its C-8 configuration could not be defined.

Eucalmainoside E (**5**) had the same molecular formula as that of **4**, $C_{24}H_{36}O_{13}$, which was established from the HRESIMS (*m*/*z* 531.2060 [M − H]$^-$, calcd 531.2077). The ^{1}H and ^{13}C NMR spectra of **5** were closely related to those of **4**, except that the two methyl groups appeared as one doublet and one triplet in **4** presented as two doublet methyls [δ 0.87, 0.85 (each 3H, d, 6.7 Hz)]. Additionally, the carbon signals [δ 209.3 (C), 54.2 (CH_2), 26.8 (CH), 23.1 (CH_3), 23.1(CH_3)] were closely related to the isovaleryl group.[26] These observations indicated that the methyl group at C-8 in **4** was shifted to C-9 in **5**; that is, compound **5** had an isovaleryl substituent in the molecule. Connectivities of the methyl and sugar moieties with the phloroglucinol unit were further confirmed by a 2D NMR experiment. Thus, the structure of eucalmainoside E was elucidated as shown in **5**.

Compounds **6**−**9**, **11**−**14**, **17**, **21**−**28**, and **32**−**34** were evaluated for their in vitro anti-herpes simplex virus type 1 (HSV-1) activity using a cytopathic effect assay and cytotoxicity on African green monkey kidney cells (Vero cells) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method. The results are shown in Table 3. Only myricetin (**6**), quercetin (**7**), and isocoriariin F (**33**) showed weak anti-HSV-1 activity with TIC values of 0.31, 0.33, and 0.12 mM, while their maximal noncytotoxic concentrations (MNCC) against Vero cells were 0.31, 0.02, and 0.25 mM, respectively. These results together with our previous report[27] suggested that C-3 and C-7 hydroxy groups in flavonols may strengthen the anti-HSV-1 activity, while both *O*-methylation of 7-OH and removal of the 3-OH group decreased their anti-HSV-1 activities.

Table 2. ^{13}C NMR Data (δ Values) of Compounds **1**−**5**

position	**1**[a]	**2**[a]	**3**[b]	**4**[c]	**5**[c]
1	157.5	153.9	108.9	154.2	154.8
2	105.3	106.2	158.7	111.5	112.5
3	156.1	154.2	110.9	160.3	160.3
4	97.3	106.3	156.6	109.4	109.1
5	156.7	153.3	98.6	161.5	161.7
6	95.4	96.4	162.1	111.3	111.1
7	8.1	8.5	194.5	213.5	209.3
8		8.4	8.9	47.0	54.2
9				26.0	26.8
10				12.6	23.1
11				18.6	23.1
12				8.4	8.3
13				9.9	9.7
1′	101.6	101.8	105.1	105.5	105.5
2′	73.9	73.6	74.1	75.7	75.7
3′	76.9	76.5	76.4	77.7	77.5
4′	70.5	70.1	69.8	72.0	71.5
5′	76.9	76.6	77.1	77.5	77.2
6′	61.8	61.4	61.0	69.5	69.3
1″				104.6	104.6
2″				74.9	74.7
3″				77.5	77.2
4″				71.2	71.1
5″				66.5	66.5

[a] In acetone-d_6 + D_2O. [b] In DMSO-d_6. [c] In CD_3OD.

Table 3. Anti-HSV-1 Activity of Compounds **6**−**9**, **11**−**14**, **17**, **21**−**28**, and **32**−**34**

compound	TIC (mM)[a]	MNCC (mM)[b]
6	0.31	0.31
7	0.33	<0.02
8	−[d]	0.04
9	−	0.70
11	−	0.41
12		0.43
13	−	0.43
14		0.45
17	−	0.37
21	−	0.59
22	−	0.03
23	−	0.40
24	−	0.10
25	−	>1.09
26	−	0.20
27	−	0.43
28	−	0.38
32	−	0.009
33	0.12	0.25
34	−	0.26
aciclovir	0.0043[c]	1.11

