

Hepatoprotective Constituents from the Roots and Stems of *Erycibe hainanensis*

Shuang Song, Yixiu Li, Ziming Feng, Jianshuang Jiang, and Peicheng Zhang*

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education), Beijing 100050, People's Republic of China

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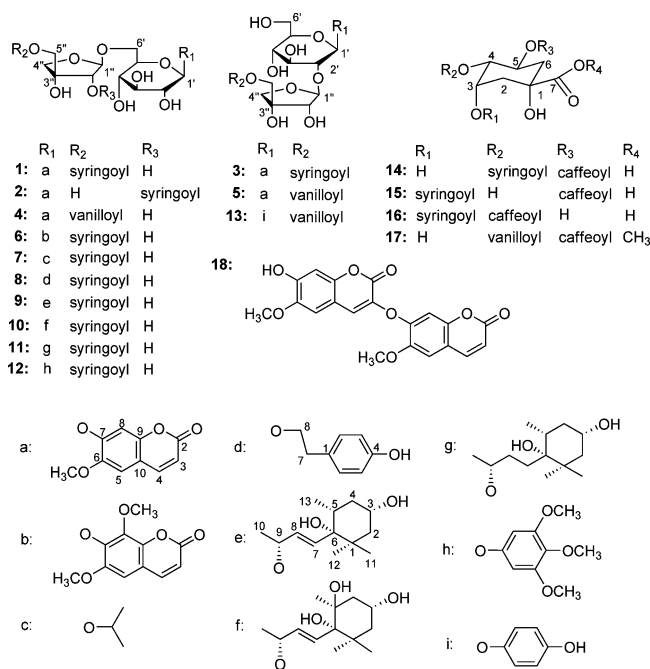
Eleven new diglycosides, ercibosides A–K (**1–11**), four new chlorogenic acid derivatives (**14–17**), and a new bis-coumarin (**18**), together with 21 known compounds, have been isolated from an EtOH extract of the roots and stems of *Erycibe hainanensis*. Their structures were elucidated by means of spectroscopic methods and chemical evidence. Inhibitory activities of some of the compounds on D-galactosamine-induced cytotoxicity in WB-F344 rat hepatic epithelial stem-like cells were screened, and compounds **2**, **6**, **10**, **18**, and **32** showed potent hepatoprotective activities at concentrations of 1×10^{-5} to 1×10^{-4} M.

The genus *Erycibe* roxb. (Convolvulaceae) consists of about 66 species, with 11 species found in China. However, only *E. obtusifolia*, *E. schmidtii*, *E. hainanensis*, *E. expansa*, and *E. elliptilimba* were chemically investigated previously. Flavonoids, coumarins, chlorogenic acids, alkaloids, and several other components were reported from *Erycibe* species.^{1–5} Some of them have been shown to exhibit anti-inflammatory, muscarinic agonistic, and cytotoxic activities.^{3,6–8} Our previous phytochemical study of *E. obtusifolia*, used in Chinese folk medicine to relieve symptoms of rheumatoid arthritis, led to the isolation of two new bis-coumarins, a new coumarin glucoside, and a new chlorogenic acid derivative, together with four known coumarins.⁹ Continuing our study on the constituents and bioactivities of the plants of the genus *Erycibe*, we investigated *E. hainanensis* Merr., a species growing in Guangdong, Hainan, and Guangxi Provinces of the People's Republic of China.¹⁰ Sixteen new compounds including 11 diglycosides, ercibosides A–K (**1–11**), four chlorogenic acid derivatives (**14–17**), and a bis-coumarin (**18**) were isolated, along with 21 known compounds, which were identified by comparison of experimental and reported spectroscopic data as 1-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-3,4,5-trimethoxybenzene (**12**),¹¹ seguinoside E (**13**),¹² caffeic acid (**19**),¹³ 3,4-dihydroxybenzoic acid (**20**),¹⁴ *trans*-*N*-feruloyltyramine (**21**),¹⁵ 7,7'-dihydroxy-6,6'-dimethoxy-3,3'-bis-coumarin (**22**),⁹ *trans*-*N*-(*p*-coumaroyl)tyramine (**23**),¹⁶ chlorogenic acid (**24**),¹⁷ methyl chlorogenate (**25**),¹⁸ methyl-3-*O*-(4'-hydroxy-3'',5''-dimethoxybenzoyl)chlorogenate (**26**),⁹ (+)-lyoniresinol 3a-*O*- β -D-glucopyranoside (**27**),¹⁹ 4-hydroxy-3-methoxybenzoic acid (**28**),²⁰ ethyl chlorogenate (**29**),²¹ 3-*O*-caffeoylquinic acid butyl ester (**30**),²² ethyl 3,4-dicaffeoylquininate (**31**),²³ 7*R*,8*R*,8'*S*-aketrilignoside B (**32**),²⁴ aketrilignoside B,²⁴ *cis*-*N*-feruloyltyramine,²⁵ syringaresinol-di-*O*- β -D-glucopyranoside,²⁶ scopoletin,²⁷ and scopolin.²⁸ Additionally, the hepatoprotective activities of compounds **1–16** and **18–32** against D-GalN-induced cytotoxicity in the primary cultured mouse hepatocytes were examined.

Results and Discussion

The EtOH extract of the roots and stems of *E. hainanensis* was suspended in H₂O and then sequentially partitioned with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH and EtOAc fractions were subjected to separation using various column chromatographic techniques to afford 16 new compounds (**1–11** and **14–18**), together with the known compounds mentioned above.

Compound **1** was obtained as a white powder, $[\alpha]_D^{20} -83.6$ (c 0.05, MeOH), and it showed blue fluorescence under UV light (365



nm), typical of a coumarin. The negative HRESIMS data of **1** indicated an $[M - H]^-$ ion at m/z 665.1704 corresponding to the molecular formula C₃₀H₃₄O₁₇ (calcd for C₃₀H₃₃O₁₇, 665.1712). In the ¹H NMR spectrum of **1**, a pair of doublets at δ 6.24 (1H, d, $J = 9.5$ Hz) and 7.86 (1H, d, $J = 9.5$ Hz), and two aromatic singlets at δ 7.20 (1H, s) and 7.13 (1H, s), indicated the presence of a 6,7-disubstituted coumarin skeleton. In addition, the characteristic signals at δ 7.15 (2H, s) and 3.78 (6H, s) suggested the existence of a syringoyl moiety, while two doublets due to anomeric protons at δ 5.06 (1H, d, $J = 7.0$ Hz, H-1') and 4.86 (1H, d, $J = 2.5$ Hz, H-1''), together with the partially overlapped signals between δ 3.08 and 5.28, showed the presence of two glycosyl groups. An apiofuranose moiety could be assumed from the occurrence of two pairs of doublets at δ 4.23 (1H, d, $J = 11.0$ Hz) and 4.19 (1H, d, $J = 11.0$ Hz) and at δ 4.04 (1H, d, $J = 9.5$ Hz) and 3.87 (1H, d, $J = 9.5$ Hz) for the two methylene groups (C-4'' and C-5''), respectively. The ¹³C NMR spectrum showed 30 signals (see Table 3). Except for 19 carbon signals assigned as a coumarin skeleton with a methoxy and a syringoyl group, the remaining 11 carbon signals were attributable to glucosyl and apiosyl moieties. Furthermore, the coupling constant ($J = 7.0$ Hz) of the anomeric proton of the glucosyl moiety as well as the chemical shift (δ 109.4) of the anomeric carbon of the apiosyl moiety demonstrated that both sugar moieties had β -anomeric configurations.²⁹ Comparison of the

* To whom correspondence should be addressed. Tel: 86-10-63165231. Fax: 86-10-63017757. E-mail: pczhang@imm.ac.cn.

