Phenolic Compounds from the Fresh Leaves of *Eucalyptus maideni*

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Four new phenolic compounds, 7-*O*-methylcatechin 5-*O*-*β*-D-glucopyranoside (**1**), 6-*O*-feruloyl-D-glucopyranose (**2**), demethylpiperitol 4-*O*-*β*-D-glucopyranoside (**3**), and 2-episesaminol 2-*O*-*β*-D-glucopyranoside (**4**) were isolated from the fresh leaves of *Eucalyptus maideni*, together with six hydrolyzable tannins, **5**–**10**, a flavonol glycoside, **11**, three simple phenolics, **12**–**14**, a monoterpene glucoside, **15**, and a rosenoside, **16**. Their structures were determined on the basis of detailed spectroscopic analysis, acidic hydrolysis, and enzymatic hydrolysis. The known compounds **10** and **13** were obtained from the genus *Eucalyptus* for the first time.

Introduction. – The genus *Eucalyptus* (Myrtaceae), mainly growing in the tropical and subtropical areas of the world, contains *ca.* 945 species, 300 of which are distributed in China [1]. In this genus, some species, *e.g.*, *E. globulus*, *E. robusta*, and *E. melliodora*, are important folk-medicinal plants [2], and a series of terpenoids [3], tannins [4], flavonoids [5], and phloroglucinol derivatives [6–9] with antiviral [8] and antibacterial [9] effects have been reported.

E. maideni F. MUELL is a tall timber tree growing widely in the southern part of China [2]. The trunks are commonly used in forestry, while its leaves are extracted for essential oil. Our previous study [10] on the fresh leaves has resulted in the isolation of five new (+)-oleuropeic acid derivatives. Further investigation on the same extract led to the isolation of a new methyl-flavan-3-ol glucoside **1**, a new glucose feruloyl ester **2**, and two new lignan glucosides, **3** and **4** (*Fig. 1*). In addition, twelve known compounds, **5**–**16** (*Fig. 2*), including six hydrolyzable tannins, **5**–**10**, a flavonol glycoside, **11**, three simple phenolics, **12**–**14**, a monoterpene glucoside, **15**, and a reoseoside, **16**, were also obtained. This article presents the structure determination of these compounds by means of NMR and MS techniques, acidic hydrolysis, and enzymatic hydrolysis.

Results and Discussion. – The 80% aqueous acetone extract of fresh leaves of *E. maideni* was partitioned with $CHCl_3$ and H_2O. The H_2O portion was then subjected to *Diaion HP20SS* column chromatography (CC) to afford seven fractions. Further CC on *Fractions 1–3*, *6*, and *7* afforded four new compounds, **1**–**4**. In addition, twelve known compounds were identified as isocoriariin F (**5**) [11], oenothein C (**6**) [11], gemin D (**7**) [12], 3-*O*-galloylglucose (**8**) [11], 4-*O*-galloylglucose (**9**) [13], punigluconin (**10**) [14], quercetin 3-*O*-*β*-D-glucuropyranoside (**11**) [15], eucalmanioside C (**12**) [16], lysidisides

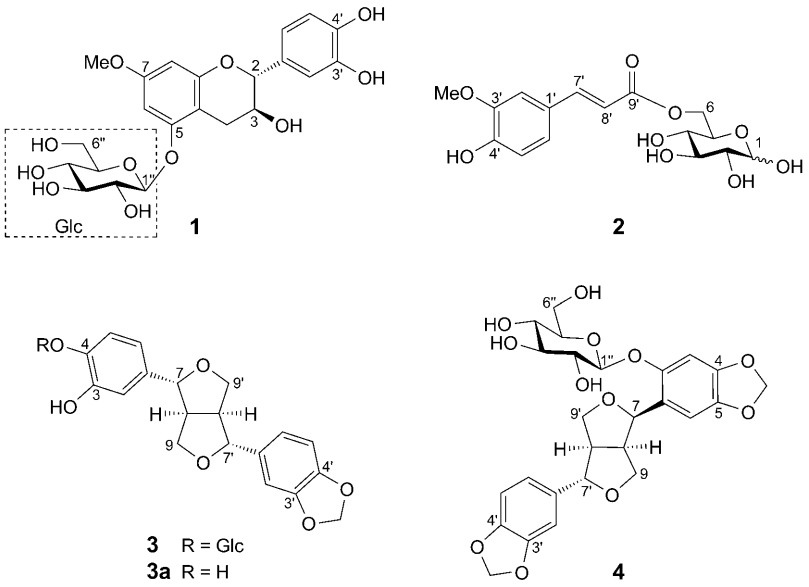

Fig. 1. *New compounds* **1**–**4** *isolated from the fresh leaves of* E. maideni

C (**13**) [17], gallic acid (**14**) [16], 1,8-cineole 2-*O*-*β*-D-glucopyranoside (**15**) [18], and (6*S*,9*R*)-reoseoside (**16**) [19], by comparison of the physical and spectral data with literature values. The known compounds **10** and **13** were obtained from the genus *Eucalyptus* for the first time.