[a] TIC: total inhibitory concentration against HSV-1. [b] MNCC: maximal noncytotoxic concentration against Vero cells. [c] IC_{50} value (concentration required to reduce 50% of cytopathic effect). [d] −: no activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 high-sensitivity polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were measured in methanol-d_4, DMSO-d_6, or acetone-d_6 solution on a Bruker DRX-500 instrument (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) at 25 °C, using TMS as an internal standard. FABMS were recorded on a VG Auto Sepc-3000 mass spectrometer using glycerol as matrix; ESIMS and HRESIMS were recorded on an API QSTAR Pular-1 mass spectrometer (for compounds **1** and **2**, 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. 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.), and Toyopearl HW-40F (fine grade) (TOSOH, Japan). Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Preparative HPLC (Waters 600) was performed using a C-18 column (Zorbax, 9.4 mm i.d. × 250 mm) and developed isocratically at room temperature with MeOH−H_2O (45/:55); detection wavelength, 203 nm. 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% $NaHCO_3$ and Hepes buffer (47.6 g of Hepes was dissolved in H_2O (200 mL) and its pH adjusted to 7.5−8.0 by 1 N NaOH).

Plant Material. The fresh fruits of *E. maideni* were collected in the Botanical Garden of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China, during May 2007, and identified by Prof. Xiao Cheng (Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (KIB-ZL-200702) 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 fruits of *E. maideni* (4.0 kg) were extracted with 80% aqueous acetone at room temperature (3 × 30 L, each 1 week). The extracts were concentrated under reduced pressure and partitioned with petroleum ether (4 × 2 L) and EtOAc (5 × 2 L) successively after filtration of the precipitate. The EtOAc extract (69 g) was subjected to Sephadex LH-20 column chromatography (CC), eluting with MeOH−H_2O (0:1−1:0) to afford eight fractions. Fraction 1 (12 g) was chromatographed over silica gel ($CHCl_3$−MeOH, 1:0−8:2) to yield four fractions: 1-1, 1-2, 1-3, and 1-4. Fraction 1-2 was chromatographed over Toyopearl HW-40 (0−40% MeOH) to yield **25** (43 mg). Fraction 1-3 was subjected to CC over MCI-gel CHP-20P (40−70% MeOH) and Toyopearl HW-40F (0−50% MeOH) to yield **26** (10 mg). Fraction 1-4 was subjected to chromatography on Toyopearl HW-40F and subsequent purification by preparative HPLC to yield **4** (8 mg) and **5** (4 mg). Fraction 2 (4.3 g) was applied to silica gel ($CHCl_3$−MeOH−H_2O, 95:5:0−75:25:2) to yield three fractions: 2-1, 2-2, and 2-3. Fraction 2-2 was subjected to silica gel ($CHCl_3$−MeOH−H_2O, 9:1:0.1−7:3:0.5), MCI-gel CHP-20P (30−70% MeOH), and Toyopearl HW-40F (20−60% MeOH) to yield **3** (31 mg), **27** (6 mg), and **28** (36 mg). Fraction 2-3 was subjected to MCI-gel CHP-20P (0−40% MeOH) and silica gel ($CHCl_3$−MeOH−H_2O, 7.5:2.5:0.2) to yield **1** (10 mg). Fraction 3 (5.9 g) was chromatographed over Sephadex LH-20 (0−50% MeOH) to yield four fractions: 3-1, 3-2, 3-3, and 3-4. Fraction 3-1 was subjected to silica gel ($CHCl_3$−MeOH−H_2O, 8:2:0.2−7:3:0.5) and MCI-gel CHP-20P (30−70% MeOH) to afford **17** (30 mg) and **18** (50 mg). Fraction 3-2 was chromatographed over Toyopearl HW-40 (0−40% MeOH), MCI-gel CHP-20P (30−70% MeOH), and silica gel ($CHCl_3$−MeOH−H_2O, 8.5:1.5:0.1−8:2:0.2) to yield **22** (30 mg) and **23** (42 mg). Fraction 3-3 was subjected to MCI-gel CHP-20P (30−70% MeOH) repeatedly to yield **24** (39 mg). Fraction 4 (4.7 g) was subjected to CC over MCI-gel CHP-20P (0−80% MeOH) and silica gel ($CHCl_3$−MeOH−H_2O, 9:1:0.1−7:3:0.2) to yield **21** (660 mg) and **17** (28 mg). Fraction 5 (3.3 g) was chromatographed over MCI-gel CHP-20P (0−100% MeOH) to yield four fractions: 5-1, 5-2, 5-3, and 5-4. Fractions 5-1 and 5-2 were subjected to silica gel ($CHCl_3$−MeOH−H_2O, 8.5:1.5:0.1−7:3:0.2) to yield **12** (130 mg), **13** (13 mg), and **14** (9 mg). Fraction 5-3 was subjected to silica gel ($CHCl_3$−MeOH−H_2O, 8.5:1.5:0.1−8:2:0.2) and MCI-gel CHP-20P (50−100% MeOH) to yield **15** (10 mg) and **16** (20 mg). Fraction 6 (3.9 g) was subjected to silica gel ($CHCl_3$−MeOH−H_2O, 9:1:0.1−7:3:0.5) and MCI-gel CHP-20P (50−100% MeOH) to yield **10** (30 mg) and **11** (14 mg). Fraction 7 (3.2 g) was subjected to MCI-gel CHP-20P (50−80% MeOH) and silica gel ($CHCl_3$−MeOH−H_2O, 9:1:0.1−8:2:0.2) to yield **7** (25 mg) and **9** (161 mg). Fraction 8 (3.3 g) was subjected to MCI-gel CHP-20P (50−80% MeOH) and silica gel ($CHCl_3$−MeOH−H_2O, 9:1:0.1−8:2:0.2) to yield **6** (142 mg) and **7** (123 mg).