Table 1. ¹H NMR Spectroscopic Data (δ) of Compounds 1–6^a

position	1	2	3	4	5	6
3	6.24, d (9.5)	6.21, d (9.5)	6.28, d (9.5)	6.25, d (9.5)	6.28, d (9.5)	6.35, d (9.5)
4	7.86, d (9.5)	7.79, d (9.5)	7.83, d (9.5)	7.88, d (9.5)	7.82, d (9.5)	7.88, d (9.5)
5	7.20, s	7.14, s	7.03, s	7.23, s	7.00, s	7.05, s
8	7.13, s	7.05, s	6.98, s	7.14, s	7.00, s	
1'	5.06, d (7.0)	5.06, d (7.0)	5.08, d (8.0)	5.06, d (5.0)	5.10, d (7.5)	5.08, d (7.0)
2'	3.11, m	3.11, m	3.62, m	3.12, m	3.62, m	3.09, m
3'	3.80, m	3.80, m	3.43, m	3.61, m	3.42, m	3.76, m
4'	3.30, m	3.30, m	3.17, m	3.25, m	3.17, m	3.24, m
5'	3.67, m	3.65, m	3.42, m	3.25, m	3.52, m	3.27, m
6'a	3.77, m	3.89, m	3.75, m ^b	3.86, m	3.72, m	3.77, m
6'b	3.50, m	3.52, m	3.52, m	3.49, m	3.45, m	3.43, m
1''	4.86, d (2.5)	5.14, d (2.5)	5.48, br s	4.85, br s	5.48, br s	4.74, d (2.5)
2''	3.83, d (2.5)	4.91, d (2.5)	3.75, br s	3.79, br s	3.75, m ^b	3.66, m ^b
4''a	4.04, d (9.5)	4.02, d (9.5)	4.26, d (10.0)	4.02, d (9.5)	4.28, d (10.0)	3.73, d (9.5)
4''b	3.87, d (9.5)	3.70, d (9.5)	3.76, d (10.0)	3.81, d (9.5)	3.82, d (10.0)	3.67, d (9.5)
5''a	4.23, d (11.0)	3.47, s	4.18, d (11.5)	4.23, d (11.0)	4.14, d (11.0)	4.08, s
5''b	4.19, d (11.0)		4.06, d (11.5)	4.19, d (11.0)	4.05, d (11.0)	
2'''	7.15, s	7.08, s	7.03, s	7.37, d (2.0)	7.23, br s	7.19, s
5'''				6.79, d (7.5)	6.67, d (7.5)	
6'''	7.15, s	7.08, s	7.03, s	7.43, dd (7.5, 2.0)	7.27, d (7.5)	7.19, s
6-OMe	3.80, s	3.80, s	3.68, s	3.79, s	3.66, s	3.79, s
8-OMe						3.88, s
3'''-OMe	3.78, s	3.78, s	3.74, s	3.77, s	3.74, s	3.79, s
5'''-OMe	3.78, s	3.78, s	3.74, s			3.79, s

^a ¹H NMR data (δ) were measured in DMSO-*d*₆ at 500 MHz. Coupling constants (*J*) in Hz are given in parentheses. ^b Overlapping signals.

Table 2. ¹H NMR Spectroscopic Data (δ) of Compounds 7–11^a

position	7	8	9	10	11
1	1.07, d (6.5)				
2a	3.82 ^b	6.99, d (8.5)	1.50, t (11.5)	1.49, t (11.5)	1.50 ^b
2b			1.20 ^b	1.27, dd (11.5, 2.5)	1.09, m
3	1.04, d (6.5)	6.62, d (8.5)	3.57, m	3.82 ^b	3.49, m
4a			1.47 ^b	1.61, dd (12.5, 4.5)	1.36 ^b
4b			1.25, q (12.0)	1.55, t (12.5)	1.25, q (11.5)
5		6.62, d (8.5)	1.75, m		1.66, m
6		6.99, d (8.5)			
7		2.68, m	5.44, d (16.5)	5.98, d (16.0)	1.47 ^b
8a		3.82, m	5.61, dd (16.5, 6.0)	5.71, dd (16.0, 7.0)	1.40 ^b
8b		3.55, m			
9			4.24, m	4.30, m	3.58, m
10			1.15, d (6.5)	1.19, d (6.0)	1.03, d (6.5)
11			0.75, s	1.07, s	0.84, s
12			0.84, s	0.72, s	0.84, s
13			0.67, d (6.5)	0.99, s	0.78, d (6.5)
1'	4.12, d (7.5)	4.14, d (8.0)	4.17, d (8.0)	4.22 ^b	4.14, d (8.0)
2'	2.88, m	2.94, m	2.93, dd (8.5, 7.5)	2.94, m	2.88, m
3'	3.11, m	3.13, m	3.11, dd (9.0, 8.5)	3.13, m	3.11, m
4'	2.95, m	2.98, m	3.02, dd (9.0, 9.0)	3.03, m	2.97, m
5'	3.24, m	3.30, m	3.18, m	3.19, m	3.22, m
6'a	3.82 ^b	3.45, m	3.81 ^b	3.82 ^b	3.80 ^b
6'b	3.43, m	3.25, m	3.44, dd (11.5, 7.0)	3.44, m	3.43, m
1''	4.92, d (2.5)	4.92, d (2.5)	4.90, d (2.5)	4.91, d (2.5)	4.91 ^b
2''	3.83 ^b	3.82, d (2.5)	3.81 ^b	3.84 ^b	3.80 ^b
4''a	3.93, d (9.5)	3.93, d (9.5)	3.93, d (9.0)	3.94, d (9.5)	3.90, d (9.5)
4''b	3.77, d (9.5)	3.86, d (9.5)	3.80, d (9.0)	3.79 ^b	3.78 ^b
5''a	4.25, d (11.5)	4.23, d (11.0)	4.25 ^b	4.20 ^b	4.25, d (11.5)
5''b	4.25, d (11.5)	4.19, d (11.0)	4.22 ^b	4.19 ^b	4.22, d (11.5)
2''' ^b , 6'''	7.23, s	7.23, s	7.24, s	7.24, s	7.23, s
3''' ^b , 5'''-OMe	3.80, s	3.79, s	3.82, s	3.82, s	3.80, s

^a ¹H NMR data (δ) were measured in DMSO-*d*₆ at 500 MHz. Coupling constants (*J*) in Hz are given in parentheses. ^b Overlapping signals.

NMR data of **1** with those of known compounds **12** and **13** suggested the presence of a 6-*O*-(5-*O*-syringoyl)-β-apiofuranosyl)-β-glucopyranosyl moiety. This was confirmed by HMBC correlations (see Figure 1) of C-6' with H-1'' and C-7''' with H-5''. The HMBC correlations of C-6 with the methoxy protons at δ 3.80 and of C-7 with H-1' indicated that the methoxy group and the sugar chain were located at C-6 and C-7, respectively, of the coumarin moiety. In addition, the glucose obtained from the hydrolysis of **1** gave a positive specific rotation, [α]_D²⁰ +47.4 (c 0.2, H₂O), suggesting that it was D-glucose. The common D-configuration for apiose was assumed. According to the above

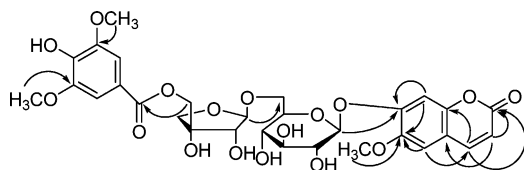
evidence, the structure of **1** was characterized as 7-*O*-[6-*O*-(5-*O*-syringoyl)-β-D-apiofuranosyl]-β-D-glucopyranosyl]-6-methoxycoumarin and named eryciboside A.