Compound **1** was obtained as a pale amorphous powder. The molecular formula of **1** was determined as $C_{22}H_{26}O_{11}$ by HR-ESI-MS (*m*/*z* 465.1410 ($[M-H]^-$)). The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) showed signals of five aromatic H-atoms of a 1,3,4-trisubstituted (δ(H) 6.81 (*d*, *J* = 2.0, H−C(2′)), 6.75 (*d*, *J* = 8.5, H−C(5′)), and 6.70 (*dd*, *J* = 8.0, 2.0, H−C(6′))) and a 1,3,4,5-tetrasubstituted (δ(H) 6.38, 6.14 (2*d*, *J* = 2.4, H−C(6), H−C(8))) phenyl group each, and of three aliphatic C-atoms at δ(C) 82.9 (C(2)), 68.4 (C(3)), and 28.3 (C(4)), corresponding to a typical catechin moiety. The larger coupling constant (*J* = 7.5 Hz) between H−C(2) and H−C(3) indicated that it was a (+)-catechin moiety [20]. In addition, signals arising from a glucopyranosyl moiety (δ(C) 102.5, C(1″), 78.2, C(3″), 78.1, C(5″), 74.8, C(2″), 71.4, C(4″), 62.5, C(6″)) and a MeO group (δ(H) 3.73 (*s*); δ(C) 55.7) were also observed. The *J* value of the anomeric H-atom (δ(H) 4.83 (*d*, *J* = 7.7)) indicated its *β*-configuration. In the ROESY spectrum of **1**, the MeO H-atoms showed correlations with both H−C(6) and H−C(8), while the anomeric H-atom was correlated with H−C(6), indicating the locations of the MeO group at C(7) and the glucosyl moiety at C(5). Thus, compound **1** was elucidated to be 7-*O*-methylcatechin 5-*O*-*β*-D-glucopyranoside[1]).

Compound **2**, a pale amorphous powder, had the molecular formula $C_{16}H_{20}O_9$, deduced from the HR-ESI-MS (*m*/*z* 391.0795 ($[M+Cl]^-$)). The ^{1}H-NMR spectrum

[1]) Absolute configuration of glucose was assumed to be D from the same biogenetic considerations.