The H_2O layer (180 g) was separated on Sephadex LH-20 (0−100% MeOH) to yield six fractions. Fraction 2 (12.6 g) was subjected to MCI-gel CHP-20P (0−50% MeOH) to yield three fractions: 2-1, 2-2, and 2-3. Fraction 2-1 was repeatedly subjected to MCI-gel CHP-20P (0−50% MeOH) and Toyopearl HW-40F (0−60% MeOH) to yield **32** (67 mg). Fraction 2-2 was subjected to Toyopearl HW-40F (0−50% MeOH), MCI-gel CHP-20P (0−50% MeOH), and silica gel ($CHCl_3$−MeOH−H_2O, 9:1:0.1−8:2:0.2) to yield **29** (3 mg), **30** (7 mg), and **31** (8 mg). Fraction 3 (3.2 g) was subjected to MCI-gel CHP-20P (0−50% MeOH) and Toyopearl HW-40F (0−60% MeOH) to yield **19** (13 mg) and **2** (4 mg). Fraction 4 (9.5 g) was repeatedly subjected

to MCI-gel CHP-20P (0−100% MeOH) and silica gel ($CHCl_3$−MeOH−H_2O, 7.5:2.5:0.2) to yield **17** (585 mg), **18** (80 mg), and **20** (4 mg). Fraction 5 (17.8 g) was repeatedly subjected to MCI-gel CHP-20P (0−50% MeOH) and Sephadex LH-20 (40−100% MeOH) to yield **33** (93 mg) and **34** (30 mg).

Eucalmainoside A (1): pale, amorphous powder; $[\alpha]_D^{27}$ −66.6 (*c* 0.25, MeOH); UV (MeOH), λ_{max} (log ε) 204 (4.54), 275 (3.29) nm; IR (KBr) ν_{max} 3383, 2924, 2864, 1613, 1478, 1384, 1073, 1038 cm^{-1}; ^1H NMR (acetone-d_6 + D_2O, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6 + D_2O, 125 MHz), see Table 2; ESIMS (negative ion mode) *m*/*z* 337 $[M + Cl]^-$, 139 $[M - H - 162]^-$; HRESIMS *m*/*z* 337.0696 $[M + Cl]^-$ (calcd for $C_{13}H_{18}O_8Cl$, 337.0690).