Compound **2** was obtained as a white powder, [α]_D²⁰ -39.3 (c 0.05, MeOH). The positive HRESIMS data of **2** showed an [M + Na]⁺ ion at *m/z* 689.1686 corresponding to the same molecular formula, C₃₀H₃₄O₁₇, as **1**. The NMR data of **2** showed close resemblance to those of **1** (see Tables 1 and 3). Comparison of the ¹H and ¹³C NMR data of **1** and **2** indicated that H-2'' and C-2'' of **2** were deshielded by Δδ_H 1.08 and Δδ_C 2.4 ppm, respectively, while H-5'' and C-5'' were shielded by Δδ_H 0.74 and Δδ_C 2.1 ppm,

Table 3. ^{13}C NMR Spectroscopic Data (δ) of Compounds **1**–**11**^a

position	1	2	3	4	5	6	7	8	9 ^b	10	11
1							23.4	128.6	39.2 ^c (40.5)	40.0 ^c	40.0 ^c
2	160.3	160.4	160.5	160.3	160.5	159.7	70.1	129.7	45.1 (45.9)	45.7	46.8
3	113.3	113.3	113.1	113.3	113.1	114.7	21.8	115.0	64.9 (67.5)	62.6	64.9
4	143.9	143.7	144.0	143.9	144.0	144.2		155.5	39.7 ^c (39.9)	45.2	40.1 ^c
5	109.6	109.5	109.0	109.7	109.0	105.4		115.0	33.6 (35.4)	75.7	33.8
6	145.8	145.8	145.5	145.9	145.5	149.4		129.7	76.0 (78.6)	77.1	73.9
7	149.7	149.7	149.2	149.7	149.3	141.6		34.8	134.4 (135.9)	131.7	30.9
8	102.9	102.8	102.5	103.0	102.5	140.3		69.9	131.6 (133.4)	132.0	32.9
9	148.8	148.9	148.7	148.8	148.7	142.3			74.5 (78.1)	74.8	74.1
10	112.3	112.3	112.1	112.3	112.1	114.7			20.9 (21.4)	20.8	19.4
11									25.5 (25.3)	25.7	24.5
12									24.5 (26.2)	27.0	25.8
13									16.1 (16.5)	26.9	16.1
1'	99.5	99.6	97.8	99.6	97.8	102.3	99.5	102.8	100.5 (102.4)	100.3	100.4
2'	72.9	72.9	77.2	72.9	77.1	73.9	73.4	73.3	73.7 (75.3)	73.6	73.4
3'	77.0	76.7	77.1	76.7	77.0	76.3	76.7	76.9	76.8 (78.2)	76.7	76.8
4'	69.9	70.0	70.0	69.8	69.9	70.0	70.3	70.2	70.0 (71.6)	70.0	70.3
5'	75.4	75.4	74.4	75.4	74.4	75.9	75.3	75.5	75.5 (77.6)	75.3	75.3
6'	68.1	67.4	60.5	67.9	60.5	67.4	67.9	67.7	67.4 (68.7)	67.5	67.7
1''	109.4	107.3	108.0	109.3	107.9	108.8	109.0	108.9	109.0 (110.8)	109.0	109.0
2''	77.0	79.4	76.9	76.9	76.9	77.0	76.8	76.6	77.1 (77.6)	76.9	76.8
3''	77.0	78.4	77.4	77.0	77.4	76.7	77.1	77.1	76.8 (79.1)	77.0	77.1
4''	73.5	74.3	73.6	73.5	73.5	73.2	73.3	73.3	73.4 (75.1)	73.4	73.3
5''	66.5	64.4	66.7	66.4	66.4	66.4	66.7	66.6	66.8 (68.2)	66.9	66.8
1'''	119.5	119.0	119.5	120.1	120.1	118.9	119.0	119.5	119.5 (121.1)	119.5	119.2
2'''	107.0	107.2	107.0	112.6	112.5	107.1	107.1	107.1	107.1 (108.5)	107.1	107.1
3'''	147.4	147.3	147.3	147.3	147.1	147.6	147.5	147.6	147.5 (149.0)	147.5	147.5
4'''	140.2	140.7	140.8	150.8	151.4	141.0	141.0	140.0	140.2 (142.2)	140.2	140.8
5'''	147.4	147.3	147.3	115.1	114.8	147.6	147.5	147.6	147.5 (149.0)	147.5	147.5
6'''	107.0	107.2	107.0	123.6	123.4	107.1	107.1	107.1	107.1 (108.5)	107.1	107.1
7'''	165.3	164.7	165.1	165.3	165.1	165.3	165.4	165.4	165.4 (167.9)	165.5	165.4
6-O-Me	56.0	56.0	55.6	55.5	55.4	56.4					
8-O-Me						61.3					
3'''-O-Me	56.0	56.0	56.0	56.0	55.4	56.1	55.5	56.1	56.1 (57.0)	56.1	56.1
5'''-O-Me	56.0	56.0	56.0			56.1	55.5	56.1	56.1 (57.0)	56.1	56.1

^a ^{13}C NMR data (δ) were measured in $\text{DMSO}-d_6$ at 125 MHz. ^b Chemical shifts in parentheses were measured in $\text{MeOH}-d_4$. ^c Signal overlapped by solvent peaks.

**Figure 1.** Selected HMBC correlations of **1**.

respectively. These data showed that the syringoyl group was linked to C-2'' of the β -D-apiofuranosyl moiety, as confirmed by an HMBC correlation from H-2'' to C-7'''. Therefore, **2** was elucidated to be 7-O-[6-O-(2-O-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-6-methoxycoumarin and named ercycoside B.

Compound **3** was obtained as a white powder, $[\alpha]_D^{20} -33.6$ (c 0.03, MeOH), and the positive HRESIMS ion at m/z 689.1682 $[\text{M} + \text{Na}]^+$ indicated it had the same molecular formula as **1**. The NMR spectroscopic data of **3** also resembled those of **1** (see Tables 1 and 3). However, the ^{13}C NMR chemical shift differences of C-2' ($\Delta\delta_C +4.3$) and C-6' ($\Delta\delta_C -7.6$) for **3** and **1** suggested that the apiofuranosyl moiety was located at C-2' in **3** instead of C-6' in **1**. This was supported by an HMBC correlation of C-1'' with H-2'. From these data, **3** was established as 7-O-[2-O-(5-O-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-6-methoxycoumarin and named ercycoside C.

Compound **4** was obtained as a white powder, $[\alpha]_D^{20} -62.4$ (c 0.05, MeOH). Its molecular formula was determined to be $\text{C}_{29}\text{H}_{32}\text{O}_{16}$ from the positive HRESIMS data ($[\text{M} + \text{Na}]^+$, m/z found 659.1583). An ABX spin system at δ 7.37 (1H, d, $J = 2.0$ Hz), 6.79 (1H, d, $J = 7.5$ Hz), and 7.43 (1H, dd, $J = 7.5, 2.0$ Hz) and a singlet for a methoxy group at δ 3.77 (3H, s), instead of the characteristic signals of the syringoyl moiety, were observed in the

^1H NMR spectrum of **4**, suggesting the presence of a vanilloyl moiety. The ^{13}C NMR spectrum of **4** showed carbon signals corresponding to the vanilloyl moiety (see Table 3). Furthermore, the HMBC spectrum displayed long-range correlations of C-7 with H-1', C-6' with H-1'', and C-7''' with H-5''. Considering these spectroscopic observations, **4** was determined as 7-O-[6-O-(5-O-vanilloyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-6-methoxycoumarin and named ercycoside D.

Compound **5** was obtained as a white powder, $[\alpha]_D^{20} -32.9$ (c 0.05, MeOH). The same molecular formula, $\text{C}_{29}\text{H}_{32}\text{O}_{16}$, as **4** was determined by the positive HRESIMS data ($[\text{M} + \text{Na}]^+$, m/z found 659.1579). Comparison of the NMR data (see Tables 1 and 3) of **5** and **3** showed that the signals of the syringoyl moiety in **3** were replaced by signals attributed to a vanilloyl moiety in **5**. Further confirmation was derived from HMBC correlations of C-3''' with the methoxy protons at δ 3.74, C-6' with the methoxy protons at δ 3.66, C-7 with H-1', C-2' with H-1'', and C-7''' with H-5''. Thus **5** was assigned as 7-O-[2-O-(5-O-vanilloyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-6-methoxycoumarin and named ercycoside E.