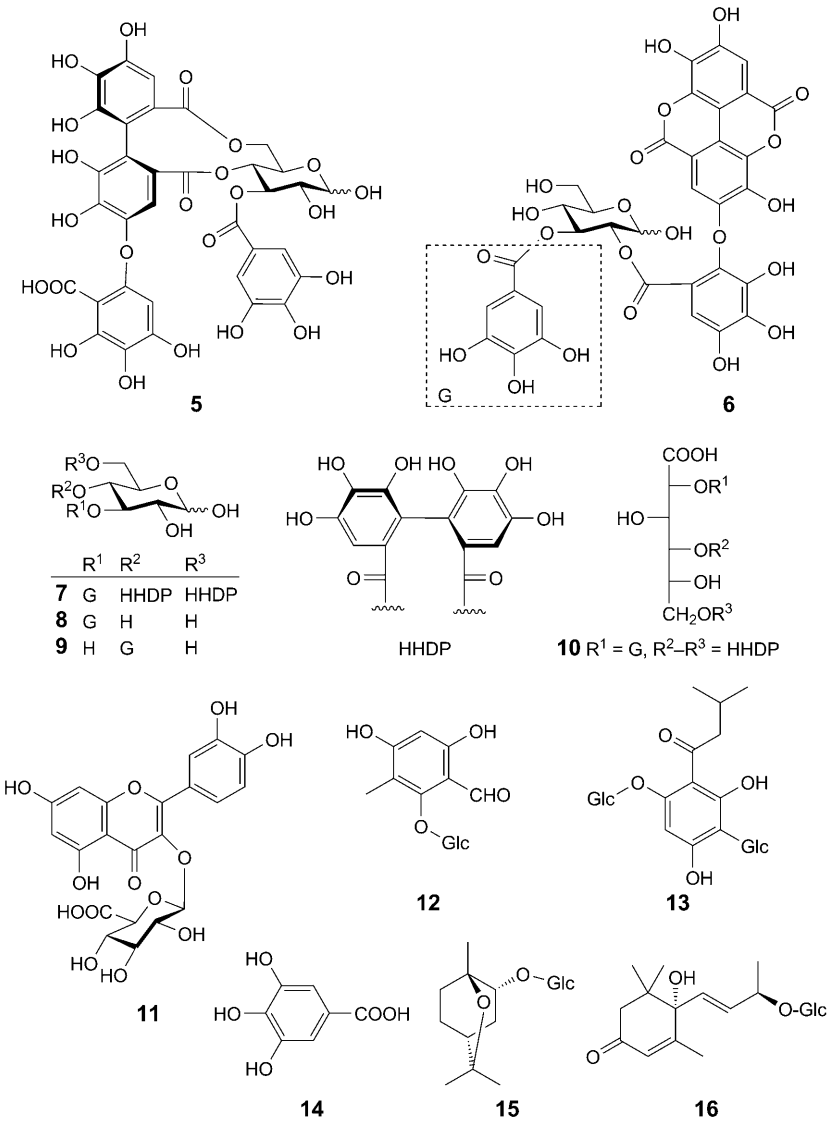

Fig. 2. *Known compounds* **5**–**16** *isolated from the fresh leaves of* E. maideni

(*Table 2*) displayed signals of two *trans*-coupled CH H-atoms at δ(H) 7.55 and 6.31 (2*d*, $J = 15.0$, H–C(7′), H–C(8′)), three aromatic H-atoms at δ(H) 7.19 (*d*, $J = 1.7$, H–C(2′)), 7.05 (*dd*, $J = 1.7$, 8.2, H–C(6′)), and 6.79 (*d*, $J = 8.2$, H–C(5′)), and a MeO group at δ(H) 3.81 (*s*), corresponding to a feruloyl group. The MeO group at C(3′) of the feruloyl group was confirmed by its ROESY correlation with H–C(2′). In addition, a set of complex H-atom signals arising from the sugar moiety, as well as the appearance of the anomeric H-atom signals at δ(H) 5.09 (0.5 H, *d*, $J = 3.6$) (α-form)

Table 1. *^{1}H- and ^{13}C-NMR Data of Compound* **1**. Recorded at 125 and 500 MHz, respectively, in CD_3OD, δ in ppm, J in Hz.

	δ(H)	δ(C)
Aglycone		
C(2)	4.61 (*d*, *J* = 7.5)	82.9
C(3)	4.01 – 3.97 (*m*)	68.4
C(4)	3.01 (*dd*, *J* = 5.0, 16.5), 2.60 (*dd*, *J* = 8.0, 16.5)	28.3
C(5)		158.0
C(6)	6.38 (*d*, *J* = 2.4)	96.0
C(7)		160.9
C(8)	6.14 (*d*, *J* = 2.4)	96.8
C(9)		156.6
C(10)		104.5
C(1′)		131.9
C(2′)	6.81 (*d*, *J* = 2.0)	115.1
C(3′)		146.2
C(4′)		146.2
C(5′)	6.75 (*d*, *J* = 8.5)	116.1
C(6′)	6.70 (*dd*, *J* = 2.0, 8.0)	119.9
*Me*O–C(7)	3.73 (*s*)	55.7
Glc		
C(1″)	4.83 (*d*, *J* = 7.7)	102.5
C(2″)	3.46 – 3.44 (*m*)	74.8
C(3″)	3.43 – 3.40 (*m*)	78.2
C(4″)	3.38 – 3.36 (*m*)	71.4
C(5″)	3.47 – 3.44 (*m*)	78.1
C(6″)	3.89 (*dd*, *J* = 2.5, 12.0), 3.68 (*dd*, *J* = 5.5, 12.0)	62.5

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2**. Recorded at 125 and 500 MHz, respectively, in (D_6)acetone and D_2O, δ in ppm, J in Hz.

	δ(H)		δ(C)	
Glc	β	α	β	α
C(1)	4.52 (*d*, *J* = 7.9)	5.09 (*d*, *J* = 3.6)	97.1	92.8
C(2)	3.18 (*t*, *J* = 8.5)	3.44 – 3.37 (*m*)	74.9	72.3
C(3)	3.44 – 3.37 (*m*)	3.66 (*t*, *J* = 9.5)	76.5	73.4
C(4)	3.40 – 3.34 (*m*)	3.40 – 3.34 (*m*)	70.5	70.5
C(5)	3.58 – 3.52 (*m*)	4.00 – 3.96 (*m*)	74.1	69.8
C(6)	4.40 (*d*, *J* = 12.0), 4.26 – 4.21 (*m*)	4.36 (*d*, *J* = 11.0), 4.28 – 4.18 (*m*)	64.1	64.2
Feruloyl				
C(1′)			126.7	
C(2′)	7.19 (*d*, *J* = 1.7)		111.2	
C(3′)			146.3	
C(4′)			148.4	
C(5′)	6.79 (*d*, *J* = 8.2)		115.8	
C(6′)	7.05 (*dd*, *J* = 1.7, 8.2)		123.6	
C(7′)	7.55 (*d*, *J* = 15.0)		146.3	
C(8′)	6.31 (*d*, *J* = 15.0)		114.5	
C(9′)			168.3	
*Me*O–C(3′)	3.81 (*s*)		56.0	