Eucalmainoside B (2): pale, amorphous powder; $[\alpha]_D^{27}$ −40.3 (*c* 0.4, MeOH); UV (MeOH), λ_{max} (log ε) 204 (4.54), 304 (3.61) nm; IR (KBr) ν_{max} 3421, 2924, 1619, 1545, 1387, 1100, 1074, 1035 cm^{-1}; ^1H NMR (acetone-d_6 + D_2O, 500 MHz), see Table 1; ^{13}C NMR (acetone-d_6 + D_2O, 125 MHz), see Table 2; ESIMS (negative ion mode) *m*/*z* 351 $[M + Cl]^-$, 153 $[M - H - 162]^-$; HRESIMS *m*/*z* 351.0842 $[M - H]^-$ (calcd for $C_{14}H_{20}O_8Cl$, 351.0846).

Eucalmainoside C (3): pink, amorphous powder; $[\alpha]_D^{27}$ +12.5 (*c* 0.1, MeOH); UV (MeOH), λ_{max} (log ε) 208 (4.13), 288 (4.08) nm; IR (KBr) ν_{max} 3462, 2886, 1636, 1510, 1429, 1380, 1275, 1074, 1022 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; FABMS (negative ion mode) *m*/*z* 329 $[M - H]^-$, 167 $[M - H - 162]^-$; HRESIMS *m*/*z* 329.0878 $[M - H]^-$ (calcd for $C_{14}H_{17}O_9$, 329.0872).

Eucalmainoside D (4): pale, amorphous powder; $[\alpha]_D^{27}$ −4.4 (*c* 0.2, MeOH); UV (MeOH), λ_{max} (log ε) 202 (3.98), 286 (3.84) nm; IR (KBr) ν_{max} 3421, 2927, 2873, 1613, 1460, 1424, 1371, 1077, 1044 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz), see Table 1; ^{13}C NMR (methanol-d_4, 125 MHz), see Table 2; FABMS (negative ion mode) *m*/*z* 531 $[M - H]^-$, 399 $[M - H - 132]^-$, 237 $[M - H - 162 - 132]^-$; HRESIMS *m*/*z* 531.2064 $[M - H]^-$ (calcd for $C_{24}H_{35}O_{13}$, 531.2077).

Eucalmainoside E (5): pale, amorphous powder; $[\alpha]_D^{26}$ +1.8 (*c* 0.3, MeOH); UV (MeOH), λ_{max} (log ε) 218 (4.23), 286 (4.10) nm; IR (KBr) ν_{max} 3424, 2925, 2870, 1616, 1465, 1424, 1370, 1077, 1044 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz), see Table 1; ^{13}C NMR (methanol-d_4, 125 MHz), see Table 2; FABMS (negative ion mode) *m*/*z* 531 $[M - H]^-$, 399 $[M - H - 132]^-$, 237 $[M - H - 162 - 132]^-$; HRESIMS *m*/*z* 531.2060 $[M - H]^-$ (calcd for $C_{24}H_{35}O_{13}$, 531.2077).

Acid Hydrolysis of Compounds 1, 3, and 4. Compounds **1**, **3**, and **4** (2.0, 2.0, and 4.0 mg) were hydrolyzed with 2 N HCl−1,4-dioxane (1:1, 4 mL) at 80 °C for 3 h. The mixture was extracted with $CHCl_3$ (3 × 4 mL). The aqueous layer was neutralized with NaOH (2 N) and 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. Trimethylsilyl imidazole (1.5 mL) was added, and the mixture was heated at 60 °C for another 30 min. An aliquot (4 μL) 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 N_2 (1 mL/min), injector and detector temp 250 °C, split ratio 1:50. The configurations of D-gluose and D-xylose for compounds **1**, **3**, and **4** were determined by comparison of the retentions times of the corresponding derivatives with standard D-glucose and D-xylose, giving single peaks at 19.208 and 13.674 min, respectively.

HSV-1 Inhibition Activity. HSV-1 inhibition activity was assayed with the plaque reduction assay,[28] with aciclovir 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 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay.[29] Vero cells were seeded into a 96-well plate. Different concentrations of samples (100 μL), 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 μL 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 μL of DMSO was added to dissolve the formazan crystals. The content in the wells was homogenized on a microplate shaker 30 min later. The OD (optical density) was then read on a microplate spectrophotometer at double wavelengths of 540 and 630 nm. The maximal noncytotoxic 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.

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

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