Compound **6** was obtained as a white powder, $[\alpha]_D^{20} -3.8$ (c 0.11, MeOH). Its molecular formula was determined as $\text{C}_{31}\text{H}_{36}\text{O}_{18}$ by the positive HRESIMS data ($[\text{M} + \text{Na}]^+$, m/z found 719.1801). The ^1H and ^{13}C NMR data (see Tables 1 and 3) of **6** also showed characteristic signals for a 6-O-(5-O-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl moiety. The ^1H NMR spectrum revealed the presence of an additional methoxy group at δ 3.88 (3H, s) and the absence of an aromatic proton observed in **1**. When compared to those of **1**, C-8 of **6** was deshielded by $\Delta\delta_C$ 37.4 ppm, while C-7, C-9, and C-5 were shielded by $\Delta\delta_C$ 8.1, 6.5, and 4.2 ppm, respectively. These data suggested that the additional methoxy group was located at C-8. This was confirmed by an HMBC correlation from this methoxy group to C-8. In addition, the HMBC spectrum

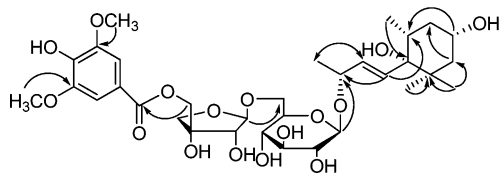


Figure 2. Selected HMBC correlations of **9**.

also showed correlations of C-7 with H-1', C-6' with H-1'', and C-7''' with H-5''. All these data indicated the structure of **6** as 7-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-6,8-dimethoxycoumarin, named ericiboside F.

Compound **7** was obtained as a white powder, $[\alpha]_D^{20} -50.6$ (*c* 0.10, MeOH), and its molecular formula was determined to be $C_{23}H_{34}O_{14}$ by the positive HRESIMS data ($[M + Na]^+$, *m/z* found 557.1841). Signals derived from a 6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl moiety were also observed in the NMR spectra of **7** (see Tables 2 and 3). However, the signals of the coumarin unit observed in **1–6** were replaced by signals attributed to isopropyl at δ_H 1.07 (3H, d, *J* = 6.5 Hz), 3.82 (1H, overlapped), and 1.04 (3H, d, *J* = 6.5 Hz) in the 1H NMR spectrum and at δ_C 23.4, 70.1, and 21.8 in the ^{13}C NMR spectrum. HMBC correlations of C-6' with H-1'' and C-7''' with H-5'' confirmed the sugar chain as 6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl. Furthermore, the connection between the isopropyl and sugar moieties was established by HMBC correlations of C-2 with H-1' and C-1' with H-2. These spectroscopic data established **7** as 2-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]isopropyl alcohol, named ericiboside G.

Compound **8** was obtained as a white powder, $[\alpha]_D^{20} -41.4$ (*c* 0.05, MeOH), and its molecular formula was determined to be $C_{28}H_{36}O_{15}$ by the positive HRESIMS data ($[M + Na]^+$, *m/z* found 635.1951). Its 1H and ^{13}C NMR data (see Tables 2 and 3) also indicated the presence of a 6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl moiety, and this was further confirmed by HMBC correlations of C-6' with H-1'' and C-7''' with H-5''. The remaining proton signals at δ 6.99 (2H, d, *J* = 8.5 Hz, H-2 and H-6), 6.62 (2H, d, *J* = 8.5 Hz, H-3 and H-5), 3.82 (1H, m, H-8a), 3.55 (1H, m, H-8b), and 2.68 (2H, m, H₂-7) in the 1H NMR spectrum were attributable to a 4-substituted phenylethyl alcohol moiety. HMBC correlations of C-8 with H-1' and C-1' with H-8 suggested that the sugar moiety was at C-8. Thus, compound **8** was determined to be 8-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-4-hydroxyphenylethyl alcohol and named ericiboside H.

Compound **9** was obtained as a white powder, $[\alpha]_D^{20} -38.7$ (*c* 0.06, MeOH), and its positive HRESIMS data ($[M + Na]^+$, *m/z* found 725.2983) indicated the molecular formula to be $C_{33}H_{50}O_{16}$. The 1H and ^{13}C NMR spectra of **9** (see Tables 2 and 3) displayed signals assignable to a 6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl moiety. The remaining signals in the 1H NMR spectrum included two methyls at δ 0.75 and 0.84 as singlets, two methyl doublets at δ 1.15 and 0.67, five aliphatic protons ranging from δ 1.20 to 1.75, two oxymethine protons as multiplets at δ 3.57 and 4.24, and two olefinic protons at δ 5.44 (d, *J* = 16.5 Hz) and 5.61 (dd, *J* = 16.5, 6.0 Hz) for a disubstituted *trans* double bond. The ^{13}C NMR spectrum displayed 13 carbon signals due to the aglycone moiety. The above spectroscopic data suggested the planar structure of the aglycone moiety was 3,6,9-trihydroxymegastigman-7-ene. This suggestion was further supported by the vicinal coupling correlations of H-9 with both H₃-10 and H-8, H-8 with H-7, H-3 with both H₂-2 and H₂-4, and H-5 with H₃-13 in the 1H - 1H COSY spectrum, together with the related HMBC correlations (see Figure 2). In addition, HMBC correlations from the anomeric proton H-1' to C-9 and from H-9 to C-1' indicated the sugar moiety was attached to C-9. The large couplings of H-2ax with H-3 (*J* = 11.5 Hz), and H-4ax with H-3 (*J* = 12.0 Hz) and

H-5 (*J* = 12.0 Hz), implied that H-3 and H-5 must be in the axial positions. The 3,6,9-trihydroxymegastigman-7-ene moiety with H-3 and H-5 in the axial positions has been reported,^{30–33} and except for C-9, their absolute configurations were determined as 3*S*, 5*R*, and 6*S*. Thus the ring system of **9** was presumed to have the same configuration. The absolute configuration of C-9 was further elucidated by comparing the ^{13}C NMR data of **9** with those of reported 9-*O*-glycosides of (3*S*,5*R*,6*S*,9*R*)-3,6,9-trihydroxymegastigman-7-ene and (3*S*,5*R*,6*S*,9*S*)-3,6,9-trihydroxymegastigman-7-ene.^{30,33} The ^{13}C NMR data of the aglycone moiety of **9** were consistent with those of (3*S*,5*R*,6*S*,9*R*)-3,6,9-trihydroxymegastigman-7-ene moiety. Therefore, **9** was established as 9-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-(3*S*,5*R*,6*S*,9*R*)-3,6,9-trihydroxymegastigman-7-ene and named ericiboside I.

Compound **10** was obtained as a white powder, $[\alpha]_D^{20} -36.9$ (*c* 0.05, MeOH). The positive HRESIMS ion of **10** at *m/z* 741.2946 ($[M + Na]^+$) proved the molecular formula to be $C_{33}H_{50}O_{17}$. The NMR spectra (see Tables 2 and 3) indicated that it was also a megastigmane derivative with a 6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl moiety. The 1H NMR spectrum (see Table 2) showed the absence of signal of H-5 in **9**, and the changes of coupling patterns of H₂-4 [δ 1.61 (1H, dd, *J* = 12.5, 4.5 Hz, H-4eq) and 1.55 (1H, t, *J* = 12.5 Hz, H-4ax)] indicated the presence of a hydroxy group at C-5. This was supported by the deshielded signal of C-5 at δ 75.7 in the ^{13}C NMR spectrum (see Table 3). The planar structure of **10** was further confirmed by the 1H - 1H COSY, HSQC, and HMBC spectra. The elucidation of the relative configuration of the aglycone moiety is based on the NOE difference experiment and on the observed 1H / 1H coupling constants. The large coupling values of H-3 with H-2ax (*J* = 11.5 Hz) and with H-4ax (*J* = 12.5 Hz) indicated H-3 must be in the axial position. The NOE difference experiment showed enhancements of both H-7 and H-3 by irradiation of H₃-11 and no enhancement of H₃-11 or H-3 by irradiation of H₃-13. This indicated H₃-11, H-3, and H-7 were on the same side of the six-membered ring, while H₃-13 was on the opposite face. In addition, as **10** differed from **9** only by an additional hydroxy group at C-5, the absolute configuration of **10** was presumed to be the same as **9**. Thus, **10** was assigned as 9-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-(3*S*,5*R*,6*R*,9*R*)-3,5,6,9-tetrahydroxymegastigman-7-ene and named ericiboside J.