and δ(H) 4.52 (0.5 H, *d*, $J = 7.9$) (β-form) in a ratio of 1:1, and the anomeric C-atom signals at δ(C) 92.8 and 97.1, indicated that **2** is an anomer mixture. Acidic hydrolysis of **2** liberated D-glucose, which was determined by GC analysis of its corresponding trimethylsilylated L-cysteine adduct. The linkage of feruloyl moiety at C(6) of D-glucose unit was established by HMBC of H–C(6) of glucose at δ(H) 4.40 and 4.22 (β-form), and 4.36 and 4.19 (α-form) with the CO group at δ(C) 168.4 (C(9′)) of the feruloyl moiety. Based on the above evidences, the structure of **2** was determined to be 6-*O*-feruloyl-D-glucopyranose.

Compound **3** was obtained as a pale amorphous powder. Its molecular formula $C_{25}H_{28}O_{11}$ was elucidated from the HR-ESI-MS (*m/z* 503.1566 ($[M-H]^-$), calc. 503.1553). The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table 3*) showed the presence of two 1,3,4-trisubstituted phenyl groups (δ(H) 6.86 (*d*, $J = 1.3$, H–C(2)), 6.76 (*d*, $J = 8.0$,

Table 3. *^{1}H- and ^{13}C-NMR Data of Compounds* **3** *and* **4**. Recorded at 125 and 500 MHz, respectively, in CD_3OD, δ in ppm, *J* in Hz.

	3		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
Aglycone				
H–C(1)		138.1		125.8
H–C(2)	6.86 (*d*, $J = 1.3$)	114.9		150.5
H–C(3)		148.6	6.87 (*s*)	100.0
CH_2(4)		146.3		149.1
H–C(5)	6.76 (*d*, $J = 8.0$)	118.6		143.8
H–C(6)	6.78 (*dd*, $J = 1.3$, 8.0)	118.7	6.90 (*s*)	106.7
H–C(7)	4.67 (*d*, $J = 5.4$)	87.3	5.16 (*d*, $J = 4.7$)	86.5
H–C(8)	3.08–3.02 (*m*)	55.6	3.14–3.09 (*m*)	55.6
CH_2(9)	4.20 (*dd*, $J = 7.0$, 9.0), 3.83 (*dd*, $J = 4.2$, 9.0)	72.8	4.37 (*dd*, $J = 7.5$, 9.0), 4.02 (*dd*, $J = 5.0$, 9.0)	74.1
H–C(1′)		136.4		137.0
H–C(2′)	6.86 (br. *s*)	107.5	6.94 (*d*, $J = 1.5$)	107.8
H–C(3′)		148.5		148.3
H–C(4′)		149.4		148.4
H–C(5′)	7.15 (*d*, $J = 8.3$)	109.0	6.86 (*d*, $J = 8.0$)	109.2
H–C(6′)	6.82 (*d*, $J = 8.3$)	120.6	6.89 (*dd*, $J = 1.5$, 8.0)	120.8
H–C(7′)	4.68 (*d*, $J = 5.0$)	86.9	4.68 (*d*, $J = 5.6$)	82.8
H–C(8′)	3.08–3.02 (*m*)	55.4	3.03–2.98 (*m*)	55.1
CH_2(9′)	4.20 (*dd*, $J = 7.0$, 9.0), 3.83 (*dd*, $J = 4.2$, 9.0)	72.6	4.23 (*dd*, $J = 7.0$, 9.0), 3.90 (*dd*, $J = 4.0$, 9.0)	72.4
OCH_2O	5.98 (*s*)	102.4	6.01 (*s*)	102.6
OCH_2O			5.98 (*s*)	102.4
Glc				
H–C(1″)	4.74 (*d*, $J = 7.5$)	102.5	4.90 (*d*, $J = 7.2$)	103.1
H–C(2″)	3.48–3.45 (*m*)	74.8	3.40 (*t*, $J = 8.5$)	74.9
H–C(3″)	3.46–3.44 (*m*)	78.2	3.44–3.42 (*m*)	78.3
H–C(4″)	3.40–3.38 (*m*)	71.4	3.34 (*t*, $J = 9.5$)	71.3
H–C(5″)	3.46–3.44 (*m*)	78.1	3.44–3.42 (*m*)	78.4
CH_2(6″)	3.84 (*dd*, $J = 2.0$, 11.2), 3.65 (*dd*, $J = 5.0$, 11.2)	62.5	3.84 (*dd*, $J = 2.0$, 12.0), 3.65 (*dd*, $J = 5.5$, 12.0)	62.4

H–C(5)), and 6.78 (*dd*, *J* = 1.3, 8.0, H–C(6)); 6.86 (br. *s*, H–C(2′)), 7.15 (*d*, *J* = 8.3, H–C(5′)), and 6.82 (*d*, *J* = 8.3, H–C(6′))), three O-bearing CH_2 groups (δ(H) 5.98 (*s*), 4.20 (*dd*, *J* = 7.0, 9.0, H–C(9a,9′a)), and 3.83 (*dd*, *J* = 4.2, 9.0, H–C(9b,9′b))), and four CH groups, including two O-bearing ones (δ(H) 4.67 (*d*, *J* = 5.5, H–C(7)), 4.68 (*d*, *J* = 5.0, H–C(7′))). These NMR data were closely related to those of demethylpiperitol [21], except for the presence of an additional glucose moiety in **3**. Signals arising from this additional glucosyl moiety (anomeric H-atom signal at δ(H) 4.74 (*d*, *J* = 7.5, H–C(1″))) were also observed. Enzymatic hydrolysis of compound **3** yielded D-glucose ($[\alpha]_D = +53.8$ (H_2O)) as sugar residue, and (−)-demethylpiperitol ($[\alpha]_D = -61.1$ ($CHCl_3$), FAB-MS *m*/*z* 341 ($[M-H]^-$)) as aglycone. In the HMBC spectrum of **3**, the glucosyl anomeric H-atom at δ(H) 4.74 correlated with the C(4) (δ(C) 146.3) determined the glucosylation position at C(4) of the demethylpiperitol unit. Thus, compound **3** was deduced to be demethylpiperitol-4-*O*-β-D-glucopyranoside.

Compound **4**, a pale amorphous powder, had a molecular formula $C_{26}H_{28}O_{12}$, as deduced from the HR-ESI-MS (*m*/*z* 567.1270 ($[M+Cl]^-$), calc. 567.1269). The ^{1}H-NMR spectrum of **4** showed the presence of one 1,3,4-trisubstituted and one 1,2,4,5-tetrasubstituted benzene ring each (δ(H) 6.94 (*d*, *J* = 1.5, H–C(2′)), 6.86 (*d*, *J* = 8.0, H–C(5′)), 6.89 (*dd*, *J* = 1.5, 8.0, H–C(6′)), and 6.87, 6.90 (*s*, H–C(3), H–C(6))), four O-bearing CH_2 groups (δ(H) 6.01, 5.98 (2*s*, 2 OCH_2O), 4.37 (*dd*, *J* = 7.5, 9.0, H–C(9a)) 4.02 (*dd*, *J* = 5.0, 9.0, H–C(9b)), 4.23 (*dd*, *J* = 7.0, 9.0, H–C(9′a)), and 3.90 (*dd*, *J* = 4.0, 9.0, H–C(9′b)), and four CH groups, including two O-bearing ones (δ(H) 5.16 (*d*, *J* = 4.7, H–C(7)) and 4.68 (*d*, *J* = 5.6, H–C(7′))). These NMR data (*Table 3*) closely resemble those of 2-episesaminol [22]. In addition, a set of signals due to a glucosyl unit was observed in the ^{1}H- and ^{13}C-NMR spectra of **4**. The HMBCs of the glucosyl anomeric H-atom at δ(H) 4.90 (*d*, *J* = 7.2) and H–C(7) (δ(H) 5.16) with C(2) (δ(C) 150.5) indicated the glucosylated position at C(2) of the 2-episesaminol unit. In the NOESY spectrum of compound **4**, both H–C(8) (δ(H) 3.14–3.09, *m*) and H–C(8′) (δ(H) 3.03–2.98, *m*) showed correlations with H–C(7) (δ(H) 5.16), indicating that the two aryl groups in **4** were located on the opposite sites of the furo[3,4-*c*]furan moiety, as in 2-episesaminol. Accordingly, compound **4** was determined as 2-episesaminol 2-*O*-β-D-glucopyranoside.

According to [22], the aglycone of compound **4**, 2-episesaminol, was easily converted to its epimer, sesaminol, in a $CHCl_3$ solution at room temperature. However, we did not find any conversion for compound **4** during the isolation and purification process, and even re-recording the ^{1}H-NMR spectrum after two-month keeping in CD_3OD at room temperature, suggesting that the glycosidation of 2-episesaminol on HO–C(2) may increase the stability of its aglycone, 2-episesaminol.

In this study, besides the new methylcatechin glucoside **1**, and lignans **3** and **4**, six hydrolyzable tannins, **2** and **5**–**9**, occurring as anomeric mixtures, were isolated from the fresh leaves of *Eucalyptus maideni*, in addition to phloroglucinol glycosides, monoterpenoids, and simple phenolics. They may arise from the hydrolysis of complex hydrolyzable tannins, such as eucalbanins. On the basis of these results, it can be stated that *E. maideni* may provide a good source for further new biologically active natural products.