Compound **11** was obtained as a white powder, $[\alpha]_D^{20} -50.7$ (*c* 0.06, MeOH). The spectroscopic data of **11** indicated that it was also a megastigmane derivative with a 6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl moiety. The molecular formula was $C_{33}H_{50}O_{17}$, as indicated by the positive HRESIMS ion ($[M + Na]^+$, *m/z* found 727.3148). The 1H and ^{13}C NMR spectra of **11** (see Tables 2 and 3) also suggested a close structural similarity to **9**, with the main difference of the replacement of signals for a double bond with an additional pair of methylenes [δ_H 1.47 (2H, overlapped, H₂-7) and 1.40 (2H, overlapped, H₂-8), and δ_C 30.9 (C-7) and 32.7 (C-8)]. This suggestion was confirmed by the 1H - 1H COSY, HSQC, and HMBC spectra. The absolute configuration of **11** was also presumed to be the same as **9**. Thus, **11** was elucidated as 9-*O*-[6-*O*-(5-*O*-syringoyl- β -D-apiofuranosyl)- β -D-glucopyranosyl]-(3*S*,5*R*,6*S*,9*R*)-3,6,9-trihydroxymegastigmane and named ericiboside K.

Compound **14** was obtained as a white powder, $[\alpha]_D^{20} -106.7$ (*c* 0.05, MeOH), and its molecular formula was determined to be $C_{25}H_{26}O_{13}$ by the negative HRESIMS data ($[M - H]^-$, *m/z* found 533.1287). Signals derived from a syringoyl moiety were observed in the 1H and ^{13}C NMR spectra (see Table 4). The 1H NMR spectrum showed an ABX system attributed to a 1,3,4-trisubstituted aromatic ring at δ 7.00 (1H, d, *J* = 1.5 Hz), 6.95 (1H, dd, *J* = 8.0, 1.5 Hz), and 6.74 (1H, d, *J* = 8.0 Hz) and an AX system assignable to a *trans* double bond at δ 7.43 (1H, d, *J* = 15.5 Hz) and 6.15 (1H, d, *J* = 15.5 Hz), which suggested the presence of a caffeoyl

Table 4. NMR Spectroscopic Data (δ) of Compounds **14**–**17**^a

position	14		15		16		17	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1		74.0		72.4		73.1		72.8
2	1.97, 2.20, m	37.8	2.19, 2.00, m	36.4	2.14, 1.99, m	40.0 ^c	1.91, 2.24, m	37.7
3	4.30, m	66.7	5.31, m	71.2	5.53, m	68.9	4.20, m	65.3
4	4.97, dd (8.0, 2.0)	75.2	3.84, m	68.3	4.92, dd (8.0, 2.0)	73.1	5.01, m	73.1
5	5.57, m	67.4	5.28, m	70.0	4.16, m	65.3	5.35, m	67.5
6	2.08, 2.20, m	37.8	2.00, 1.98, m	34.7	1.99, 1.91, m	36.5	2.00, 2.26, m	36.2
7		175.0		175.3		177.4		173.3
1'		125.6		125.0		125.4		125.2
2'	7.00, d (1.5)	115.2	7.04, br s	114.3	7.01, d (1.5)	115.8	7.00, br s	114.7
3'		145.9		145.0		145.6		145.7
4'		148.8		147.8		148.5		148.6
5'	6.74, d (8.0)	116.0	6.77, d (8.0)	115.2	6.74, d (8.0)	113.9	6.75, d (8.0)	115.1
6'	6.95, dd (8.0, 1.5)	121.7	6.99, d (8.0)	120.7	6.93, dd (8.0, 1.5)	121.4	6.95, d (8.0)	121.4
7'	7.43, d (15.5)	145.9	7.44, d (16.0)	144.4	7.43, d (15.5)	145.4	7.40, d (15.6)	145.6
8'	6.15, d (15.5)	113.9	6.18, d (16.0)	113.8	6.24, d (15.5)	114.0	6.12, d (15.6)	113.2
9'		166.0		165.2		165.9		165.2
1''		119.6		119.8		119.7		120.4
2''	7.21, s	107.4	7.31, s	106.8	7.20, s	106.9	7.44, br s	112.8
3''		147.8		146.9		147.4		147.3
4''		141.1		139.9		140.5		151.6
5''		147.8		146.9		147.4	6.85, d (8.0)	114.7
6''	7.21, s	107.4	7.31, s	106.8	7.20, s	106.9	7.49, d (8.0)	123.7
7''		165.5		164.7		164.7		164.9
7-OMe							3.52, s	52.0
3''-OMe	3.79, s	56.3	3.81, s	55.5	3.72, s	55.9	3.76, s	55.6
5''-OMe	3.79, s	56.3	3.81, s	55.5	3.72, s	55.9		

^a NMR data (δ) were measured in DMSO-*d*₆ at 500 or 600 MHz for ¹H NMR and at 125 MHz for ¹³C NMR. ^b Overlapping signals. ^c Signal overlapped by solvent peaks.

moiety. The remaining signals of three oxygenated methine protons at δ 4.30 (1H, m, H-3), 4.97 (1H, dd, J = 8.0, 2.0, H-4), and 5.57 (1H, m, H-5) and four methylene protons at δ 2.20, 1.97 (2H, m, H₂-2) and 2.20, 2.08 (2H, m, H₂-6) indicated the presence of a quinic acid moiety. This was supported by a set of characteristic signals in the ¹³C NMR spectrum at δ 175.0, 75.2, 74.0, 67.4, 66.7, 37.8, and 37.8. Analyses of the HSQC spectrum of **14** led to unambiguous assignment of proton and corresponding carbon signals in the NMR spectra. The linkages between the units were established by HMBC correlations from H-4 to C-7'' and from H-5 to C-9'. Additional support for the location of the caffeoyl moiety was obtained from hydrolysis of **14** under alkaline conditions to afford 5-*O*-caffeoylquinic acid. Consequently, the structure of **14** was determined to be 5-*O*-caffeoyl-4-*O*-syringoylquinic acid.

Compound **15** was obtained as a white powder, $[\alpha]_{\text{D}}^{20}$ -96.4 (c 0.05, MeOH), and its negative HRESIMS and NMR data (see Experimental Section and Table 4) were similar to those of **14**. Comparison of the NMR data of **15** and **14** indicated that C-4 and H-4 of **15** were shielded by $\Delta\delta_{\text{C}}$ 6.9 and $\Delta\delta_{\text{H}}$ 1.13 ppm, respectively, whereas C-3 and H-3 were deshielded by $\Delta\delta_{\text{C}}$ 5.2 and $\Delta\delta_{\text{H}}$ 1.01 ppm, respectively. These data suggested that the ester substituents were located at C-3 and C-5 in **15** instead of C-4 and C-5 in **14**. The HMBC spectrum could not confirm the locations of the ester linkages because of the overlap of the H-3 and H-5 resonance. However, the alkaline hydrolysis of **15** gave 5-*O*-caffeoylquinic acid, which suggested the caffeoyl moiety was located at C-5, while the syringoyl was attached to C-3. This was corroborated by the comparison of the NMR data of **15** with those of 5-*O*-caffeoyl-3-*O*-syringoylquinic acid methyl ester⁹ isolated from *E. obtusifolia*. Hence, **15** was identified as 5-*O*-caffeoyl-3-*O*-syringoylquinic acid.

Compound **16** was obtained as a white powder, $[\alpha]_{\text{D}}^{20}$ -83.5 (c 0.05, MeOH), and its negative HRESIMS data ($[\text{M} - \text{H}]^{-}$, m/z found 533.1287) indicated that it possessed the same molecular formula as those of **14** and **15**. The ¹H and ¹³C NMR spectra (see Table 4) of **16** also displayed signals for syringoyl, caffeoyl, and quinic acid moieties. The locations of the caffeoyl and syringoyl moieties were determined to be at C-4 and C-3, respectively, on

the basis of HMBC correlations of C-9' with H-4 and C-7'' with H-3. Thus, **16** was defined as 4-*O*-caffeoyl-3-*O*-syringoylquinic acid.

Compound **17** was obtained as a white powder, $[\alpha]_{\text{D}}^{20}$ -108.2 (c 0.04, MeOH), and its negative HRESIMS data ($[\text{M} - \text{H}]^{-}$, m/z found 517.1337) indicated the molecular formula to be C₂₅H₂₆O₁₂. Comparison of the ¹H and ¹³C NMR of **17** and **14**–**16** revealed that the signals for the syringoyl unit in **14**–**16** were replaced by the signals attributed to the vanilloyl moiety (see Table 4). HMBC correlation of C-7'' with H-4 demonstrated that the vanilloyl moiety was located at C-4. Although the correlation of C-9' with H-5 was not observable in the HMBC spectrum, the alkaline hydrolysis of **17** gave 5-*O*-caffeoylquinic acid, which suggested the location of the caffeoyl moiety at C-5. An additional methyl ester group in **17** was deduced from its ¹H NMR signals at δ 3.52 (3H, s) and the HMBC correlation between the methoxy protons and the carbonyl carbon. On the basis of the above results, **17** was elucidated as 5-*O*-caffeoyl-4-*O*-vanilloylquinic acid methyl ester.

Compound **18** was obtained as a yellowish powder, and its molecular formula was determined to be C₂₀H₁₄O₈ by negative HRESIMS data ($[\text{M} - \text{H}]^{-}$, m/z found 381.0592). The compound exhibited blue fluorescence under UV light (365 nm). The ¹H NMR spectrum (see Experimental Section) displayed a typical pair of doublets at δ 6.41 (1H, d, J = 9.5 Hz, H-3') and 8.03 (1H, d, J = 9.5 Hz, H-4') and five singlets at δ 6.85 (1H, s, H-8), 7.18 (1H, s, H-5), 7.23 (1H, s, H-8'), 7.48 (1H, s, H-5'), and 7.61 (1H, s, H-4) for aromatic protons. The ¹³C NMR spectrum (see Experimental Section) exhibited 18 carbon signals in the downfield region, including two conjugated ester carbonyls at δ 160.1 and 156.6, which indicated that **18** possessed a dimeric coumarin skeleton. Analysis of the above proton and carbon signals led to the construction of a 6,7-*O*-disubstituted coumarin unit and a 3,6,7-*O*-trisubstituted coumarin unit, aided by the HMBC spectrum (see Figure 3). In addition, the substituents at C-6, C-6', and C-7 were established by HMBC correlations of C-6 and C-6' with the methoxy groups at δ 3.79 (3H, s) and 3.88 (3H, s), respectively, and C-7 with the OH proton at δ 10.21 (1H, s). Given the fact that there were only two oxygen-substituted positions remaining, it can

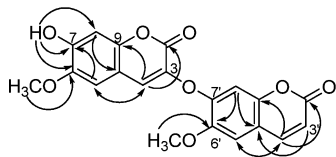


Figure 3. Selected HMBC correlations of **18**.

Table 5. Hepatoprotective Effects of Compounds **2**, **6**, **10**, **16**, and **32** against D-Galactosamine-Induced Toxicity in WB-F344 Cells^a

compound	cell survival rate (% of normal)	inhibition (% of control)
normal	100 ± 8.6	
control	30 ± 1.6	
bicyclol ^b	38 ± 2.3**	11.2
2	47 ± 0.7***	19.8
6	44 ± 4.8*	15.5
10	45 ± 2.9**	17.4
16	61 ± 0.7***	42.2
32	45 ± 3.6**	17.4

^a Results are expressed as means ± SD ($n = 3$; for normal and control, $n = 6$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **2** was tested at 1×10^{-5} M due to its poor solubility, while other compounds were tested at 1×10^{-4} M. ^b Positive control substance.

be inferred that the two coumarin units were linked by an ether bridge between C-3 and C-7'. Therefore, the structure of **18** was deduced as 7-hydroxy-6,6'-dimethoxy-3,7'-*O*-bis-coumarin. This is the first report of a bis-coumarin with a C–O–C linkage in the family of Convolvulaceae.

The hepatoprotective activities against D-galactosamine-induced toxicity of compounds **1–16** and **18–32** were examined in WB-F344 cells. Compounds **2**, **6**, **10**, **18**, and **32** showed potent hepatoprotective activities, without any obvious cytotoxic effects (see Table 5), while the other compounds tested were inactive at 1×10^{-4} M.

Experimental Section

General Experimental Procedures. The optical rotations were measured on a Jasco P-2000 polarimeter. The UV spectra were scanned by a Jasco V650 spectrophotometer. IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. ¹H NMR (500 or 600 MHz), ¹³C NMR (125 MHz), and 2D-NMR spectra were run on INOVA 500 and 600 MHz spectrometers. HRESIMS were performed on a Finnigan LTQ FT mass spectrometer. The ESI mass spectra were recorded on an Agilent 1100 series LC/MSD TOF from Agilent Technologies. Column chromatography was performed with macroporous resin (Diaion HP-20, Mitsubishi Chemical Corp., Tokyo, Japan), Rp-18 (50 μm, YMC, Kyoto, Japan), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden), and silica gel (100–200, 200–300 mesh, Qingdao Marine Chemical Inc. Qingdao, People's Republic of China). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with an SPD-20A detector, using a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm). HPLC-DAD analysis was performed using an Agilent 1200 series system (Agilent Technologies, Waldbronn, Germany) with an Apollo C18 column (250 mm × 4.6 mm, 5 μm; Grace Davison). Precoated silica gel GF-254 plates (Yantai Jiangyou Silica Gel Exploitation Company) were used for analytical TLC.

Plant Material. The roots and stems of *E. hainanensis* were collected in Hainan Province, People's Republic of China, in March 2008. The plant material was identified by Mr. Huanqiang Chen (Jianfengling National Nature Reserve of Hainan Province). A voucher specimen (ID-21741) was deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China.