Experimental Part

General. Coloumn chromatography (CC): *Diaion HP-20SS* (*Mitsubishi Chemical Co.*), *MCI*-gel *CHP-20P* (75–150 μm; *Mitsubishi Chemical Co.*), *Sephadex LH-20* (25–100 μm; *Pharmacia Fine Chemical Co. Ltd.*), *Toyopearl HW-40F* (*TOSOH*, Japan), and silica gel (SiO_2; 200–300 mesh, *Qingdao Haiyang Chemical Co. Ltd.*). TLC: SiO_2 plates; detection by spraying with 2% $FeCl_3$ in EtOH or 10% H_2SO_4 reagents. Optical rotations: *JASCO-20* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrometer; MeOH solns.; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-400* and *-DRX-500* spectrometers; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *VG-Auto-Spec-3000* spectrometer with matrix as glycerol for FAB-MS; *API-QSTAR-Pulsar-1* spectrometer for ESI-MS and HR-ESI-MS; in *m*/*z*.

Plant Material. The fresh leaves of *E. maideni* were collected in the Botanical Garden of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, P. R. China, in May 2007. A voucher specimen (KIB-ZL-200701) has been deposited with 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% aq. acetone at r.t. (3 × 10 l, each 1 week). After removal of the org. solvent under reduced pressure and of precipitate by filtration, the concentrated H_2O portion was partitioned with $CHCl_3$ (6 × 2 l). As described in [3], the H_2O layer was then subjected to CC (*Diaion HP-20SS*; H_2O/MeOH 1:0–0:1) to give seven fractions. *Fr. 1* (38 g) was applied to CC (*Sephadex LH-20*; H_2O/MeOH 1:0–0:1), to give fractions *1A–1E*. *Frs. 1B*, *1C*, and *1D* were subjected to CC (*MCI*-gel *CHP-20P*, H_2O/MeOH 1:0–7:3; and *Sephadex LH-20*, H_2O/MeOH 1:0–4:6) to yield **8** (18 mg), **9** (48 mg), **10** (68 mg), and **14** (30 mg), resp. *Fr. 2* (38 g) was applied to CC (*Sephadex LH-20*, H_2O/MeOH 1:0–1:0), to give *Frs. 2A–2C*. *Fr. 2B* was subjected to CC (*Sephadex LH-20*; H_2O/MeOH 1:0–6:4; *MCI*-gel *CHP-20P*, H_2O/MeOH 1:0–3:7) to yield **5** (6 mg), **6** (8 mg), and **7** (57 mg). *Fr. 3* (40 g) was subjected to CC (*Sephadex LH-20*, H_2O/MeOH 1:0–1:0), to give *Frs. 3A–3D*. *Fr. 3A* was subjected to CC (*MCI*-gel *CHP-20P*, H_2O/MeOH 7:3–0:1; *Sephadex LH-20*, H_2O/MeOH 1:0–6:4; and SiO_2, $CHCl_3$/MeOH 9:1–8:2) to yield **15** (238 mg) and **16** (6 mg). Similarly, *Fr. 3B* was subjected to CC (*MCI*-gel *CHP-20P*, H_2O/MeOH 1:0–3:7; *Sephadex LH-20*, H_2O/MeOH 1:0–4:6; and SiO_2, $CHCl_3$/MeOH 8.5:1.5–7:3) to yield **2** (30 mg), **12** (18 mg), and **11** (30 mg). *Fr. 3C* was subjected to CC (*MCI*-gel *CHP-20P*, H_2O/MeOH 1:0–3:7; *Toyopearl HW-40F*, H_2O/MeOH 1:0–2:8; and SiO_2, $CHCl_3$/MeOH 8:2–7:3) to yield **1** (3 mg) and **13** (4 mg). *Fr. 6* (1.92 g) was applied to CC (SiO_2, $CHCl_3$/MeOH 9:1–8:2; *MCI*-gel *CHP-20P*, H_2O/MeOH 6:4–3:7) to yield **3** (9 mg). *Fr. 7* was applied to CC (*Sephadex LH-20*, H_2O/MeOH 3:7–0:1; *MCI* gel *CHP-20P*, H_2O/MeOH 6:4–0:1) to yield **4** (16 mg).