Extraction and Isolation. The dried roots and stems of *E. hainanensis* (22.5 kg) were extracted with 95% EtOH under reflux (3×1.5 h). The EtOH extract was concentrated under reduced pressure to give a residue (1.3 kg), which was suspended in H₂O (7500 mL) with the suspension sequentially partitioned with petroleum ether (3×6000

mL), EtOAc (3×6000 mL), and *n*-BuOH (3×5000 mL), successively. After evaporation of the solvent under reduced pressure, the *n*-BuOH extract (450 g) was subjected to column chromatography over macroporous resin, eluting successively with H₂O, 15% EtOH, 30% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH (20 L each). After removing the solvent, the 30% EtOH fraction (30 g) was subjected to chromatography over Sephadex LH-20 with H₂O as the mobile phase to yield eight fractions (A1–A8) on the basis of HPLC-DAD analysis. Fraction A3 (1.0 g) was subjected to reversed-phase preparative HPLC, using MeOH–H₂O (33:67) as the mobile phase, to give **1** (300 mg), **2** (9 mg), and **6** (15 mg). Fraction A4 (1.1 g) was chromatographed over reversed-phase silica gel, eluting with a gradient of increasing MeOH (0–45%) in H₂O, to yield five subfractions (A4-1–A4-5). Subfractions A4-3 (50 mg) and A4-4 (200 mg) were further separated by reversed-phase preparative HPLC, using MeOH–H₂O (30:70 and 38:62) as the mobile phase, respectively, to afford **4** (10 mg), and **9** (30 mg), **10** (11 mg), and **11** (50 mg). Fractions A5 (200 mg) and A8 (150 mg) were separately subjected to reversed-phase preparative HPLC, for fraction A5 using MeOH–H₂O (33:67) as the mobile phase, to afford **3** (10 mg), **5** (10 mg), and **7** (12 mg), for fraction A8 using MeOH–H₂O (35:65) as the mobile phase, to afford **8** (15 mg). After removal of solvent, the EtOAc extract (100 g) was applied to a normal-phase silica gel column. Successive elution of the column with a gradient of increasing acetone (0–100%) in petroleum ether afforded six fractions (B1–B6) on the basis of HPLC-DAD analysis. Fraction B3 (3.5 g) was further chromatographed over a normal-phase silica gel column eluting with a gradient of increasing EtOAc (0–100%) in petroleum ether, to afford five subfractions (B3-1–B3-5). Subfraction B3-4 (500 mg) was purified by reversed-phase preparative HPLC, using a mobile phase of MeOH–H₂O (50:50), to yield **18** (25 mg). Fraction B5 (20 g) was subjected to chromatography over Sephadex LH-20 with a gradient of increasing MeOH (0–100%) in H₂O as the mobile phase, to give five subfractions (B5-1–B5-5). Subfractions B5-3 (1000 mg) and B5-4 (500 mg) were separated by reversed-phase preparative HPLC, for subfraction B5-3 using MeOH–H₂O (34:66) as the mobile phase, to afford **14** (200 mg), **15** (30 mg), and **16** (20 mg), for subfraction B5-4 using MeOH–H₂O (40:60) as the mobile phase, to afford **17** (10 mg).

Eryciboside A (1): white powder; [α]_D²⁰ –83.6 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 282 (4.16), 341 (3.89) nm; IR ν_{\max} 3429, 1734, 1615, 1567, 1516, 1459, 1280, 1222, 1069, 867, 822, 763 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) data, see Table 1; ¹³C NMR (DMSO-*d*₆, 125 MHz) data, see Table 3; (–)-ESIMS m/z 665 [M – H][–]; (–)-HRESIMS m/z 665.1704 (calcd for C₃₀H₃₃O₁₇, 665.1712).

Eryciboside B (2): white powder; [α]_D²⁰ –39.3 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 283 (4.16), 340 (3.87) nm; IR ν_{\max} 3382, 1710, 1613, 1565, 1513, 1462, 1279, 1223, 1114, 863, 826, 761 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) data, see Table 1; ¹³C NMR (DMSO-*d*₆, 125 MHz) data, see Table 3; (+)-ESIMS m/z 689 [M + Na]⁺; (+)-HRESIMS m/z 689.1686 (calcd for C₃₀H₃₄O₁₇Na, 689.1688).

Eryciboside C (3): white powder; [α]_D²⁰ –33.6 (c 0.03, MeOH); UV (MeOH) λ_{\max} (log ϵ) 282 (4.12), 340 (3.86) nm; IR ν_{\max} 3368, 1703, 1614, 1568, 1514, 1465, 1279, 1207, 1104, 859, 817, 760 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) data, see Table 1; ¹³C NMR (DMSO-*d*₆, 125 MHz) data, see Table 3; (–)-ESIMS m/z 665 [M – H][–]; (+)-HRESIMS m/z 689.1682 (calcd for C₃₀H₃₄O₁₇Na, 689.1688).

Eryciboside D (4): white powder; [α]_D²⁰ –62.4 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 260 (4.06), 290 (4.02), 340 (3.85) nm; IR ν_{\max} 3395, 1711, 1611, 1564, 1514, 1281, 1072, 820, 763 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) data, see Table 1; ¹³C NMR (DMSO-*d*₆, 125 MHz) data, see Table 3; (+)-ESIMS m/z 637 [M + H]⁺; (+)-HRESIMS m/z 659.1583 (calcd for C₂₉H₃₂O₁₆Na, 659.1583).

Eryciboside E (5): white powder; [α]_D²⁰ –32.9 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 261 (4.15), 290 (4.11), 343 (3.95); IR ν_{\max} 3407, 1685, 1611, 1565, 1513, 1461, 1284, 1074, 864, 822, 760 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) data, see Table 1; ¹³C NMR (DMSO-*d*₆, 125 MHz) data, see Table 3; (–)-ESIMS m/z 635 [M – H][–]; (+)-HRESIMS m/z 659.1579 (calcd for C₂₉H₃₂O₁₆Na, 659.1583).

Eryciboside F (6): white powder; [α]_D²⁰ –3.8 (c 0.11, MeOH); UV (MeOH) λ_{\max} (log ϵ) 285 (4.24), 338 (3.77) nm; IR ν_{\max} 3416, 1709, 1610, 1568, 1514, 1462, 1337, 1223, 1116, 850, 763 cm⁻¹; ¹H NMR (DMSO-*d*₆, 500 MHz) data, see Table 1; ¹³C NMR (DMSO-*d*₆, 125 MHz) data, see Table 3; (+)-ESIMS m/z 719 [M + Na]⁺; (+)-HRESIMS m/z 719.1801 (calcd for C₃₁H₃₆O₁₈Na, 719.1794).

Eryciboside G (7): white powder; $[\alpha]_D^{20}$ -50.6 (c 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 217 (4.46), 277 (4.09) nm; IR ν_{\max} 3399, 1702, 1610, 1515, 1462, 1335, 1220, 1112, 763 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) data, see Table 2; ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 3; (+)-ESIMS m/z 557 $[\text{M} + \text{Na}]^+$; (+)-HRESIMS m/z 557.1841 (calcd for $\text{C}_{23}\text{H}_{34}\text{O}_{14}\text{Na}$, 557.1841).

Eryciboside H (8): white powder; $[\alpha]_D^{20}$ -41.4 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 219 (4.60), 268 (4.20), 284 (sh) (4.17); IR ν_{\max} 3390, 1697, 1613, 1515, 1461, 1336, 1226, 1114, 830, 763 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) data, see Table 2; ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 3; (+)-ESIMS m/z 635 $[\text{M} + \text{Na}]^+$; (+)-HRESIMS m/z 635.1951 (calcd for $\text{C}_{28}\text{H}_{36}\text{O}_{15}\text{Na}$, 635.1946).

Eryciboside I (9): white powder; $[\alpha]_D^{20}$ -38.7 (c 0.06, MeOH); UV (MeOH) λ_{\max} (log ϵ) 216 (sh) (4.52), 278 (4.09) nm; IR ν_{\max} 3403, 1701, 1610, 1515, 1461, 1335, 1218, 1113, 764 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) data, see Table 2; ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 3; (-)-ESIMS m/z 701 $[\text{M} - \text{H}]^-$; (+)-HRESIMS m/z 725.2983 (calcd for $\text{C}_{33}\text{H}_{50}\text{O}_{16}\text{Na}$, 725.2991).

Eryciboside J (10): white powder; $[\alpha]_D^{20}$ -36.9 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 216 (sh) (4.46), 278 (4.06) nm; IR ν_{\max} 3401, 1699, 1611, 1515, 1462, 1336, 1223, 1116, 764 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) data, see Table 2; ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 3; (-)-ESIMS m/z 717 $[\text{M} - \text{H}]^-$; (+)-HRESIMS m/z 741.2946 (calcd for $\text{C}_{33}\text{H}_{50}\text{O}_{17}\text{Na}$, 741.2940).