7-O-*Methylcatechin 5*-O-*β*-D-*Glucopyranoside* (=(2R,3S)-*3,4-Dihydro-2-(3,4-dihydroxyphenyl)-3-hydroxy-7-methoxy-2*H-*chromen-5-yl β*-D-*Glucopyranoside*; **1**). Pale amorphous powder. $[\alpha]_D^{27} = -19.8$ ($c = 0.15$, MeOH). UV: 205 (4.7), 280 (3.97). IR: 3422, 2922, 2851, 1621, 1597, 1515, 1442, 1074, 1047. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (neg.): 465 ($[M-H]^-$). HR-ESI-MS (neg.): 465.1410 ($[M-H]^-$, $C_{22}H_{25}O_{11}^-$; calc. 465.1396).

6-O-*Feruloyl*-D-*glucopyranose* (=*6*-O-*[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]*-D-*glucopyranose*; **2**). Pale amorphous powder. $[\alpha]_D^{27} = 22.7$ ($c = 0.58$, MeOH). UV: 238 (4.07), 327 (4.20). IR: 3404, 2923, 2844, 1696, 1600, 1516, 1284, 1028. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS (neg.): 355 ($[M-H]^-$). HR-ESI-MS (neg.): 391.0795 ($[M+Cl]^-$, $C_{16}H_{20}ClO_9^-$; calc. 391.0795).

Demethylpiperitol 4-O-*β*-D-*glucopyranoside* (=*4-[(1*R,*3a*S,*4*R,*6a*S)-*4-(1,3-benzodioxol-5-yl)tetrahydro-1*H,*3*H-*furo[3,4-*c*]furan-1-yl]-2-hydroxyphenyl β*-D-*Glucopyranoside*; **3**). Pale amorphous powder. $[\alpha]_D^{28} = -72.9$ ($c = 0.50$, MeOH). UV: 204 (4.75), 282 (3.89), 376 (3.34). IR: 3397, 2925, 2874, 1710, 1596, 1506, 1443, 1247, 1038. ^{1}H- and ^{13}C-NMR: *Table 3*. FAB-MS (neg.): 503 ($[M-H]^-$), 341 ($[M-Glc]^-$). HR-ESI-MS (neg.): 503.1566 ($[M-H]^-$, $C_{25}H_{27}O_{11}^-$; calc. 503.1553).

2-Episesaminol 2-O-*β*-D-*glucopyranoside* (=*6-[(1*R,*3a*R,*4*S,*6a*R)-*4-(1,3-Benzodioxol-5-yl)tetrahydro-1*H,*3*H-*furo[3,4-*c*]furan-1-yl]-1,3-benzodioxol-5-yl β*-D-*Glucopyranoside*; **4**). Pale amorphous. $[\alpha]_D^{27} = -37.3$ ($c = 0.30$, MeOH). UV: 203 (4.66), 236(3.98), 290 (3.91). IR: 3407, 2918, 2880, 1631, 1504, 1485, 1250, 1073, 1037. ^{1}H- and ^{13}C-NMR: *Table 3*. FAB-MS (neg.): 531 ($[M-H]^-$), 369 ($[M-Glc]^-$). HR-ESI-MS (neg.): 567.1270 ($[M+Cl]^-$, $C_{26}H_{28}ClO_{12}^-$; calc. 567.1269).

Acid Hydrolysis of Compound **2**. Compound **2** (4.0 mg) were hydrolyzed with 2N HCl/1,4-dioxane 1:1 (4 ml) at 80° for 3 h. The mixture was passed through a *MCI*-gel *CHP-20P* column (1.5 × 14 cm), developing with H_2O. The H_2O eluate was neutralized with NaOH (1N) and was evaporated to dryness. The dry powder was dissolved in pyridine (2.0 ml), and then L-cysteine methyl ester hydrochloride (1.5 mg) was added. After heating at 60° for 1 h, 1-(trimethylsilyl)-1*H*-imidazole (1.5 ml) was added. The mixture was heated at 60° for another 30 min. An aliquot (4 µl) of the supernatant was removed and directly subjected to GC analysis: column temp., 180–280° at 3 deg/min; carrier gas, N_2 (1 ml/min); injector and detector temp., 250°; split ratio, 1:50. The configurations of D-glucose moieties of **2** were determined by comparison of the retentions times (t_R) of the corresponding derivatives with those of standard D/L-glucose. The t_R values of the standard D- and L-derivatives were 19.450 and 19.943 min, resp.

Enzymatic Hydrolysis of **3**. A mixture of **3** (8.0 mg) and β-glucosidase (6.0 mg, sigma) in H_2O (1.5 ml) was kept in a H_2O bath at 37° for 5 d. The mixture was subjected CC (*MCI*-gel *CHP-20P*; H_2O, and 70 and 100% MeOH). The H_2O eluates were concentrated to give D-glucose (2.0 mg) ($[\alpha]_D = +50.8$ ($c = 0.1$, H_2O)). The 100% MeOH eluates were dried under reduced pressured to give an aglycone, *demethylpiperitol* (**3a**; 3.0 mg). Pale amorphous powder. $[\alpha]_D = -61.1$ ($c = 0.3$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 3.06 (*m*, H–C(8), H–C(8′)); 3.86 (*m*, H–C(9a), H–C(9′a)); 4.23 (*m*, H–C(9b), H–C(9′b)); 4.68 (*d*, *J* = 5.2, H–C(7)); 4.71 (*d*, *J* = 5.2, H–C(7′)); 5.96 (*s*, OCH_2O); 6.75 (*dd*, *J* = 7.8, 1.5, H–C(6), H–C(6′)), 6.77 (*s*, H–C(2′)); 6.80 (*d*, *J* = 7.8, H–C(5), H–C(5′)); 6.82 (*s*, H–C(2)). ^{13}C-NMR ($CDCl_3$): 147.9 (C(3′)); 147.1 (C(4′)); 143.8 (C(3)); 143.4 (C(4)); 134.8 (C(1′)); 133.5 (C(1)); 119.4 (C(5′)); 118.6 (C(6)); 115.3 (C(5)); 113.3 (C(2)); 108.2 (C(6′)); 106.5 (C(2′)); 101.1 (OCH_2O); 85.8 (C(7′)); 85.6 (C(7)); 71.7 (C(9′)); 71.6 (C(9)); 54.1 (C(8′)); 53.9 (C(8)). FAB-MS (neg.): 341 ($[M-H]^-$).

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REFERENCES

[1] R. G. Zhang, S. G. Li, Y. D. Jiang, *Xibu Linye Kexue* **2007**, *36*, 97.
[2] 'Flora of Yunnannica', Institutum Botanicum Kunmingense Academiae Science Press, Beijing, 1997, Vol. 7, p. 102.
[3] S. Begum, Farhat, B. S. Siddiqui, *J. Nat. Prod.* **1997**, *60*, 20.
[4] T. Yoshida, T. Maruyama, A. Nitta, T. Okuda, *Chem. Pharm. Bull.* **1992**, *40*, 1750.
[5] H. Okamura, A. Mimura, M. Niwano, Y. Takahara, H. Yasuda, H. Yoshida, *Phytochemistry* **1993**, *33*, 512.
[6] E. L. Ghisalberti, *Phytochemistry* **1996**, *41*, 7.
[7] I. P. Singh, S. B. Bharate, *Nat. Prod. Rep.* **2006**, *23*, 558.
[8] M. Takasaki, T. Konoshima, K. Fujitani, S. Yoshida, H. Nishimura, H. Tokuda, H. Nishino, A. Iwashima, M. Kozuka, *Chem. Pharm. Bull.* **1990**, *38*, 2737.
[9] M. Murata, Y. Yamakoshi, S. Homma, K. Aida, K. Hori, Y. Ohashi, *Agric. Biol. Chem.* **1990**, *54*, 3221.
[10] L.-W. Tian, Y.-J. Zhang, Y.-F. Wang, C.-C. Lai, C.-R. Yang, *J. Nat. Prod.* **2009**, *72*, 1608.
[11] T. Hatano, N. Ogawa, R. Kira, T. Yasuhara, T. Okuda, *Chem. Pharm. Bull.* **1989**, *37*, 2083.
[12] T. Yoshida, Y. Maruyama, M. U. Memon, T. Shingu, T. Okuda, *Phytochemsitry* **1985**, *24*, 1041.
[13] J.-H. Lin, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.* **1990**, *38*, 1218.
[14] T. Tanaka, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.* **1986**, *34*, 656.
[15] Y. H. Gong, L. S. Ding, 'Carbon Nuclear Magnetic Resonance Analyses', Yunnan Technology Press, Yunnan, 2006, p. 555.
[16] L.-W. Tian, Y.-J. Zhang, C. Qu, Y.-F. Wang, C.-R. Yang, *J. Nat. Prod.* **2010**, *73*, 160.
[17] S. Gao, J. Liu, G.-M. Fu, Y.-C. Hu, S.-S. Yu, L.-H. Fan, D.-Q. Yu, J. Qu, *Planta Med.* **2007**, *73*, 163.

[18] T. Hasegawa, F. Takano, T. Takata, M. Niiyama, T. Ohta, *Phytochemistry* **2008**, *69*, 747.
[19] Y. Yamano, M. Ito, *Chem. Pharm. Bull.* **2005**, *53*, 541.
[20] S. C. Santos, P. G. Waterman, *Fitoterpia* **2001**, *72*, 316.
[21] O. Pérez, L. N. Almonacid, J. M. Trujillo, A. G. González, S. J. Alonso, E. Navarro, *Phytochemistry* **1995**, *40*, 1511.
[22] M. Nagata, T. Osawa, N. Namiki, Y. Fukuda, T. Ozaki, *Agric. Biol. Chem.* **1987**, *51*, 1285.

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