Eryciboside K (11): white powder; $[\alpha]_D^{20}$ -50.7 (c 0.06, MeOH); UV (MeOH) λ_{\max} (log ϵ) 217 (4.49), 278 (4.14) nm; IR ν_{\max} 3360, 1701, 1609, 1515, 1461, 1334, 1216, 1111, 763 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) data, see Table 2; ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 3; (-)-ESIMS m/z 703 $[\text{M} - \text{H}]^-$; (+)-HRESIMS m/z 727.3148 (calcd for $\text{C}_{33}\text{H}_{50}\text{O}_{16}\text{Na}$, 727.3170).

5-O-Caffeoyl-4-O-syringoylquinic acid (14): white powder; $[\alpha]_D^{20}$ -106.7 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 249 (4.10), 289 (4.32), 331 (4.25) nm; IR ν_{\max} 3374, 1695, 1605, 1516, 1461, 1346, 1278, 1223, 1114, 764 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 4; (-)-ESIMS m/z 533 $[\text{M} - \text{H}]^-$; (-)-HRESIMS m/z 533.1287 (calcd for $\text{C}_{25}\text{H}_{25}\text{O}_{13}$, 533.1290).

5-O-Caffeoyl-3-O-syringoylquinic acid (15): white powder; $[\alpha]_D^{20}$ -96.4 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 249 (4.11), 290 (4.34), 330 (4.26) nm; IR ν_{\max} 3421, 1693, 1608, 1517, 1462, 1334, 1278, 1233, 1115, 763 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 4; (-)-ESIMS m/z 533 $[\text{M} - \text{H}]^-$; (-)-HRESIMS m/z 533.1291 (calcd for $\text{C}_{25}\text{H}_{25}\text{O}_{13}$, 533.1290).

4-O-Caffeoyl-3-O-syringoylquinic acid (16): white powder; $[\alpha]_D^{20}$ -83.5 (c 0.05, MeOH); UV (MeOH) λ_{\max} (log ϵ) 248 (4.10), 290 (4.32), 330 (4.25) nm; IR ν_{\max} 3414, 1699, 1608, 1516, 1461, 1334, 1278, 1234, 1116, 762 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 4; (-)-ESIMS m/z 533 $[\text{M} - \text{H}]^-$; (-)-HRESIMS m/z 533.1287 (calcd for $\text{C}_{25}\text{H}_{25}\text{O}_{13}$, 533.1290).

5-O-Caffeoyl-4-O-vanilloylquinic acid methyl ester (17): white powder; $[\alpha]_D^{20}$ -108.2 (c 0.04, MeOH); UV (MeOH) λ_{\max} (log ϵ) 254 (4.16), 298 (4.28), 330 (4.26) nm; IR ν_{\max} 3407, 1694, 1600, 1516, 1432, 1282, 1217, 1155, 763 cm^{-1} ; ^1H NMR (DMSO- d_6 , 600 MHz) and ^{13}C NMR (DMSO- d_6 , 125 MHz) data, see Table 4; (-)-ESIMS m/z 517 $[\text{M} - \text{H}]^-$; (-)-HRESIMS m/z 517.1337 (calcd for $\text{C}_{25}\text{H}_{25}\text{O}_{12}$, 517.1341).

7-Hydroxy-6,6'-dimethoxy-3,7'-O-bis-coumarin (18): yellowish powder; UV (MeOH) λ_{\max} (log ϵ) 286 (3.95), 351 (4.09) nm; IR ν_{\max} 3406, 1716, 1571, 1511, 1456, 1281, 1135, 1013, 861 cm^{-1} ; ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.61 (1H, s, H-4), 7.18 (1H, s, H-5), 6.85 (1H, s, H-8), 6.41 (1H, d, $J = 9.5$ Hz, H-3'), 8.03 (1H, d, $J = 9.5$ Hz, H-4'), 7.48 (1H, s, H-5'), 7.23 (1H, s, H-8'), 3.79 (3H, s, OMe-6), 3.88 (3H, s, OMe-6'), 10.21 (1H, s, OH-7); ^{13}C NMR (DMSO- d_6 , 125 MHz) δ 156.6 (C-2), 137.0 (C-3), 127.3 (C-4), 109.2 (C-5), 145.6 (C-6), 149.8 (C-7), 102.7 (C-8), 146.5 (C-9), 110.2 (C-10), 160.1 (C-2'), 114.57 (C-3'), 144.0 (C-4'), 110.8 (C-5'), 146.8 (C-6'), 147.9 (C-7'), 106.1 (C-8'), 148.4 (C-9'), 114.61 (C-10'), 56.3 (OMe-6), 56.0 (OMe-6'); (-)-ESIMS m/z 381 $[\text{M} - \text{H}]^-$; (-)-HRESIMS m/z 381.0592 (calcd for $\text{C}_{20}\text{H}_{13}\text{O}_8$, 381.0605).

Acid Hydrolysis of 1. A solution of **1** (20 mg) in 0.1 N HCl (5 mL) was refluxed for 20 min under N_2 atmosphere. On cooling, the reaction mixture was cryodesiccated, and the residue was subjected to reversed-phase preparative HPLC, using MeOH-H₂O (30:70) as the mobile phase, to give scopolin (5 mg), which was identified by

comparing with an authentic standard on HPLC-DAD. The scopolin dissolved in 1 N HCl (5 mL) was refluxed for 3 h. After cooling, the reaction mixture was extracted with EtOAc (3 \times 5 mL). The aqueous layer was cryodesiccated to afford D-glucose, which was identified by comparison with an authentic sample on TLC (CH_2Cl_2 -MeOH-HOAc-H₂O, 14:6:2:1, R_f 0.27) and by its specific rotation, $[\alpha]_D^{20}$ $+47.4$ (c 0.2, H₂O).

Alkaline Hydrolysis of 14, 15, and 17. To each solution of **14**, **15**, and **17** (1.0 mg) in MeOH (1.0 mL) was added one drop of 1 N NaOH, and the mixture was stirred at room temperature. After 15 min the reaction mixture was neutralized with 0.1 N HCl and filtrated through a 0.45 μm filter for injection. HPLC-DAD analysis was performed on a C18 column using MeOH-0.2% HOAc (35:65) as mobile phase. 5-O-Caffeoylquinic acid was identified by comparing the retention time and UV spectrum with the authentic standard.

Protective Effect on Cytotoxicity Induced by D-Galactosamine in WB-F344 Cells. The hepatoprotective effects of compounds **1-16** and **18-32** were determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay in WB-F344 cells.³⁴ Each cell suspension of 1×10^4 cells in 200 μL of Dulbecco's modified Eagle's medium containing fetal calf serum (3%), penicillin (100 units/mL), and streptomycin (100 $\mu\text{g}/\text{mL}$) was placed in a 96-well microplate and precultured for 24 h at 37 $^\circ\text{C}$ under a 5% CO_2 atmosphere. Fresh medium (200 μL) containing bicyclol and test samples was added, and the cells were cultured for 1 h. The cultured cells were exposed to 40 mM D-galactosamine for 24 h. The cytotoxic effects of test samples were measured simultaneously in the absence of D-galactosamine. The medium was changed into a fresh one containing 0.5 mg/mL MTT. After 3.5 h incubation, the medium was removed and 150 μL of DMSO was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm. Inhibition (%) was obtained by the following formula: Inhibition (%) = $[(\text{OD}_{(\text{sample})} - \text{OD}_{(\text{control})}) / (\text{OD}_{(\text{normal})} - \text{OD}_{(\text{control})})] \times 100$.

Statistical Analysis. The Student's t -test for unpaired observations between normal and tested samples was carried out to identify statistical differences; p values less than 0.05 were considered significantly different.

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Supporting Information Available: NMR spectra of compounds **1-11** and **14-18**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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